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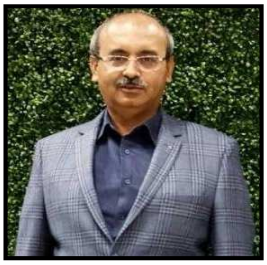

IDENTIFYING KETOREDUCTASES THAT PREFERENTIALLY SYNTHESIZE PHARMACEUTICALLY RELEVENT (S)-1-HYDROXY-2, 3-DIHYDRO-1H-INDENE-4-CARBONITRILE

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ABSTRACT

An efficient Stereoselective reduction of 4-cyanoindanone for the synthesis of 1-hydroxy-2,3 dihydro-1H-indene-4-carbonitrile, has been developed. (S)-1-hydroxy-2,3 dihydro-1H-indene-4-carbonitrileplays an important role in synthesis of IDO1 inhibitors that are used for cancer treatment and chronic viral infections; synthesis of GPR40 receptor modulators for treating metabolic diseases, especially diabetes type 2; synthesis of pyrethroid compounds with insecticidal, nematocidal and acaricidal properties. Single step conversion was achieved using ketoreductase enzymes with better enantioselectivity and yield when compared to chemical processes. >96% chiral purity was obtained for (S)-alcohol using different ketoreductase enzymes, with 100% yield way better than that obtained in chemical route. This was further confirmed by TLC and HPLC.

KEYWORDS

4-cyanoindanone, 1-indanol, IDO1 Inhibitors, Cancer treatment, GPR40 receptor modulators, Diabetes Type II, Pyrethroid compounds, Insecticides, Anti-parasitic, Acaricides, Ketoreductases, 4-cyano-1-indanol, (S)-1-hydroxy-2 and 3 dihydro 1H-indene-4-carbonitrile.

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INTRODUCTION

Indoleamine 2, 3-dioxygenase-1 (IDO1) is a heme-containing enzyme physiologically expressed in number of tissues and cells and a therapeutic target for the treatment of cancer, chronic viral infections, and other diseases characterized by pathological immune suppression. O-benzylhydroxylamine, a commercially available compound, is a potent inhibitor of IDO1. 2, 3-dihydro-indanol is used in

the synthesis of target compound, a O-benzylhydroxylamine derivative in 100% yield[1].

1-indanol could be used in coupling reactions for synthesis of various internal alkenes[2]. Dehydration of alcohols have been widely studied and applied in simple manufacture of olefins and other valuable intermediates[3].

Diabetes mellitus is a disease-state of chronic hyperglycemia associated with metabolic dysfunction and organ damages. Diabetes mellitus type II typically occurs in older age and is associated with insulin resistance in liver and skeletal muscles and partially in islet of Langerhans. The free fatty acid receptor GPR40 is a cell-surface receptor and a member of the gene super family of G-protein coupled receptors. GPR40 is found to be highly expressed in several cell types, but particularly in the pancreatic β cells and insulin-secreting cell lines.

The fatty acids that serve as activators for GPR40 increase the elevated plasma glucose-induced secretion of insulin through GPR40 receptors that are expressed in the insulin secreting cells. This might chronically lead to development of insulin resistance/ imbalances that may lead to Type II DM. The therapeutic agents capable of modulating GPR40 function could be useful for the treatment of diabetes and conditions associated with the disease, including insulin resistance, obesity and cardiovascular disease.

Chiral alcohols are important intermediates for synthesis of several of chiral compounds and medicines such as Synthetic pyrethroids; a group of ester compounds with insecticidal properties[4] as well as for the preparation of hormones, flavors and fragrances. Synthetic pyrethroids also possess combating activity against parasites of vegetation, acaricidic properties and also incorporated in food compositions combined with nutritive mixtures suitable for animal fodder.

Scheme No.1

4-cyanoindanone undergoes reduction reaction to racemic 4-cyano-indanol using by Potassium borohydride. The racemic alcohol is acylated in the presence of acetic anhydride leading to the formation of 4-cyano-1-indanyl acetate. 4-cyano-1-indanyl acetate further resolved using Lipase enzyme to produce (S)-ester and (R)-alcohol. The (S)-ester was hydrolyzed using Sodium hydroxide to produce (S)-alcohol (40.58% yield)[5].

Scheme No.2

4-cyanoindanone reduction involves use of harmful reagents for conversion to alcohol that require extreme reaction conditions and expensive reagents (78% yield). Moreover, chemical methods most often do not comply with the principles of green chemistry, while biocatalytic process along with mild conditions could obtain up to 100% theoretical yield with high enantioselectivity[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. and can be prepared from (S)-1-hydroxy-2, 3-dihydro-1H-indene-4-carbonitrile.

Ketoreductases are the most commonly used enzymes in industrial pharmaceutical synthesis. They convert variety of ketones into chiral alcohols with high stereoselectivity[7]. Ketoreductase enzymes require no special conditions (operate at a wide range of temperature, 20-45°C)[8], the cost of the enzymes is also lower compared to other enzymes and large quantities can be economically produced by microbial fermentations. Cost effective methods for regeneration of NAD- cofactors have also been developed[9].

Although progresses in production of chiral alcohols using ketoreductase enzymes is widely looked upon in the industry, there still exists some drawback for usage of the same. Ketoreductases are not stable in 100% organic solvent systems and also during the processes involved in product alcohol isolation, the ketoreductase enzyme gets deactivated preventing the reuse of catalyst[10].

Immobilization of biocatalysts has long since been employed to enable multiple reuse and recycling of enzymes. But the immobilization of Ketoreductases remain challenging as even the few cases reported, could not maintain the activity and stability under operational conditions[11].

In our study, we conducted KRED-catalyzed reactions in ~90% aqueous solvent system and

studied the reusability capacity of ketoreductases in successive experiments.

Our aim was in employing an enzymatic approach for synthesis of chiral-1-hydroxy-2, 3-dihydro 1H-indene-4-carbonitrile, using Ketoreductase 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. Ketoreductase enzymes were obtained from Enzyme Works, China, and Iosynth Labs Private Limited, India.

Methodology

Experimental Section

Step I: Preparation of Buffer Solution

In a flask, was prepared ~pH 7 Phosphate Buffer [Mix 61.5ml of 43.55g/L K_2HPO_4 with 38.5ml of 34.02g/L KH_2PO_4]. To 5ml of above buffer, the following were charged, $MgSO_4.7H_2O$ (2.4mg), $NADP^+$ (4.2mg), NAD^+ (3.6mg), Glucose (72mg), Glucose Dehydrogenase (1mg). The contents were mixed well and used for the following reactions

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

To the buffer solution (5ml) from Step 1, Ketoreductase enzyme (6mg) was added. 4-cyano indanone (3mg) and Dimethyl sulfoxide (0.10ml) were charged. Temperature was raised to 40°C and maintained at same temperature for 96 hrs. Reaction was monitored by TLC. After completion of the reaction, the resulting reaction mass was extracted with ethyl acetate and distilled under vacuum to yield (S)-1-hydroxy-2, 3-dihydro-1H-indene-4-carbonitrile (5mg), which was further confirmed by TLC and sending the sample for chiral purity.

REUSE OF KETOREDUCTASE ENZYME

Step A: Preparation of Buffer Solution

In a flask charged Aq layer of previous batch, then charged $MgSO_4.7H_2O$ (2.4mg), $NADP^+$ (4.2mg), NAD^+ (3.6mg), Glucose (72mg), Glucose Dehydrogenase (1mg). The contents were mixed well and used for the following reactions

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

To the above buffer solution was added 4-cyanonindanone (3mg) and Dimethyl sulfoxide (0.10ml). Temperature was raised to 40°C and maintained at same for 96 hrs. Reaction was monitored by TLC. No product formation was observed.

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- Ethyl Acetate: Hexane= 3:7 respectively. The plate was then viewed under UV light of 254nm/ after spraying $KMnO_4$ solution.

RESULTS AND DISCUSSION

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

Required (S)-alcohol/(S)-Isomer was formed with 96.37% chiral purity with ketoreductase enzyme KRED-CN102-LP094 under studied reaction conditions.

Reuse of ketoreductase enzyme did not result in product formation under studied reaction conditions.

Product conversion observed by TLC as shown below

To the TLC plate, were applied spots of our keto compound and final product which was immersed in a mobile phase of following composition- Ethyl Acetate: Hexane = 3:7 respectively[12]. The plate was then viewed under UV light of 254nm/viewed after sprayed with $KMnO_4$ solution.

For 96.37% Chiral purity of desired (S)-alcohol compound prepared using ketoreductase enzyme, the Chiral HPLC data is as follows in Figure No.4.

(S)-1-hydroxy-2,3 dihydro-1H-indene-4-carbonitrile was synthesized in single step with 100% yield using ketoreductase enzyme.

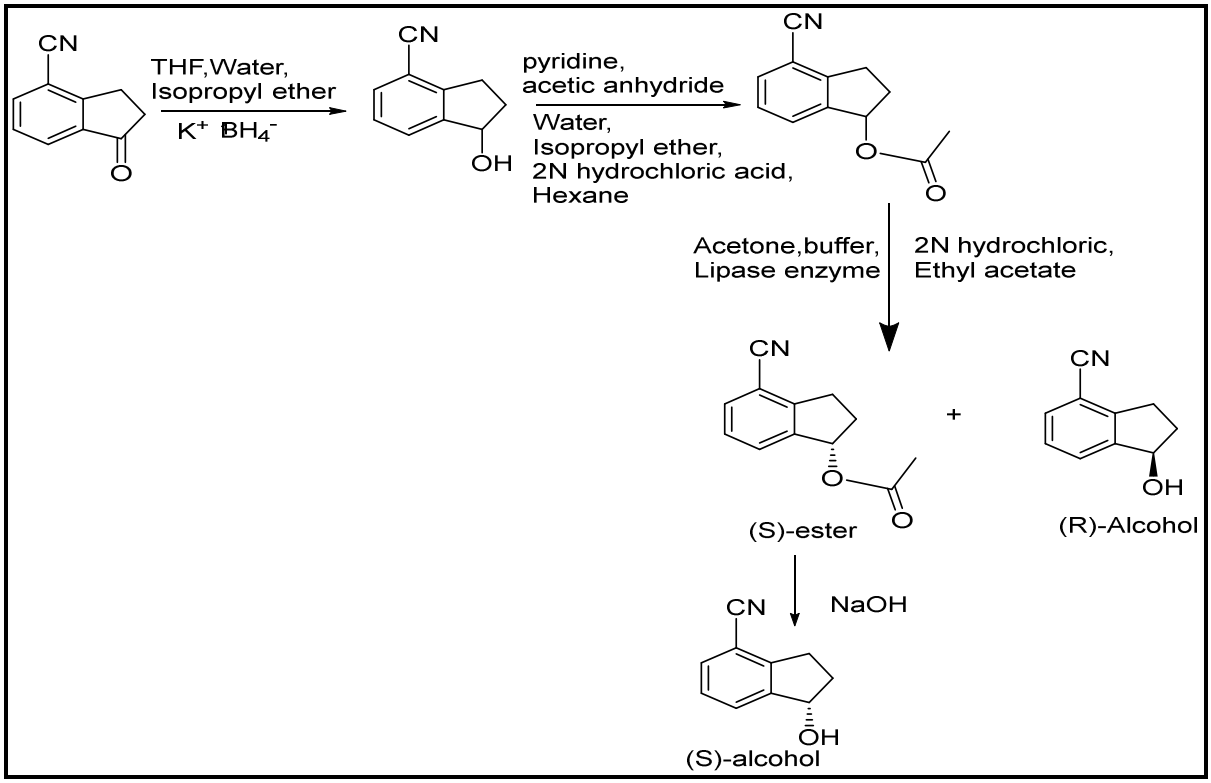
Mass Data (Figure No.5) for (S)-1-hydroxy-2, 3-dihydro-1H-indene-4-carbonitrile is as follows.

Table No.1: Ketoreductase enzyme variants with dominant (S)-1-hydroxy-2, 3-dihydro-1H-indene-4-carbonitrile formation under studied reaction conditions

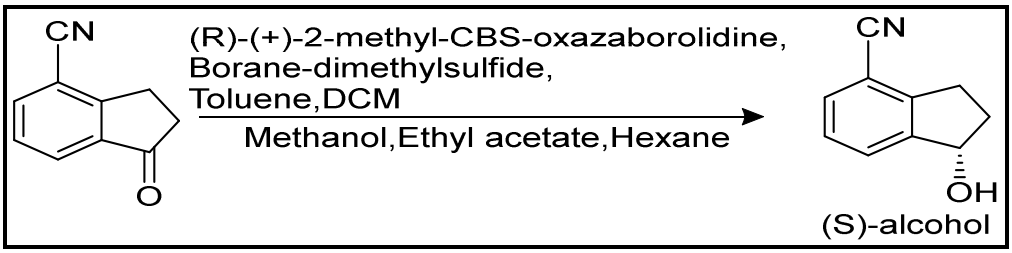
S.No	Enzyme Variant	Enzyme Qty	Batch Size	Chiral Purity	TLC Results
1	KRED-CN102-LP094	2 T	3mg	(S)-Isomer 96.37% (R)-Isomer 3.63%	Product formed. Starting material not found
2	KRED-CN102-LP128	2 T	3mg	(S)-Isomer 83.56% (R)-Isomer 16.44%	Product formed. Starting material not found
3	KRED-CN102-LP078	2 T	3mg	(S)-Isomer 81.72% (R)-Isomer 18.28%	Product formed. Starting material not found
4	KRED-CN102-LP121	2 T	3mg	(S)-Isomer 74.73% (R)-Isomer 25.27%	Product formed. Starting material not found
5	KRED-CN102-LP151	2 T	3mg	(S)-Isomer 68.37% (R)-Isomer 31.63%	Product formed. Starting material not found

Table No.2: Ketoreductase Enzyme variants with no product formation under studied reaction conditions

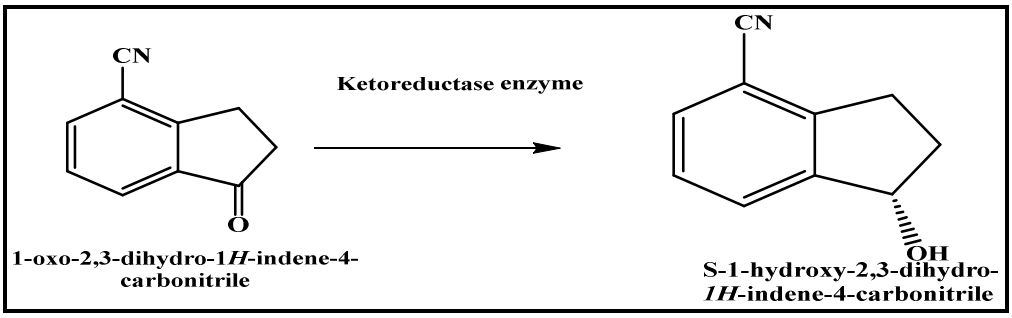
S.No	Enzyme Variant	Enzyme Qty	Batch Size	TLC Results
1	KRED-CN102-LP152	2 T	3mg	No Product formed. Starting material present
2	KRED-CN102-LP153	2 T	3mg	No Product formed. Starting material present
3	KRED-CN102-LP154	2 T	3mg	No Product formed. Starting material present
4	KRED-CN102-LP155	2 T	3mg	No Product formed. Starting material present
5	KRED-CN102-LP156	2 T	3mg	No Product formed. Starting material present



Scheme No.1: Chemoenzymatic preparation of (S)-hydroxy-2, 3-dihydro-*1H*-indene-4-carbonitrile



Scheme No.2: Chemical Route of synthesis of (S)-1-hydroxy-2, 3-dihydro-1H-indene-4-carbonitrile



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

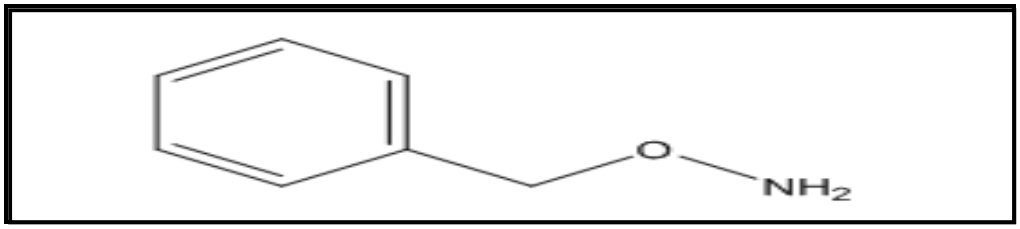


Figure No.1: O-benzylhydroxylamine; IDO1 inhibitor

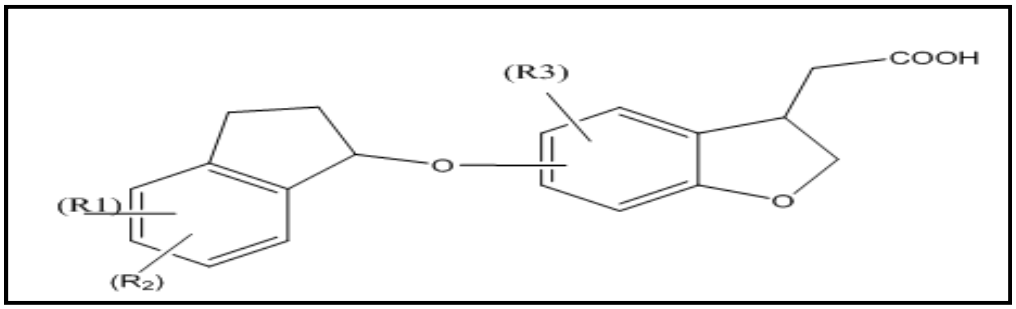


Figure No.2: GPR40 receptor modulator

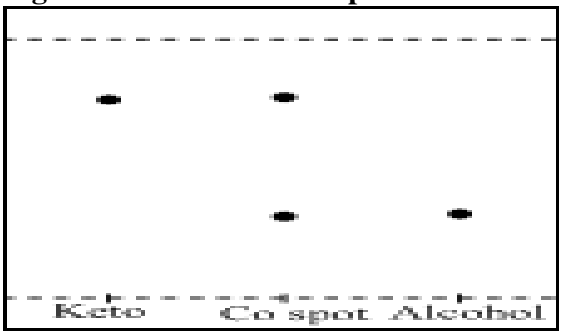


Figure No.3: TLC Pattern of starting material and product conversion

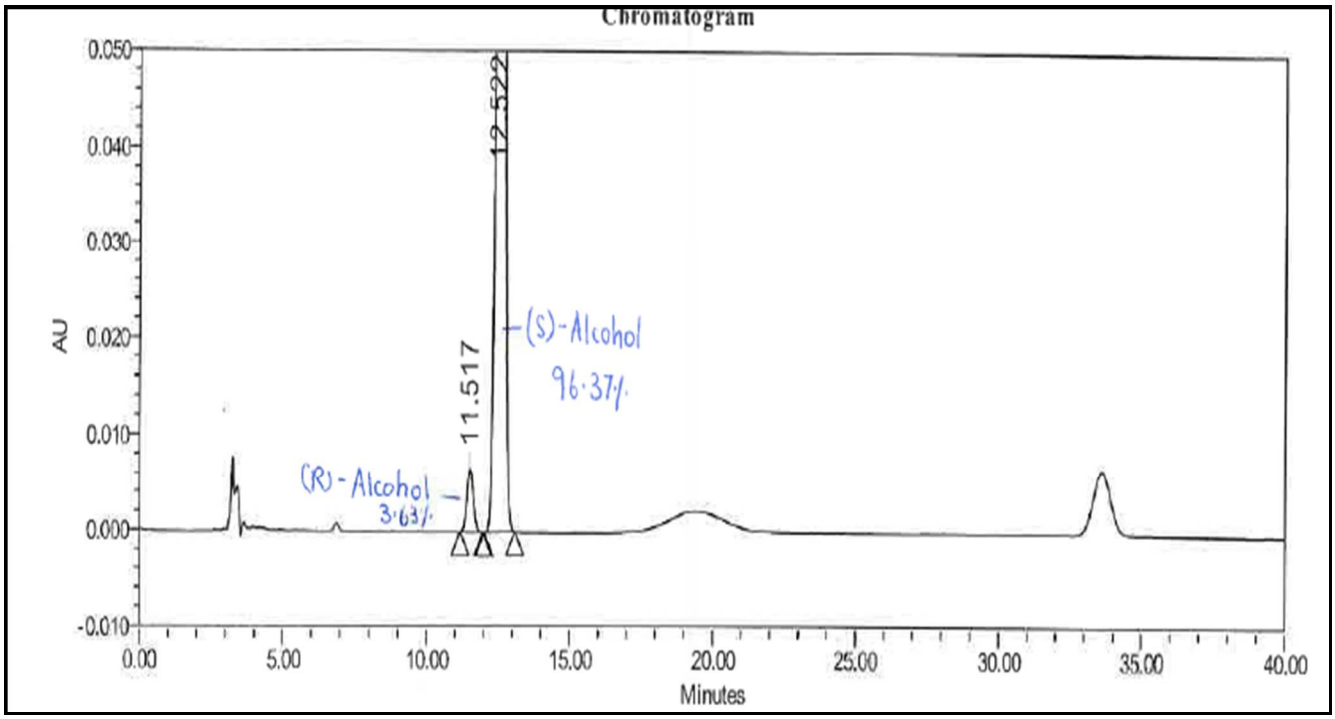


Figure No.4: Chromatogram of chiral purity by HPLC of (S)-hydroxy-2, 3-dihydro-1H-indene-4-carbonitrile

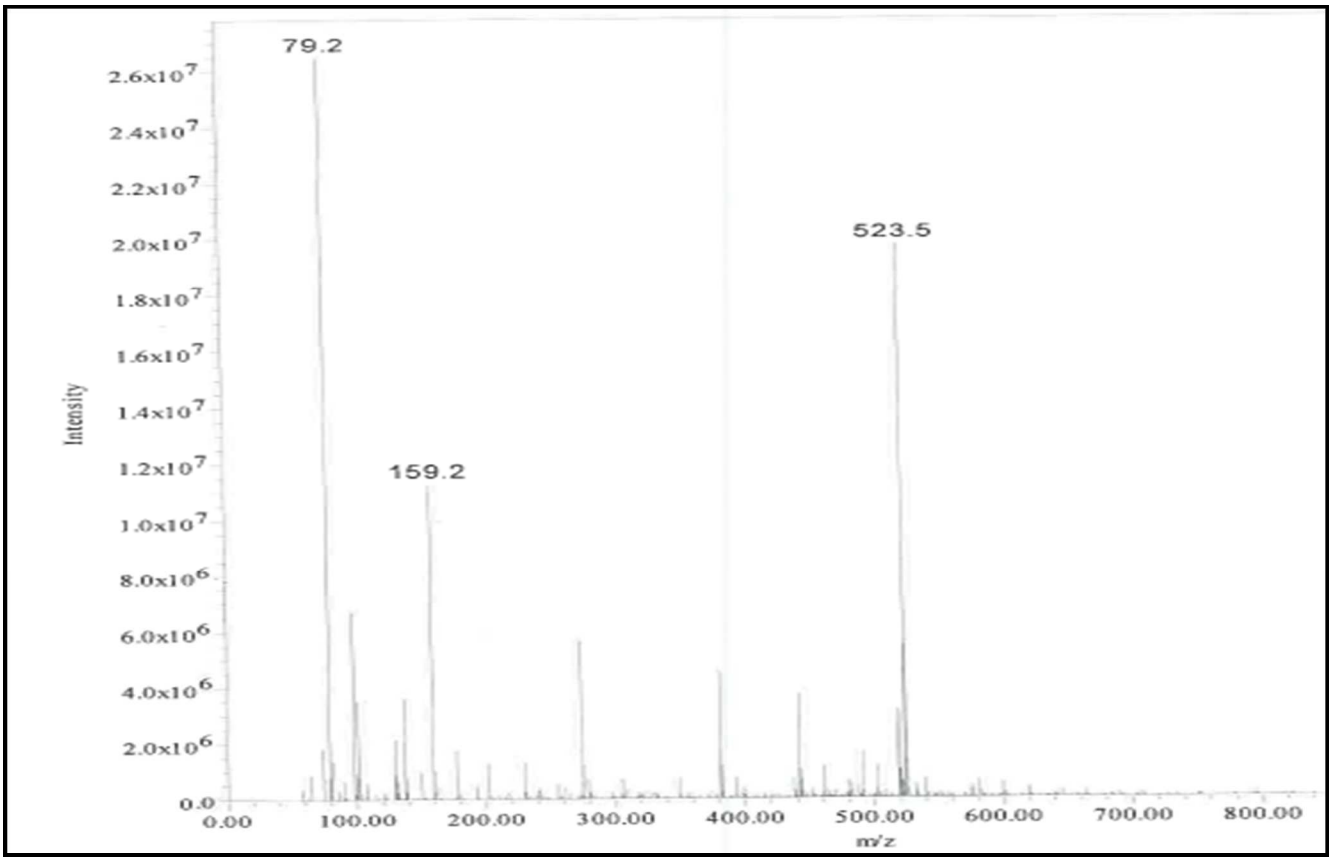


Figure No.5: Mass spectrum of (S)-1-hydroxy-2, 3-dihydro-1H-indene-4-carbonitrile

CONCLUSION

It can be determined from the above study that, (S)-alcohol compounds which are important intermediates for synthesis of several chiral compounds and receptor modulators GPR40 receptor modulators that are in use in treatment of diabetes Type II and IDO1 inhibitors that are used in cancer treatment, and pyrethroid compounds that are of major use in agricultural domain, can be prepared using Ketoreductase enzymes with high enantioselectivity and 100% yield in a single step compared 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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