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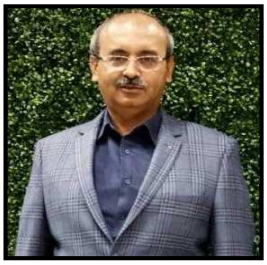

STEREOSELECTIVE SYNTHESIS OF (S)-1-AMINO-2, 3-DIHYDRO-1H-INDENE-4-CARBONITRILE USING TRANSAMINASE ENZYMES

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ABSTRACT

An improved process for amination of 4-cyanoindanone for the synthesis of (S)-1-amino-2, 3 dihydro 1H-indene-4-carbonitrile, an important intermediate of Sphingosine-1-Phosphate receptor modulators, that are used for treatment of various neurodegenerative diseases has been developed. 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, Mass and HPLC.

KEYWORDS

S1P1 Receptor Modulators, Transaminase enzymes, COVID-19, 4-cyanoindanone, (S)-1-amino-2, 3 dihydro 1H-indene-4-carbonitrile, Enantioselectivity [(S)- Isomer/ (S)-Amine] (R)-1-amino-2, 3 dihydro 1H-indene-4-carbonitrile and Enantioselectivity [(R)- Isomer/(R)-Amine].

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INTRODUCTION

Sphingolipids belong to a large group of eukaryotic cellular lipids, whose common structure is sphingosine (2-amino-1, 3-dihydroxy-octadec-4-ene). Sphingolipids are essential components of eukaryotic cell membranes, but they are localized to specific organs and tissues[1]. Nervous system has the highest number of sphingolipids where it is mainly expressed in neurons and oligodendrocytes.

Sphingosine-1-Phosphate (S1P) is an active phospholipid which results by the phosphorylation

of Sphingosine by Sphingosine Kinase-1 or 2. It is abundant in erythrocytes, brain, spleen and eyes. Sphingosine-1-Phosphate exerts positive control on cellular survival and cell growth and proliferation in glial cells[2]. But most acute/ chronic neurodegenerative diseases are associated with changes in total levels and composition of sphingolipids in different areas in nervous system.

A particular S1P threshold concentration could increase basal calcium in neurons which impairs presynaptic assembly[3]. S1P is essential for proper brain development but its cytotoxic and destructive effects on certain neuronal types have also been demonstrated.

S1P receptors have 7 transmembrane segments and are coupled to G-protein. In lymph nodes, where S1P concentration is low, lymphocytes upregulate their S1P receptor expression[4]. Dysregulation of S1P metabolism is emerging a major causative factor in various neurodegenerative diseases like Multiple Sclerosis, Alzheimers disease, Parkinson's disease, etc.

Multiple Sclerosis is a chronic inflammatory disease of the Central Nervous system which is associated with destruction of myelin sheath in neurons causing appearance of large focal lesions which in turn lead to axonal damage[5]. In MS, activated myelin-reactive T helper cells, are brought from periphery to the CNS which activates microglial cells and causes secondary demyelination. S1PR modulators are drugs that act as non-selective agonists for S1P receptors causing irreversible internalization and degradation of bound receptors, preventing them from returning to cell surface and binding excessive S1Ps that cause neurodestruction at high concentration[6].

(S)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile is one of the most important intermediates in the synthesis of S1PR modulator drugs like Ozanimod, Ponesimod etc.

1-amino-2, 3-dihydro-1H-indene-4-carbonitrile is also important for the preparation of pyridinyl-containing carboxamides as β2-adrenoceptor agonists for the treatment of inflammatory, allergic; Lung diseases; Respiratory distress syndrome[7] and COVID-19.

In Scheme No.1, racemic 1-amino-2, 3 dihydro-1H-indene-4-carbonitrile formed needs to be further resolved to obtain the required (S)-1-amino-2, 3 dihydro-1H-indene-4-carbonitrile[8]. This reaction involves multiple steps and extreme reaction conditions and high catalytic loading. This encourages us to look upon the biocatalytic approach for the preparation of the above S1PR drug intermediate.

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.

The effect of a transaminase from *Vibrio fluvialis* (VF-TA), L-alanine, GDH using glucose as a reducing agent was studied in synthesis of the aforementioned chiral intermediate which provided an ee of 25%[9].

Our aim was in developing an improved process for synthesis of (S)-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

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 (S)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile

To the buffer solution (2.69ml), transaminase enzyme (30 mg) 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 (S)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile (4.68mg), which was further confirmed by sending the sample for chiral purity.

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 254 nm 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 (S)-1-amino-2, 3 dihydro-1H-indene-4-carbonitrile and the results are as follows in Table No.1.

Required (S)-amine compound was formed with 100% Chiral Purity with the following three enzymes, ATA.EW.127, ATA.EW.135, CN102-ATA-LP034 for 6.4 T enzyme quantity.

Mass result for the transaminase enzyme ATA.EW.127 produced (S)-amine compound is as follows: $C_{10}H_{10}N_2$ = 158; found 142.2 $[M\text{-}NH_2]$[10] (Figure No.2).

Product conversion observed by TLC as shown below (Figure No.3)

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[11]. The plate was then viewed under UV light of 254 nm after sprayed with Ninhydrin (1% in ethanol).

The chiral HPLC data for the intermediate prepared through synthetic route is as follows (Figure No.4).

For 100% chiral purity of desired (S)-amine compound prepared using transaminase enzymes, the Chiral HPLC data is as follows (Figure No.5).

(S)-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[12]. 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[13].

A recent study demonstrated successful immobilization of two stereocomplementary 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[14].

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[15] 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 batch

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 -20ºC and reuse it for subsequent cycles until it is active). 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 B: Preparation of (S)-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 (S)-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 enzyme variants with Product formation and no starting material present in studied reaction conditions

S.No	Enzyme Variant	Enzyme Qty	Chiral Purity	TLC Results
1	ATA.EW.127	6.4 T	(S) Isomer 100%	Product formed. Starting material not found
2	ATA.EW.135	6.4 T	(S) Isomer 100%	Product formed. Starting material not found
3	CN102-ATA-LP034	6.4 T	(S) Isomer 100%	Product formed. Starting material not found
4	ATA.EW.126	6.4 T	(S) Isomer 97.45% (R) Isomer 2.55%	Product formed. Starting material not found
5	ATA.EW.128	6.4 T	(S) Isomer 62.18% (R) Isomer 37.82%	Product formed. Starting material not found

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

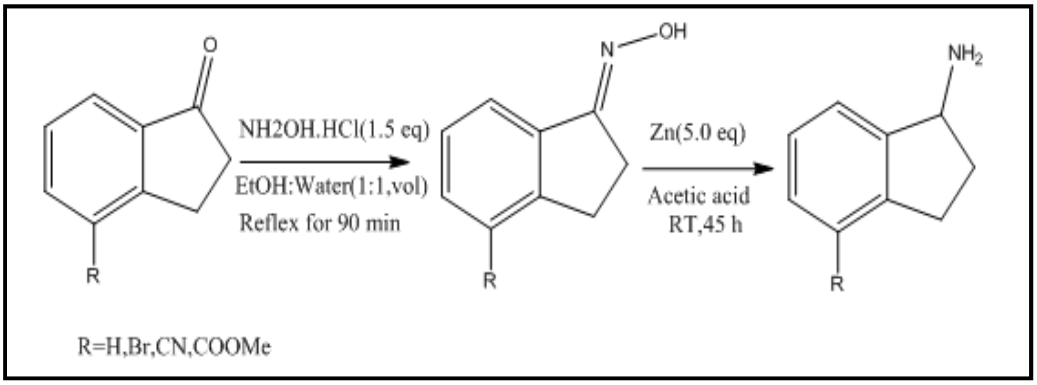
S.No	Enzyme Variant	Enzyme Qty	Results
1	ATA.EW.124	6.4 T	~10% SM present
2	ATA.EW.130	6.4 T	~10% SM present
3	ATA.EW.131	6.4 T	~30% SM present
4	evo 1.1.116	6.4 T	~10% SM present
5	evo 1.1.134	6.4 T	~20% SM present

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

S.No	Enzyme Variant	Enzyme Qty	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

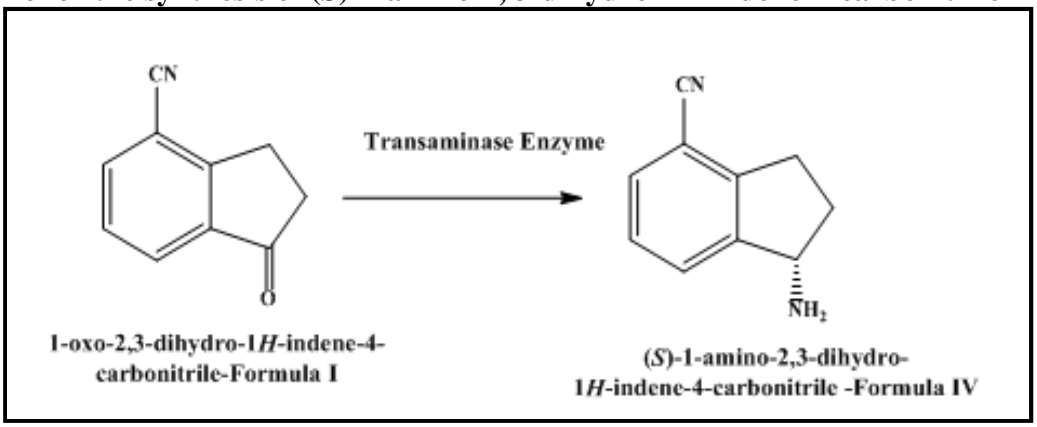
Table No.4: Enzyme variants with no product formation in studied reaction conditions

S.No	Enzyme Variant	Enzyme Qty	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: Chemical synthesis of racemic 1-amino-2, 3 dihydro-1H-indene-4 carbonitrile

Reaction scheme for the synthesis of (S)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile



Scheme No.2: Enzymatic synthesis of (S)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile

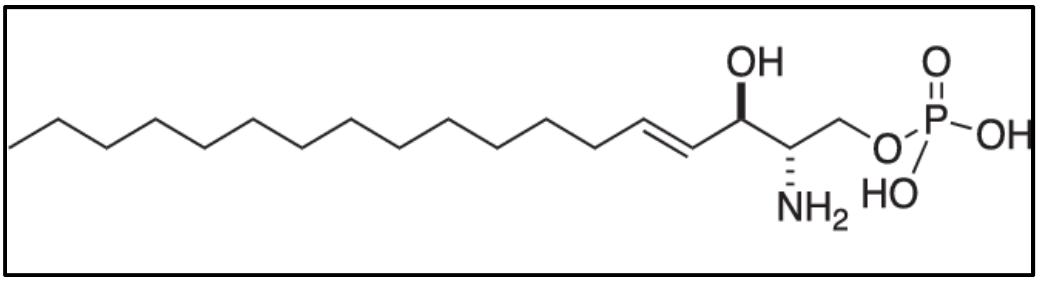


Figure No.1: Sphingosine-1-Phosphate structure

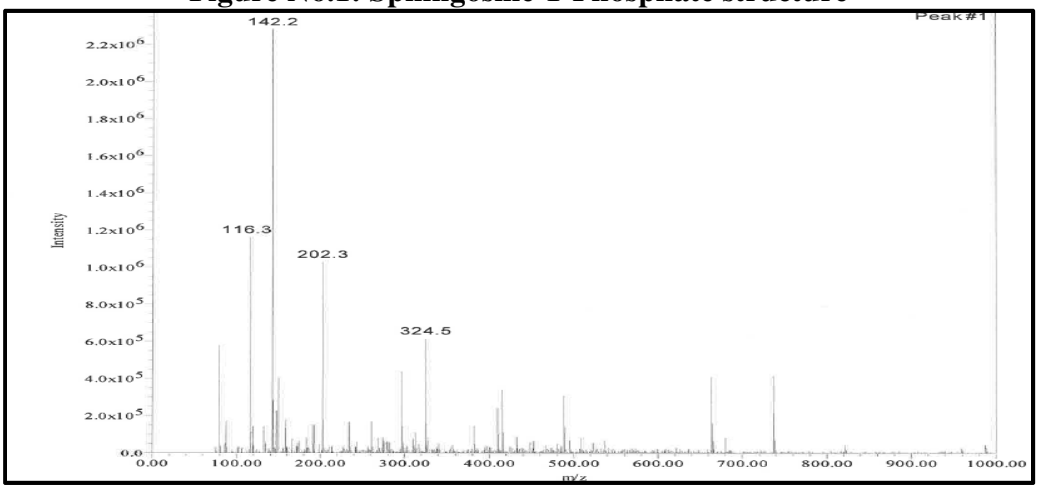


Figure No.2: Mass spectrum of (S) 1-amino-2, 3 dihydro-1H-indene-4-carbonitrile

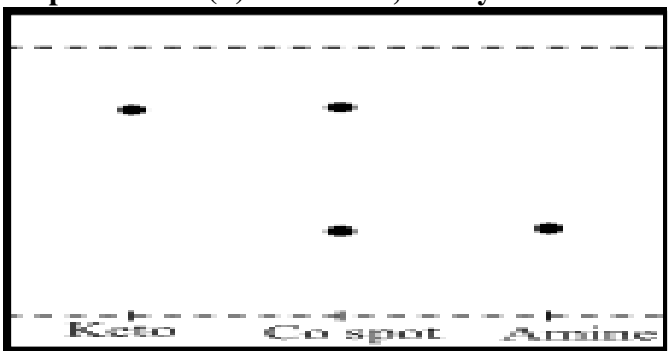


Figure No.3: TLC Pattern of starting material and product conversion

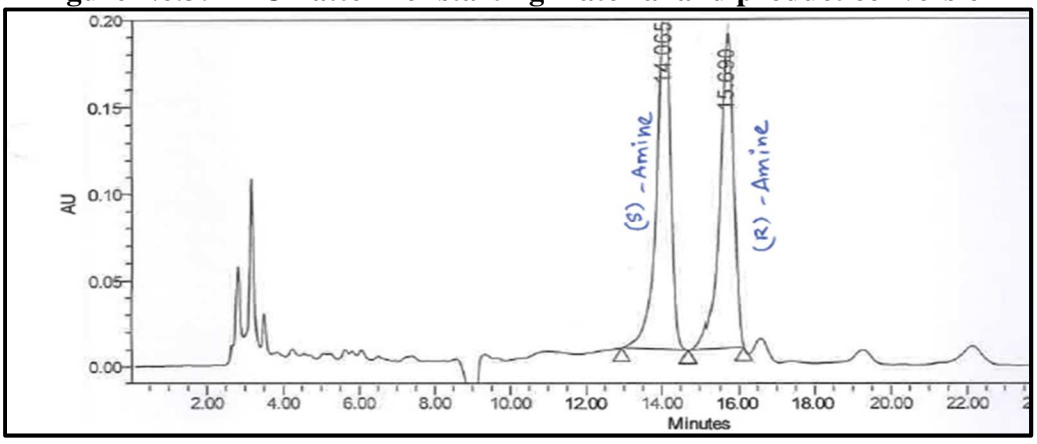


Figure No.4: HPLC chromatogram of racemic 1-amino-2, 3 dihydro-1H-indene-4-carbonitrile

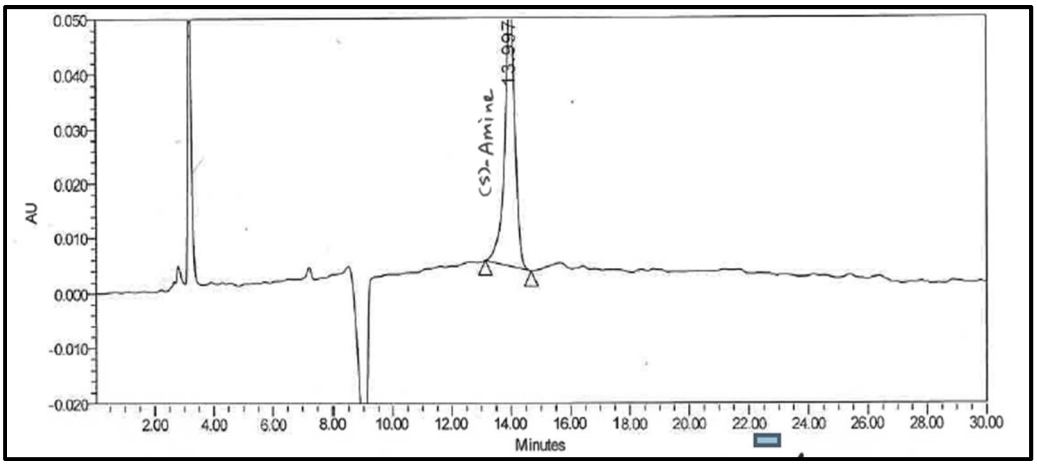


Figure No.5: HPLC chromatogram of (S)-1-amino-2, 3 dihydro-1H-indene-4-carbonitrile

CONCLUSION

It can be determined from the above study that, (S)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile which is the key chiral intermediate of several of the S1PR drugs that are used for treatment against neurodegenerative diseases can be prepared using transaminase enzyme with 100% chiral purity and good yield in a single step opposed to conventional chemical processes involving multiple steps with poor yields and low enantioselectivity.

NOTE

Since (R)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile has potential use different from it (S) isomers and (R)-1-amino-2, 3-dihydro-1H-indene-4-carbonitrile be prepared using different transaminase enzyme with 100% chiral purity and good yield in a single step; hence it is published separately.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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