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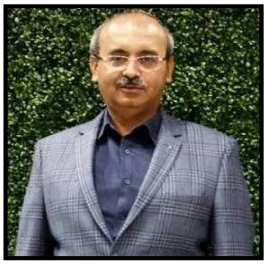

STEREOSELECTIVE SYNTHESIS OF (R)-AMINO INDANS USING TRANSAMINASE ENZYMES

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ABSTRACT

An improved process for amination of indanones for the synthesis of (R)-1-amino-2, 3 dihydro 1H-indene-4-carbonitrile, an important intermediate of pyridinyl-containing carboxamides as β2-adrenoceptor agonists for the treatment of inflammatory, allergic; Lung diseases; Respiratory distress syndrome[1] and an important intermediate of Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and (R)-1-aminoindan which is key intermediate for Rasagiline (Azilect®) is a selective and irreversible monoamine oxidase B inhibitor, which is well tolerated, safe, improves motor symptoms, and prevents motor complications in Parkinson's disease (PD)[2], in a Single step complete conversion was achieved using transaminase enzymes with high enantioselectivity and better yield in comparison with chemical processes. 100% chiral purity has been obtained for the enantiomerically pure intermediate, confirmed by TLC and HPLC.

KEYWORDS

Pyridinyl-containing carboxamides, β2-adrenoceptor agonists, Inflammatory, Allergic, Lung diseases, Respiratory distress syndrome, Coronavirus disease 2019 (COVID-19), Severe acute respiratory syndrome coronavirus 2, (SARS-CoV-2), Transaminase enzymes, 4-cyano indanone, (R)-1-amino-2, 3 dihydro 1H-indene-4-carbonitrile, Rasagiline and R-1-aminoindan enantioselectivity.

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INTRODUCTION

(R)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile is one of the most important intermediates of pyridinyl containing carboxamides as β2-adrenoceptor agonists for the treatment of inflammatory, allergic; Lung diseases; Respiratory distress syndrome and COVID-19. (R)-1-

aminoindan is the intermediate of Rasagiline which is used to treat Parkinson's disease.

In both processes, racemic 1-amino-2,3-dihydro-1H-indene-4-carbonitrile (Scheme No.1) and 1-aminoindan (Scheme No.2) formed needs to be further resolved to obtain the required (R)-1-amino-2,3-dihydro-1H-indene-4-carbonitrile and 1-aminoindan. These reactions are involves multiple steps and extreme reaction conditions and high catalytic loading[3,4]. This encourages us to look upon the biocatalytic approach for the preparation of the above drug intermediates.

Transaminases catalyze the transfer of an amino group from a primary amine to a prochiral ketone. Transaminases are highly enantioselective, so they can be used for synthesizing chiral amines from inexpensive ketones. The ketone compound can be directly aminated in a single step with better enantiomeric purity and good yields than the chemical processes.

Our aim was in developing an improved process for synthesis of (R)-1-amino-2, 3-dihydro 1H-indene-4-carbonitrile, using transaminase enzymes with high yield and enantiomeric purity.

MATERIAL AND METHODS

Reagents and Chemicals

In the experimental section, unless and otherwise stated, all reagents and solvents used in this study are commercially obtained. Transaminase enzyme were obtained from Enzyme Works, China, evoXX and Iosynth Labs Private Limited, India.

METHODOLOGY

Experimental Section, All reaction were carried out under similar conditions as mentioned below.

Step I: Preparation of Buffer Solution

In a flask, secondary butylamine (0.2g) Water (1.8ml) Triethanolamine (0.05g) and Pyridoxal phosphate (0.70mg) were taken at 25°C and cooled to 10-15°C. pH was adjusted to 8 using conc. HCl and the temperature was raised to 20-25°C and Water (0.5ml) was added.

Step II: Preparation of (R)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile

To the buffer solution (2.69ml), Transaminase enzyme (30mg) was added. 4-cyano indanone (4.68mg) and Dimethyl sulfoxide (0.3ml) were charged. Temperature was raised to 40°C and maintained at same for 96 hrs. Reaction was monitored by TLC. After completion of the reaction, to the reaction mixture, 10% sodium hydroxide solution (0.2ml) was added and stirred for 30 mins at 20-25°C. The resulting reaction mass was extracted with ethyl acetate and distilled under vacuum to yield (R)-1-amino-2,3-dihydro-1H-indene-4-carbonitrile (4.68mg), which was further confirmed by sending the sample for chiral purity.

Reaction scheme for the synthesis of (R) 1-amino-2,3-dihydro-1H-indene-4-carbonitrile (Scheme No.3)

TLC Conditions

To the TLC plate, were applied spots of our keto compound and final product which was immersed in a mobile phase of following composition-Dichloromethane: Methanol = 9:1 respectively. The plate was then viewed under UV light of 254nm after sprayed with Ninhydrin (1% in ethanol).

RESULTS AND DISCUSSION

Several variants of transaminase enzymes were screened in the following study for conversion of keto compound to our product (R)-1-amino-2,3 dihydro-1H-indene-4-carbonitrile and the results are as follows in Table No.1.

Required (R)-amine compound was formed with 100% Chiral Purity with the following enzymes. CN102-ATA-LP049, CN102-ATA-LP050, CN102-ATA-LP051, CN102-ATA-LP052, CN102-ATA-LP053, CN102-ATA-LP054, CN102-ATA-LP055, CN102-ATA-LP056, ATA.EW.134.

Mass result for the transaminase enzyme ATA.EW.134 produced (R)-amine compound is as follows (Figure No.1).

$C_{10}H_{10}N_2$ = 158; found 142.2 $[M\text{-}NH_2]^5$

Product conversion observed by TLC as shown below (Figure No.2)

To the TLC plate, were applied spots of our keto compound and final product which was immersed

in a mobile phase of following composition- Dichloromethane: Methanol = 9:1 respectively[6]. The plate was then viewed under UV light of 254nm after sprayed with Ninhydrin (1% in ethanol).

The chiral HPLC data for the intermediate prepared through synthetic route is as follows (Figure No.3).

For 100% Chiral purity of our desired (R)-amine compound prepared using transaminase enzymes, the Chiral HPLC data is as follows (Figure No.4).

(R)-1-amino-2, 3 dihydro-1H-indene-4-carbonitrile was synthesized in single step with complete conversion using transaminase enzyme.

Recycle and reuse of enzyme

Transaminase can be recycled by following ways: Adjusting pH of aqueous layer of reaction mass to pH 8 and it used for similar consecutive reactions until enzyme is active; by using immobilized enzymes in which after completion of reaction, enzyme can be filtered and recycled till enzyme shows activity. Immobilization of enzymes enhances the catalytic performance of an enzyme and enables its reuse in successive reactions[7]. But the moderate functional stability upon immobilization dampens the reuse of transaminases in organic synthesis, although several studies have been reported on successful immobilization of transaminases aimed at improving its overall catalytic performance[8].

A recent study demonstrated successful immobilization of two stereo complementary ω-transaminases from Arthrobacter sp. (AsR-ωTA) and Chromobacterium violaceum (Cv-ωTA) onto polymer-coated controlled porosity glass beads (EziG™), which retained increased activity in single batch experiments and could be recycled for at least 16 consecutive cycles without compromise in conversion[9].

Usually the lyophilized non immobilized form of transaminase enzymes was completely denatured and showed no activity under the same conditions. However recent reports show Transaminase enzymes[10] can be recovered and recycled in subsequent reactions by filtration of enzyme at the end of the reaction till enzyme shows activity.

Experimental section for reuse of enzyme precipitate of Previous Experiments

Step A: Preparation of Buffer Solution

In a flask charge precipitate of previous batch (After completion of reaction using fresh enzyme Centrifuge the reaction mixture and decant reaction mass and store the precipitate below -20°C and reuse it for subsequent cycles until it is active).Then charge secondary butylamine (0.2 g), Triethanolamine (0.05g) and Pyridoxal phosphate (0.70mg) were taken at 25°C and cooled to 10-15°C. pH was adjusted to 8 using conc. HCl and the temperature was raised to 20-25°C.

Step B: Preparation of (R)- 1-amino-2, 3-dihydro-1H-indene-4-carbonitrile

To the above buffer solution added. 4-cyano indanone (4.68mg) and Dimethyl sulfoxide (0.3ml) were charged. Temperature was raised to 40°C and maintained at same for 96 hrs. Reaction was monitored by TLC. Reaction was incomplete (Observed only ~ 10% of the product).

Experimental section for reuse of lyophilized enzyme in aqueous layer

Step C: Preparation of Buffer Solution

In a flask charge Aq layer of previous batch and adjust pH to 8.0 using Conc hydrochloric acid, then charge secondary butylamine (0.2g), Triethanolamine (0.05g) and Pyridoxal phosphate (0.70mg) were taken at 25°C and cooled to 10-15°C. pH was adjusted to 8 using conc. HCl and the temperature was raised to 20-25°C.

Step D: Preparation of (R)- 1-amino-2, 3-dihydro-1H-indene-4-carbonitrile

To the above buffer solution added. 4-cyano indanone (4.68mg) and Dimethyl sulfoxide (0.3ml) were charged. Temperature was raised to 40°C and maintained at same for 24-96 hrs. Reaction was monitored by TLC. Reaction was incomplete (Observed only ~ 10% of the product).

Table No.1: Transaminase enzymevariants with Product formation and no starting material present in studied reaction conditions

S.No	Enzyme Variant	Enzyme Qty	Chiral Purity	TLC Results
1	CN102-ATA-LP049	6.4 T	100%	Product formation observed and No starting material
2	CN102-ATA-LP050	6.4 T	100%	Product formation observed and No starting material
3	CN102-ATA-LP051	6.4 T	100%	Product formation observed and No starting material
4	CN102-ATA-LP052	6.4 T	100%	Product formation observed and No starting material
5	CN102-ATA-LP053	6.4 T	100%	Product formation observed and No starting material
6	CN102-ATA-LP054	6.4 T	100%	Product formation observed and No starting material
7	CN102-ATA-LP055	6.4 T	100%	Product formation observed and No starting material
8	CN102-ATA-LP056	6.4 T	100%	Product formation observed and No starting material
9	ATA.EW.134	6.4 T	100%	Product formation observed and No starting material

Table No.2: Enzyme variants with slight Product formation and presence of Starting material in studied reaction conditions

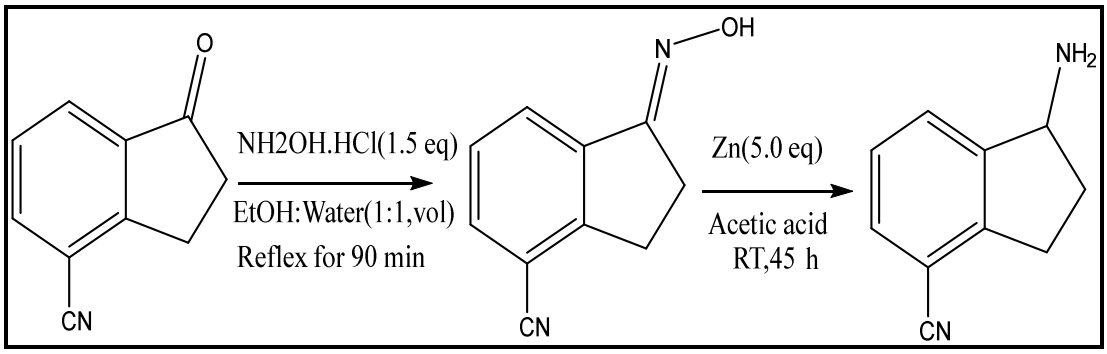
S.No	Enzyme Variant	Enzyme Qty	TLC Results
1	ATA.EW.124	6.4 T	~10% SM present
2	ATA.EW.130	6.4 T	~10% SM present
3	ATA.EW.131	6.4 T	~30% SM present
4	evo 1.1.116	6.4 T	~10% SM present
5	evo 1.1.134	6.4 T	~20% SM present

Table No.3: Enzyme variants with very little product formation in studied reaction conditions

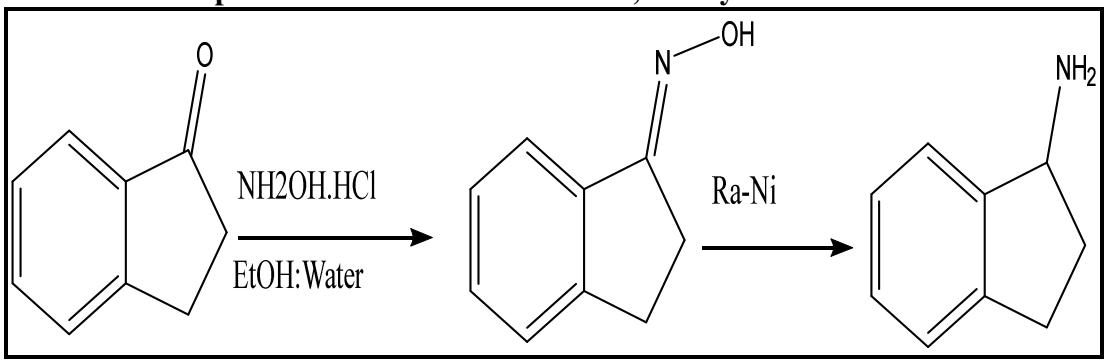
S.No	Enzyme Variant	Enzyme Qty	TLC Results
1	CN102-ATA-LP003	1.2 T	Very little product formed
2	CN102-ATA-LP004	1.2 T	Very little product formed
3	evo 1.2.128	7.0 T	Very little product formed
4	evo 1.2.116	7.0 T	Very little product formed
5	evo 1.2.129	7.0 T	Very little product formed

Table No.4: Enzyme variants with no product formation in studied reaction conditions

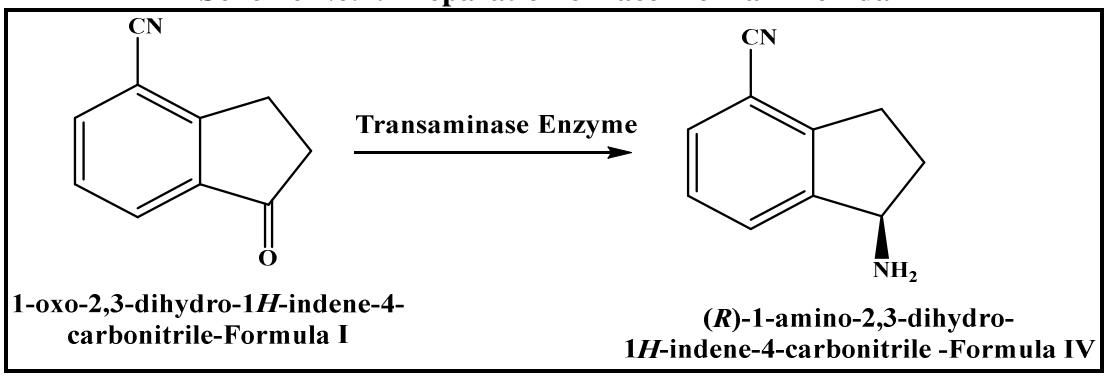
S.No	Enzyme Variant	Enzyme Qty	TLC Results
1	CN102-ATA-LP035	6 T	No product formation
2	CN102-ATA-LP036	6 T	No product formation
3	CN102-ATA-LP037	6 T	No product formation
4	CN102-ATA-LP038	6 T	No product formation
5	CN102-ATA-LP039	6 T	No product formation
6	CN102-ATA-LP041	6 T	No product formation
7	CN102-ATA-LP042	6 T	No product formation
8	CN102-ATA-LP043	6 T	No product formation
9	CN102-ATA-LP044	6 T	No product formation
10	CN102-ATA-LP045	6 T	No product formation



Scheme No.1: Preparation of racemic-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile



Scheme No.2: Preparation of racemic 1-aminoindan



Scheme No.3: Preparation of (R)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile using Transaminase

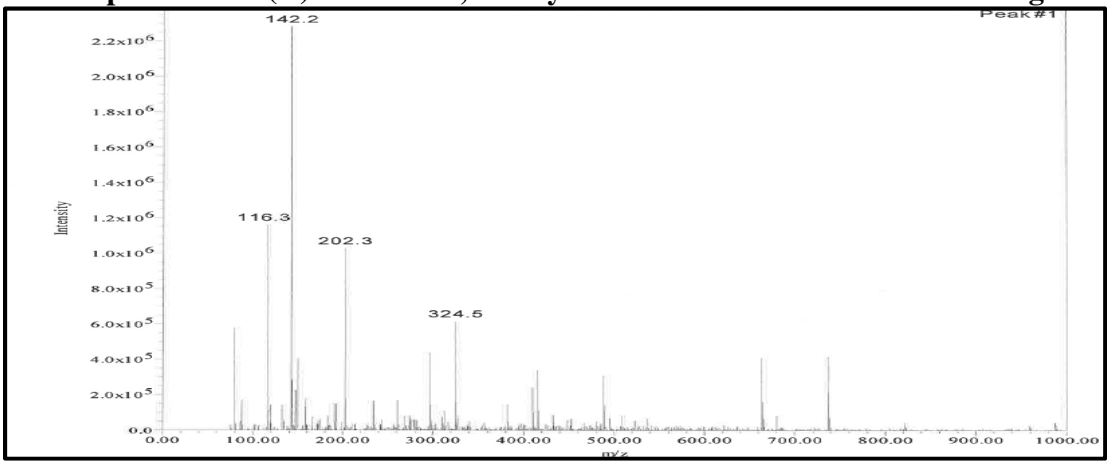


Figure No.1: Mass spectrum of (R)-1-amino-2, 3 dihydro-1H-indene-4-carbonitrile

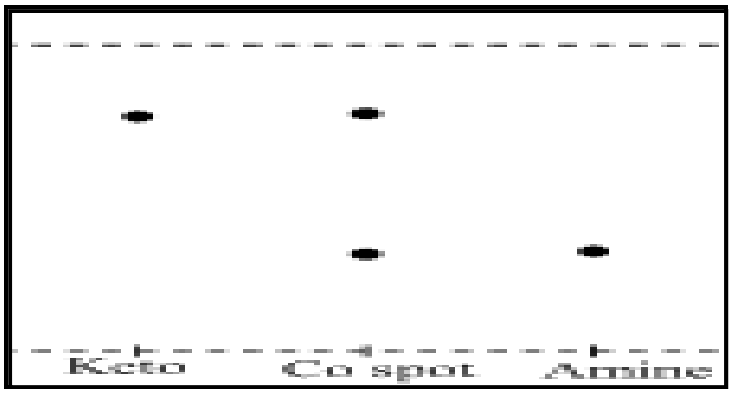


Figure No.2: TLC Pattern of starting material and product conversion

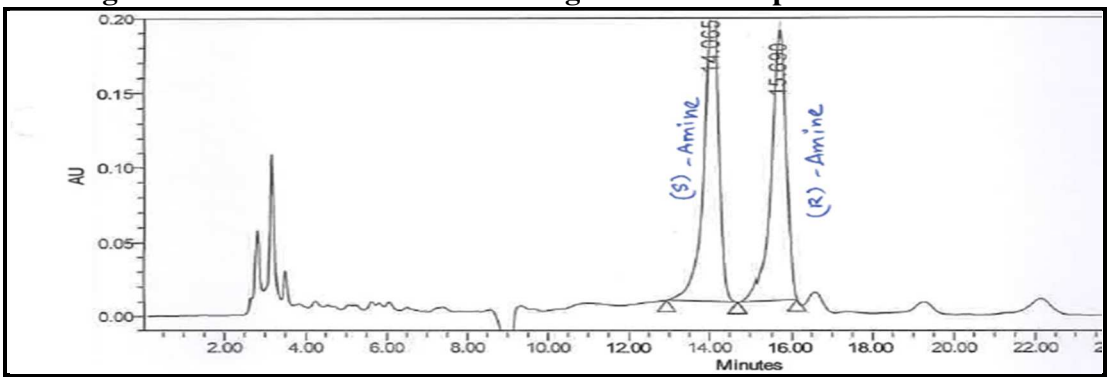


Figure No.3: HPLC chromatogram of racemic 1-amino-2, 3 dihydro-1H-indene-4-carbonitrile

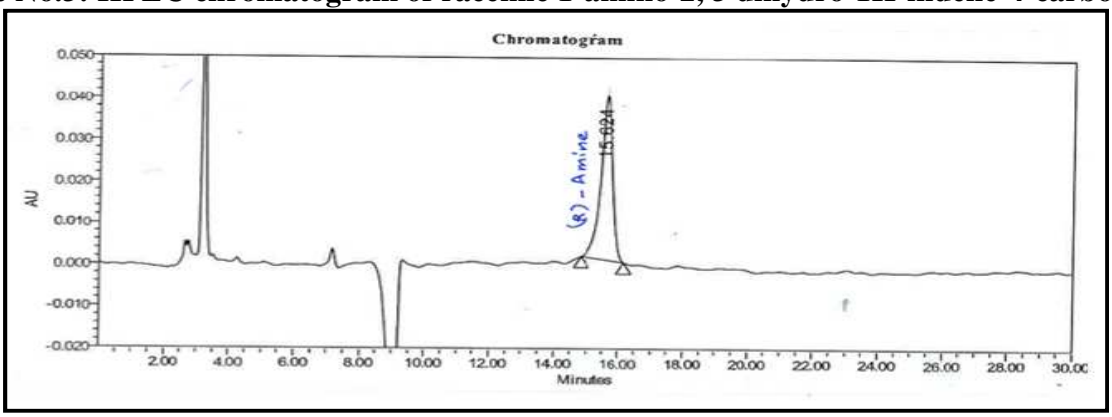


Figure No.4: HPLC chromatogram of (R)-1-amino-2, 3 dihydro-1H-indene-4-carbonitrile

CONCLUSION

It can be determined from the above study that, (R)-1-amino-2,3-dihydro-1H-indene-4-carbonitrile which is the key chiral intermediate of several of the diseases can be prepared using transaminase enzyme with 100% chiral purity and 100% yield in a single step opposed to conventional chemical processes involving multiple steps with poor yields and low enantioselectivity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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