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STEREO SELECTIVE REDUCTION OF 5-(2, 5-DIFLUOROPHENYL)-3, 4-DIHYDRO-2H-PYRROLE BY APPLYING IMINE REDUCTASE AND ITS COMPARATIVE STUDIES USING PLUG FLOW REACTOR

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ABSTRACT

Imine reductases having great importance in the synthesis of enantiomerically rich enantiomers which are used for the synthesis of chiral intermediates. A novel process for imine reduction of 5-(2, 5-difluorophenyl)-3, 4-dihydro-2H-pyrrole was achieved to synthesis (R)-2-(2, 5-difluorophenyl) pyrrolidine, which is key intermediate for the synthesis of a highly selective TRK inhibitor, is a CNS active small molecule tyrosine kinase inhibitor of the three TRK protein kinases[1-5] and (S)-2-(2, 5-difluorophenyl) pyrrolidine using imine reductase enzyme. High enantioselective reaction was accomplished and confirmed by TLC, DIP mass and HPLC. Further to value addition the imine reductase reaction was performed in plug flow reactor and verified its advantages like reduce in time cycle; impurities; reduction of quantity of catalysts/enzymes; less solvent consumption and avoiding damages of catalyst/enzymes.

KEYWORDS

Imine reductase, Plug flow reactor, (R)-2-(2, 5-difluorophenyl) pyrrolidine, (S)-2-(2, 5-difluorophenyl) pyrrolidine, Chiral purity, Chiral intermediates, Flow reactor and Flow chemistry.

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INTRODUCTION

Substituted pyrazolo [l, 5-a] pyrimidine compounds as TRK kinase inhibitors (Figure No.1) is a selective inhibitor of neurotrophin receptor kinase (NTRK) that is used in the therapy of solid tumors harboring NTRK gene fusions. TRK inhibitor is associated with a high rate of serum aminotransferase elevations during therapy but has

not been linked to instances of clinically apparent liver injury with jaundice.

Substituted pyrazolo [l, 5-a] pyrimidine compound is an orally available, small molecule inhibitor of the tropomyosin receptor kinases (TRK-A, -B and -C). The genes of TRK are found to be oncogenic drivers in many solid tumors including gliomas and sarcomas. The tumors harbor a fusion product of the proto-oncogene family and over express the neurotrophin receptor kinases which results in abnormal cell growth. Substituted pyrazolo [l, 5-a] pyrimidine compound has activity against all three forms of TRK and has been shown to be effective in prolonging progression free survival in children and adults with various solid tumors harboring an NTRK gene fusion. Substituted pyrazolo [l, 5-a] pyrimidine compound received accelerated approval for use in the United States in 2018 for the treatment of advanced or metastatic solid tumors that have NTRK gene fusion. Substituted pyrazolo [l, 5-a] pyrimidine compound is available in capsules of 25 and 100mg and as an oral solution of 20mg/mL under the brand name VITRAKVI. The recommended dose in adults is 100mg orally twice daily, the dose in children and small adults being adjusted to body surface area. Side effects are common and include fatigue, nausea, vomiting, constipation, diarrhea, dizziness and cough. Uncommon, but potentially serious side effects include neurotoxicity (delirium, dysarthria, dizziness, gait disturbance, tremor), hepatotoxicity (ALT and AST elevations) and embryo-fetal toxicity.

In early clinical trials in a total of 176 patients with various forms of solid tumors which had an NTRK gene fusion, elevations in serum aminotransferase levels occurred in 45% of patients treated with TRK inhibitor. Serum aminotransferase levels rose to above 5 times ULN in 6% of patients and led to early discontinuation in 2 %. Serum aminotransferase elevations typically arose after 4 to 12 weeks of treatment, but usually without jaundice or alkaline phosphatase elevations. Most elevations resolved within 4 to 8 weeks and discontinuations were uncommon. Restarting TRK inhibitor at a reduced dose after resolution of the aminotransferase abnormalities was generally well tolerated and did not lead to recurrence of liver injury. Cases with jaundice and symptoms during TRK inhibitor therapy have not been reported, but the clinical experience with this kinase inhibitor has been limited and prelicensure clinical trials were carried out with careful clinical monitoring.

Likelihood score: E* (unproven but suspected cause of clinically apparent liver injury).

The cause of the liver injury due to TRK inhibitor is unknown. TRK inhibitor is metabolized in the liver largely via CYP 3A4 and is highly susceptible to drug-drug interactions with CYP 3A modulators, such that the dose modifications are recommended if there is concurrent use of CYP 3A inducers (dose increase) or inhibitors (dose decrease by half).

Routine monitoring of liver tests is recommended for patients starting TRK inhibitor, including serum ALT, AST and bilirubin every 2 weeks for the first month and monthly thereafter and as clinically indicated. Serum aminotransferase elevations above 5 times the upper limit of normal should lead to dose interruption. If changes persist, are severe, or reoccur on restarting, TRK inhibitor should be discontinued. There have been no reports of acute liver failure, chronic hepatitis or vanishing bile duct syndrome due to TRK inhibitor.

Imine reductases are great importance in the synthesis of enantiomerically rich enantiomers which are used for the synthesis API/Chiral intermediates. Imine reductases (IREDs) are nicotinamide adenine dinucleotide phosphate (NADPH)–dependent enzymes that have been applied to the stereo selective synthesis of chiral amines through asymmetric imine reduction and reductive amination[6,7].

(R)-2-(2. 5-difluorophenyl) pyrrolidine preparation by chemical method[8] (Figure No.2) using very expensive ligand and harsh chemical like tri-t-butyl phosphonium tetrafluoroborate and reaction temperature is very low < -78^{o}C and chiral purity of the compound is < 95% and ~ 61 % Yield, these reaction chemicals and conditions encourages us to look alternate methods of preparation using imine reductase enzyme (Figure No.3) to prepare (R)-2-(2,

5-difluorophenyl) pyrrolidine with high enantio selective isomer > 98% ee with 100% Yield.

Flow chemistry explains chemical processes that occurs in a continuous flowing stream instead of a regular batch mode. Flow chemistry relies on concept of pumping reagents to a tubular reactor and allow the reagents to react by the pressure applied through pumps. In the field of chemical synthesis, they are used mostly in pharmaceutical chemistry for efficient synthesis of small amounts of active substances. The main concept of this work is to show the overlapping of development trends in the design of instrumentation and various ways of the utilization of specificity of chemical operations under flow conditions, especially for synthetic and analytical purposes, with a simultaneous presentation of the still rather limited correspondence between these two main areas of flow chemistry[9].

Plug flow reactors are alternatives to regular batch chemical reactors, which are evolved from flow chemistry concepts. Flow reactors widely accepted as green chemistry approach towards reducing waste generation from chemical and pharmaceutical industries. Continuous or flow process chemistry was widely incorporated in Petrochemical industry. In enzymatic synthesis damage of enzymes are major disadvantages during stirring or shaking of reaction mass which eventually leads to lesser conversion rates of desired products and high surface are in flow reactions reactors controls temperatures. To overcome these concerns, we have applied tubular column by packing the enzymes in tubular columns and circulated the liquid medium of reaction mass through pumps. As expected, enzyme was not damaged and leads to complete the reaction in shorter time cycle when compared to regular batch mode reaction[10].

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. Imine reductase enzymes were obtained from Enzyme Works, China, Iosynth Labs Private Limited, India.

Methodology

Experimental Section

Preparation of (R)-2-(2, 5-Difluorophenyl) Pyrrolidine

In a flask, charge 10ml Potassium phosphate buffer (~pH 7.0), Charge D-Glucose 40mg, GDH 20mg, NADPH 4mg, mixed thoroughly until dissolution. To this solution charge 5-(2, 5-difluorophenyl)-3, 4-dihydro-2H-pyrrole (25mg) dissolved in Dimethyl sulfoxide (0.5ml). Charge Imine reductase enzyme (50mg). Raise the temperature of the reaction mass to 40-45°C and maintained at same temperature for 24-48 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 (R)-2-(2, 5-Difluorophenyl) Pyrrolidine (25mg), which was further confirmed by TLC and chiral purity by HPLC.

Preparation of (S)-2-(2, 5-Difluorophenyl) Pyrrolidine in batch reactor

In a flask, charge 20ml Potassium phosphate buffer (~pH 7.0), Charge D-Glucose 80mg, GDH 40mg, NADPH 8mg, mixed thoroughly until dissolution. To this solution charge 5-(2, 5-difluorophenyl)-3, 4-dihydro-2H-pyrrole (50mg) dissolved in Dimethyl sulfoxide (1.0ml). Charge Imine reductase enzyme (300mg). Raise the temperature of the reaction mass to 40-45°C and maintained at same temperature for 2 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)-2-(2, 5-Difluorophenyl) Pyrrolidine (50mg), which was further confirmed by TLC and chiral purity by HPLC.

Preparation of (S)-2-(2, 5-Difluorophenyl) Pyrrolidine in plug flow reactor

Imine reductase enzyme (200mg) was packed tubular column of flow reactor, mixture of 40ml Potassium phosphate buffer (~pH 7.0), 5-(2, 5-difluorophenyl)-3, 4-dihydro-2H-pyrrole (100mg) dissolved in Dimethyl sulfoxide (2ml), D-Glucose 160mg, GDH 80mg, NADPH 16mg were pumped through syringe pump at 40-45°C at the flow rate of 1ml/min. Collected the reaction mass from outlet of flow reactor and monitored by TLC, the resulting reaction mass was extracted with ethyl acetate and

distilled under vacuum to yield (S)-2-(2, 5-Difluorophenyl) Pyrrolidine (100mg), which was further confirmed by TLC.

Product conversion observed by TLC as shown below (Figure No.8)

To the TLC plate, were applied spots of our 5-(2, 5-difluorophenyl)-3, 4-dihydro-2H-pyrrole compound and final product 2-(2, 5-difluorophenyl) pyrrolidine 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.

RESULTS AND DISCUSSION

Several variants of Imine reductase enzymes were screened in the following study for conversion of 5-(2, 5-difluorophenyl)-3, 4-dihydro-2H-pyrrole compound to (R)-2-(2, 5-difluorophenyl) pyrrolidine and (R)-2-(2, 5-difluorophenyl) pyrrolidine and the results are as follows in Table No.1.

To the TLC plate, were applied spots of our 5-(2, 5-difluorophenyl)-3, 4-dihydro-2H-pyrrole compound and final product 2-(2, 5-difluorophenyl) pyrrolidine 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.

Table No.1: Screening data of 2-(2, 5-difluorophenyl) pyrrolidine formation using different variants of Imine reductase

S.No	Enzyme Variant	Enzyme Qty	Chiral Purity	TLC observation
1	EW-IR-205	2 Times	98.9% R-Isomer 1.1 % S-Isomer	Starting Material: Not Detected
2	ECS-IRED-04	5 Times	98.93% R-Isomer. 1.07% S-Isomer	Starting Material: ~ 10 %
3	ECS-IRED-02	6 Times	100% S-Isomer	Starting Material: Not Detected
4	ECS-IRED-02 in Flow reactor	2 Times	100% S-Isomer	Starting Material: Not Detected
5	CN102-IRED-LP001	1.4 Times	4.79% R-Isomer. 95.21 % S-Isomer	Starting Material: Not Detected
6	CN102-IRED-LP003	1.4 Times	100 % S-Isomer.	Starting Material: Not Detected
7	CN102-IRED-LP008	1.4 Times	20.88% R-Isomer. 79.12 % S-Isomer	Starting Material: Not Detected
8	CN102-IRED-LP009	1.4 Times	70.88% R-Isomer. 29.12% S-Isomer	Starting Material: Not Detected
9	CN102-IRED-LP0010	1.4 Times	1.9% R-Isomer. 98.1 % S-Isomer	Starting Material: Not Detected
10	CN102-IRED-LP0011	1.4 Times	25.43% R-Isomer. 74.57% S-Isomer	Starting Material: Not Detected
11	CN102-IRED-LP0012	1.4 Times	77.69% R-Isomer. 22.31% S-Isomer	Starting Material: Not Detected
12	CN102-IRED-LP0012	1.4 Times	68.72% R-Isomer. 31.28 % S-Isomer	Starting Material: Not Detected

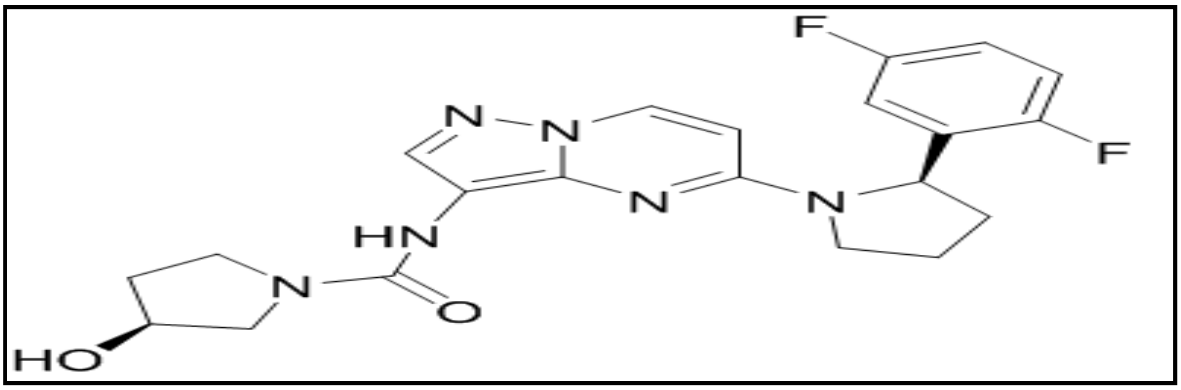

Figure No.1: Structure of substituted pyrazolo [l, 5-a] pyrimidine compound (TRK inhibitor)

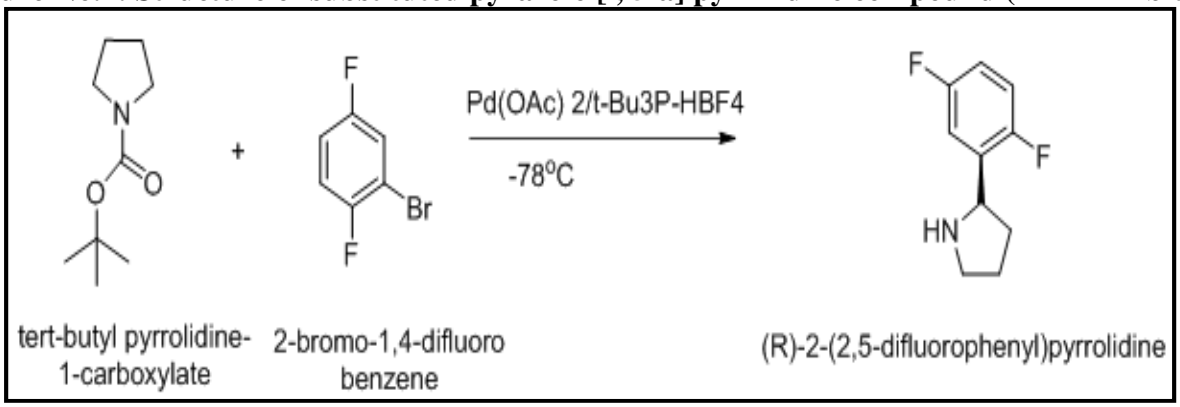

Figure No.2: (R)-2-(2, 5-difluorophenyl) pyrrolidine preparation by chemical method

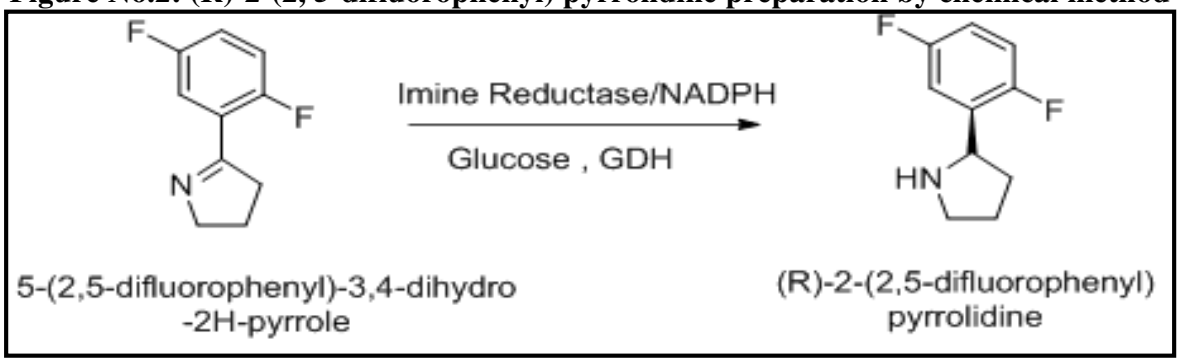

Figure No.3: (R)-2-(2, 5-difluorophenyl) pyrrolidine preparation by enzymatic method

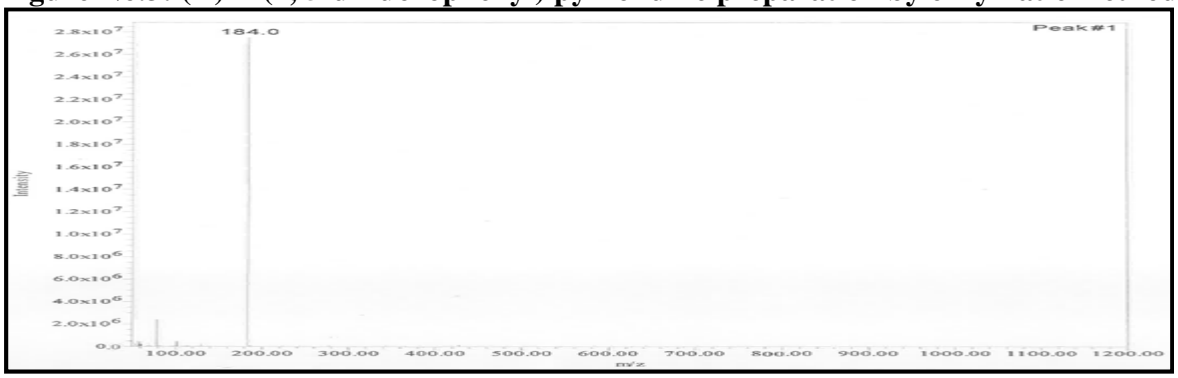

Figure No.4: Mass chromatogram of (R)- 2-(2, 25-difluorophenyl) pyrrolidine prepared by Using Imine reductase enzyme EW-IR-205

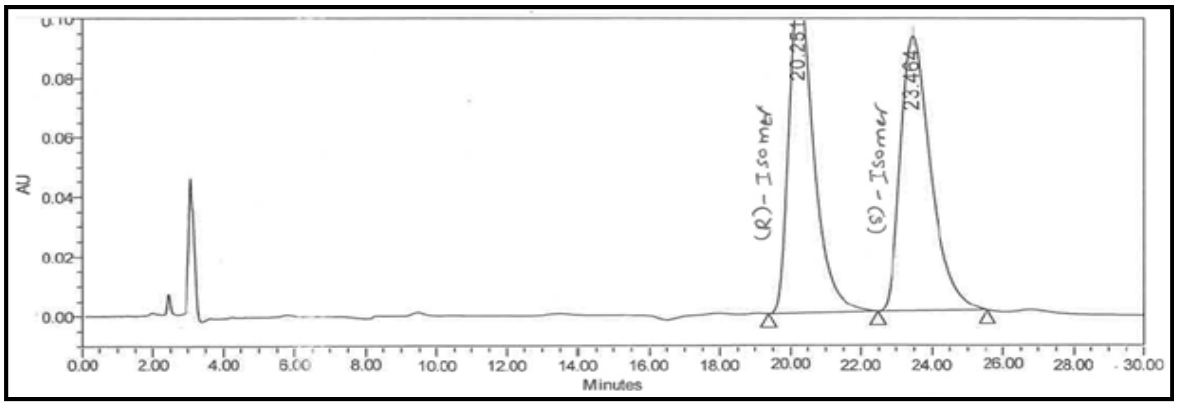

Figure No.5: HPLC chromatogram of racemic 2-(2, 5-difluorophenyl) pyrrolidine

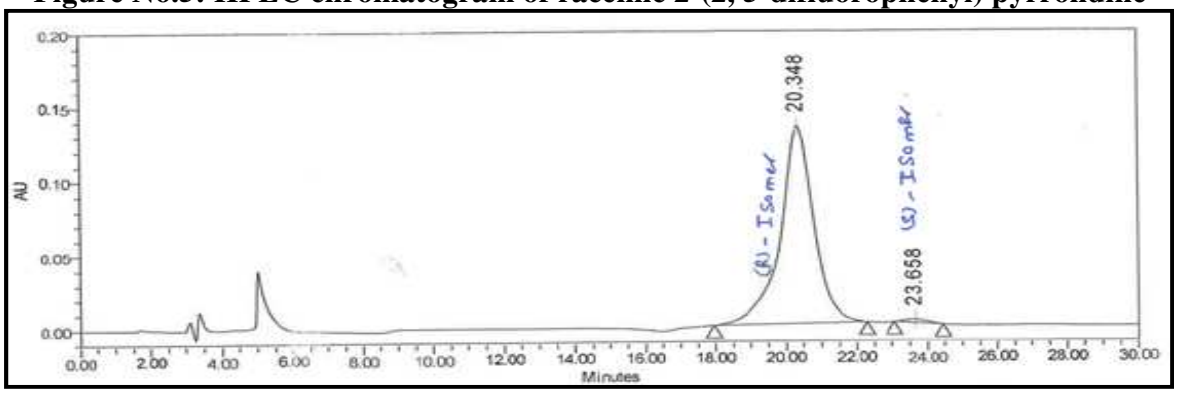

Figure No.6: HPLC chromatogram of (R)- 2-(2, 5-difluorophenyl) pyrrolidine prepared by Using Imine reductase enzyme EW-IR-205

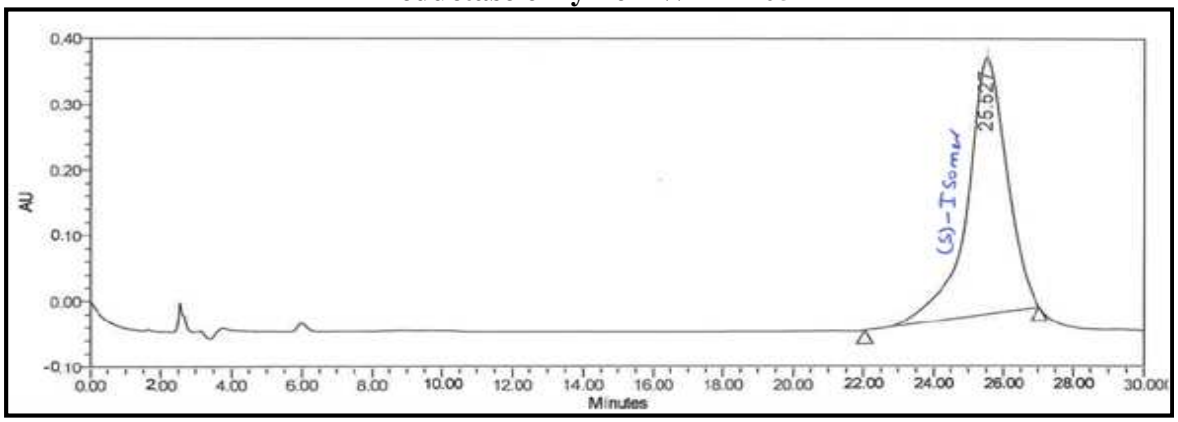

Figure No.7: HPLC chromatogram of (S)- 2-(2, 5-difluorophenyl) pyrrolidine prepared by Using Imine reductase enzyme ECS-IRED-02

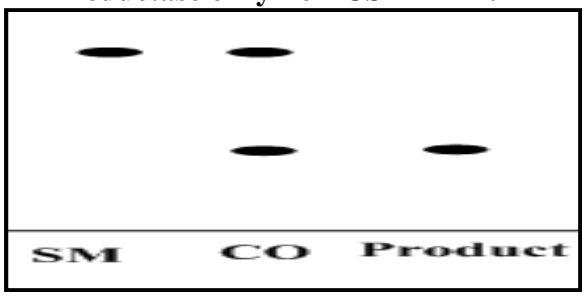

Figure No.8: TLC of Starting material and product conversion

CONCLUSION

The prescribed above-mentioned studies confirmed stereoselective conversions 5-(2, 5-difluorophenyl)-3, 4-dihydro-2H-pyrrole into both (R)-2-(2, 5-difluorophenyl) pyrrolidine and (S)-2-(2, 5-difluorophenyl) pyrrolidine chiral intermediates. Further the conversions were studied in plug flow reactor to reduce the time cycle; impurities; reduction of quantity of catalysts/enzymes; less solvent consumption and avoiding damages of catalyst/enzymes.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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