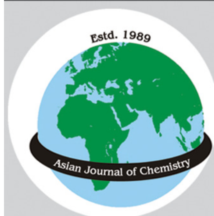

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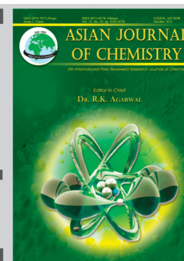

Asymmetric Reduction of *N*-[4-(2-Bromoacetyl)phenyl]methanesulfonamide by Employing *Candida viswanathii* MTCC 5158

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Enantioselective synthesis of chiral drugs by chemoenzymatic processes have attracted attention in which the crucial stereogenic step involves an enzymatic reaction by *virtue* of *chemo*-, *regio*- and *enantio*- selectivity and eco-friendly nature of biocatalysis, (S)-sotalol a β-blocker, belongs to class-III antiarrhythmic agents. To avoid the side effects of racemic sotalol, efforts have been put forward to synthesize a key intermediate for the synthesis of (*S*)-enantiomer of sotalol, which is more potent than the (*R*)-enantiomer. The aryl ketone, *N*-(4-acetyl-phenyl) methanesulfonamide was synthesized from the initial substrate 4-aminoacetophenone. Further, the bromination of aryl ketone was carried out to obtain *N*-[4-(2-bromo-acetyl)-phenyl]-methanesulfonamide. The chemical reduction as well as biological reduction of aryl ketone was carried out to obtain a racemate alcohol *N* [4-(2-bromo-1-hydroxy-ethyl) (4-methanesulfonamide)]ethanol and chiral (*S*)-*N*-[4-(2-bromo-1-hydroxy-ethyl)(4-methanesulfonamide)]ethanol, respectively. (*S*)-*N*-[-4-(2-bromo-1-hydroxy-ethyl)(4-methanesulfonamide)]ethanol is a key intermediate for the synthesis of (*S*)-sotalol. The biological reduction was carried out by using whole cells and found that 96 % conversion was obtained at 12th h of reaction after that the percentage conversion decreased.

Key Words: Chemoenzymatic synthesis, *Candida viswanathii* MTCC 5158, (*S*)-Sotalol

INTRODUCTION

The asymmetric reduction of ketones is one of the most important, fundamental and practical reactions for producing chiral alcohols, which can be transformed into various functionalities, without racemization, for the synthesis of many industrially important chemicals such as pharmaceuticals, agrochemicals and natural products[1,2]. Bioreduction catalyzed by isolated dehydrogenases or whole cells provides an attractive approach to selectively reducing a broad range of ketones[3]. The use of microbial whole cells as biocatalysts is particularly advantageous for carrying out the desired reduction, since they contain multiple dehydrogenases, which are able to accept a broad spectrum of nonnatural substrates, all the necessary cofactors and the metabolic pathways for their regeneration. Furthermore, all the enzymes and cofactors are well protected within their natural cellular environment[4,5]. Lipases and esterases have been used extensively for the kinetic resolution of chiral secondary alcohols and carboxylic acids/esters, respectively. However, one drawback to such processes lies in the fact that the maximum yield which can be obtained by kinetic resolution is 50 %, which is economically and ecologically unattractive[6]. On the other hand, asymmetric synthesis is carried out by another class of enzyme, oxidoreductases. These enzymes catalyze the asymmetric reduction of prochiral ketones to form chiral alcohols. The main advantage of these oxidoreductase-mediated bioreductions is that they can provide upto 100 % theoretical yield along with an almost absolute enantioselectivity[7-9].

Saccharomyces cerevisiae, *Rhizopus arrhizus*, white-rot fungus *Merulius tremellosus*, *Geotrichum candidum*, *Rhodococcus rubber* and *Trichothecium sp*. and many other microorganisms were also used for the preparation of enantiomerically pure alcohols and even plant cell cultures may be employed for this purpose. However, in many instances the use of these microorganisms gives a product with an unsatisfactory enantiopurity due to the coexistence of several reductases with different specific activities and stereoseletivities[10]. There are still some problems, which remain to be solved, such as moderate enantiomeric purity, narrow substrate spectrum, a long time for fermentation and reaction and incomplete transformation[10,11].

Synthetic routes of (*S*)-sotalol involving racemate resolution using chiral mandelic acid or through chiral homogeneous hydrogenation, CBS reduction of aromatic ketone or sharpless asymmetric dihydroxylation of 4-nitrostyrene.

Amongst biocatalytic methods there is only one report of the preparation of (*S*)-sotalol that relates to the use of microbial strain *Geotrichium sp*. 702 in the stereoselective reduction of a keto precursor[12].

EXPERIMENTAL

Various chemicals used in were 4-aminoacetophenone (Lancaster, Morecambe, England), methyl sulphonyl chloride, pyridine, liquid bromine, glacial acetic acid, sodium borohydrate, methyl alcohol. Solvents used were hexane, ethyl acetate. All chemicals were purchased from Aldrich Chemical Ltd. (Milwaukee, WI, USA). Growth media components were obtained from Hi-Media Inc. (Mumbai, India). Inorganic salts and other buffer salts were purchased from Qualigens Inc. (Mumbai, India). Solvents, mineral acids and other chemicals of analytical grade were procured from Ranbaxy Fine Chemicals Ltd. (Mohali, India) and S.D. Fine Chemicals Ltd. (Boisar, India).

Instruments used were U.V. chamber, silica gel-coated (E. Merck, Darmstadt, Germany, 60 F254, 0.2 mm thickness) aluminum sheets for TLC, rotary evaporator (Buchi), Bruker FT-NMR (Advance DPX300) spectrometer, FT-IR (Nicolet), Maldi (Bruker Daltonics, Ultraflex TOF/TOF), Shimadzu QP 5000 GCMS spectrometer. A Centrifuge (Sigma 6K15, Germany), pH meter (Mettler Toledo, Switzerland) and Incubator shaker (Infors AG, Switzerland) were used.

Microorganism: The microorganism used in the present work has been isolated from the National Institute for Pharmaceutical Education and Research (NIPER) soil after several days of enrichment using acetophenone as the sole source of carbon and energy. *Candida viswanathii* has been identified by MTCC 5158, Institute of Microbial Technology, Chandigarh.

Culture medium: *Candida viswanathii* MTCC 5158 was maintained on a nutrient agar medium (pH 7) containing peptic digest of animal tissue (5.0 g/L), beef extract (3.0 g/L), sodium chloride (8.0 g/L) and agar (15.0 g/L). Initially, seed culture was developed by inoculating single colony of *Candida viswanathii* into 20 mL nutrient broth (pH 7) containing peptic digest of animal tissue (5.0 g/L), yeast extract (1.50 g/L), beef extract (1.50 g/L) and sodium chloride (5.0 g/L) and 20 mM acetophenone along with glucose solution (15 % w/v) as inducers for 18 h at 30 ºC in a rotary shaker at 200 rpm. It (3 %, v/v) was transferred to production medium of same composition containing 2 mM acetophenone and glucose 1 % w/v, for 36 h at 25 ºC in a rotary shaker at 200 rpm.

Preparation of crude extracts: Cells were harvested by centrifugation at 7,000 xg for 20 min and washed with phosphate buffer (50 mM, pH 7) and centrifuged at 12,000 xg for 10 min. The pellet was re-suspended in the same buffer.

Optimization of biocatalytic reaction time: The resting cell suspension of *C. viswanathii* was used to reduce *N*-[4-(2-bromo-1-hydroxyethyl)phenyl]methanesulfonamide to 2-bromo-1-(4-methanesulfonamide)ethanol. Substrate reactivity and enantioselectivity of the reaction are strongly dependent upon reaction conditions as pH, temperature and reaction time. Therefore, the influence of these parameters on above reduction reaction was studied by using the resting cells. Furthermore, substrate and resting cell concentration were optimized to achieve maximum conversion along with excellent enantioselectivity. Time course study is one of the most important factors, which determines the efficacy of the process along with product formation. In order to optimize bioreduction time, resting cells of *C. viswanathii* were incubated with the substrate at 30 ºC for 48 h. Samples were taken at different time interval and conversion as well as was monitored by reverse-phase and chiral HPLC, respectively.

Synthetic schemes for aryl ketone

Synthesis of *N*-(4-acetylphenyl)methanesulfonamide: *N*-(4-acetylphenyl) methanesulfonamide was synthesized by reaction of 4-amino acetophenone with the methane sulphonyl chloride in the presence of pyridine for 12 h. (Fig. 1).

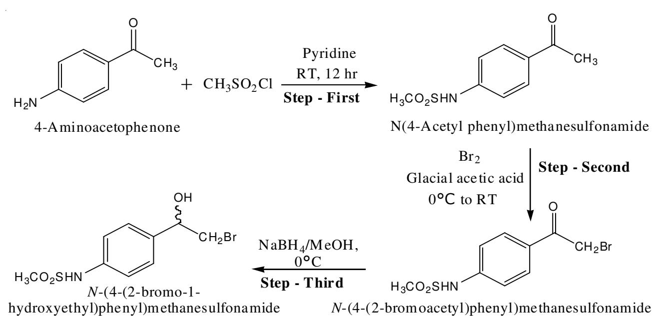

Fig. 1. Reaction scheme for synthesis of key intermediate for the synthesis of (*S*)-sotalol

Bromination of *N*-(4-acetylphenyl)methanesulfonamide: Bromination was carried out by addition of bromine in the solution of the *N*-(4-acetylphenyl) methanesul-fonamide with glacial acetic acid at 0 ºC and reaction mixture was kept at room temperature for overnight.

Chemical reduction of *N*-[4-(2-bromoacetyl)phenyl]-methanesulfonamide: The chemical reduction of *N*-[4-(2-bromoacetyl)phenyl)]methanesulfonamide was carried out by addition of sodium borohydrate in cooled and stirred solution of *N*-[4-(2- bromoacetyl)phenyl]methanesulfonamide in dry methanol.

Synthesis of *N*-(4-acetylphenyl)methanesulfonamide: Methane sulphonyl chloride (4.25 mL, 37.03 mM) was slowly added to a stirred solution of 4-aminoacetophenone (4 g, 29.62 mM) in pyridine (5 mL), as a solvent. After addition of the methyl sulphonyl chloride, the contents were allowed to stir overnight. The reaction mixture was monitored with thin layer chromatography (TLC). The reaction mixture was diluted with the ice-water and precipitated out by adding the HCl. Finally, the reaction mixture was filtered and concentrated under the reduced pressure to give a crude product. For the recrystallization, the reaction mixture was dissolved in ethyl acetate and evaporated up to half to get the pure crystals of the product.

Synthesis of *N*-[4-(2-bromoacetyl)phenyl]methanesulfonamide: Bromine (0.5 mL, 9.38 mM) was added slowly to a stirred solution of *N*-(4-acetyl-phenyl) methanesulfonamide (2 g, 9.38 mM) in glacial acetic acid in a round bottom flask at the temperature 0 ºC. After addition of the bromine, the reaction mixture was kept at room temperature for overnight. The reaction mixture was diluted by adding ice-water and extracted with the ethyl acetate. The organic mixture was washed with brine, dried over anhydrous sodium sulfate and

filtered under the reduced pressure to get the crude product that was purified by column chromatography.

Synthesis of 2-bromo-1-(4-methanesulfonamide)ethanol: Sodium borohydrate (20 mg, 0.353 mM) was added in to a cooled and stirred solution of *N*-(4-(2-bromoacetyl)phenyl) methanesulfonamide (400 mg, 1.369 mM) in dry methanol (20 mg). The contents were further stirred for 4 h till the reaction was complete. The reaction was monitored by TLC. After the completion of reaction methanol was evaporated and the resulting residue was further diluted with water and extracted with dichloromethane (4 × 75 mL). The separated organic phase was washed with brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield as a solid mixture containing some impurities. The impurities were separated by column chromatography over silica gel to get the pure solid product. Finally, the solid product was triturated with the hexane to get the white pure product.

RESULTS AND DISCUSSION

Spectral data of *N*-(4-acetylphenyl)methanesulfonamide: Yield 80 %; solid, ^{1}H NMR (300 MHz, CD_3OD), δ (ppm) 2.56 (s, 3H), 3.05 (s, 3H,), 7.31(d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.5Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm) 26.30, 39.61, 117.92, 130.22, 142.218; IR (KBr, ν_{max}, cm^{-1}) 1330, 1600, 1667, 3291; MS (APCI) m/z 213.9 (M^{1+}+1).

Spectral data of *N*-(4-(2-bromoacetyl)phenyl)methanesulfonamide: Yield 52 %, yellowish white solid, ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm) 3.08 (s, 3H), 4.44 (s, 2H), 7.31 (d, *J* = 9.34 Hz), 7.97(d, *J* = 8.4 Hz, 2H); IR (KBr, ν_{max}, cm^{-1}) 1601, 1686, 2924, 3275; MALDI (TOF/TOF) 291 $(M)^+$ -1.

Spectral data of 2-bromo-1-(4-methanesulfonamide)-ethanol: Yield 25 %, white solid, ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 2.99 (s, 3H), 3.55(t, *J* = 9.28 Hz, 24), 5.01 (s, 1H), 7.22 (d, *J* = 6.8 Hz, 2H), 7.36 (d, *J* =6.8 Hz, 2H); IR (KBr, ν_{max}, cm^{-1}) 1612, 2928, 3305.

Reverse phase HPLC method development: Method was developed for reverse phase HPLC by using C_{18} column (250 × 4.6 mm, 5 µM particle size) and the mobile phase used was acetonitrile and 20 mM phosphate buffer (30:70) at flow rate 1 mL/min and the detection wavelength was 230 nm for alcohol and 270 nm for ketone.

Chiral HPLC method development for alcohol: Method was developed for chiral HPLC by using Chiral ODH column (250 × 0.46 mm, 5 µM) mobile phase used was hexane and isopropanol (85:15), flow rate 1 mL/min, at a detection wavelength 230 nm.

Calibration curves for standard ketone and alcohol: Calibration curves for standard ketone and alcohol was made by reverse phase HPLC using C_{18} column (250 × 4.6 mm, 5 µM particle size) mobile phase used was acetonitrile and 20 mM phosphate buffer (30:70) at flow rate 1 mL/min and detected at 250 nm wavelength. The samples of 50, 100, 250, 500 and 1000 µL from 5 mL stock solution of ketone and alcohol were made with methanol.

Conversion profile of biological product: Time-course study is one of the most important factors, which determines the efficacy of the process along with product formation. In order to optimize bioreduction time, resting cells of *C. viswanathii* were incubated with the substrate at 30 ºC for 12 h. Samples were taken at different time interval and conversion as well as was monitored by reverse phase and chiral HPLC, respectively.

Conclusion

It was found that 96 % conversion was obtained at 12th h of reaction after that the percentage conversion decreased. It may be due to the removal of bromine from the substrate [*N*-{4-(2-bromoacetyl)phenyl}methanesulfonamide]. The enantiomeric excess of the product was not able to be determined due to the peak overlapping of one of the enantiomer with the residual substrate. The method for the determination of enantiomeric excess of the product may be developed by changing the flow rate of the solvent, type of the solvent, retention time *etc*. With lots of effort, we were not able to separate the peaks of substrate and one of the enantiomers. It has been noticed by several experiments that the chemically reduced product did not match with the biologically reduced product. The whole cells of the *C. viswanathii* may have some type of bioreactions with the substrate leading to products other than the desired product. This indicates that one will have to start the fresh screening of microorganisms for the successful reduction of *N*-[4-(2-bromoacetyl)phenyl]methanesulfonamide to the (*S*)-alcohol [*N*-{4-(2-bromo-1-hydroxyethyl)-phenyl}methanesulfonamide].

REFERENCES

1. K. Stoschitzky, W. Lindner and G. Zering, *J. Clin. Bas. Cardiol.*, **1**, 15, (1998).
2. K. Munish, A. Naveen, A. Khursheed, K. Surrinder, S. Swapandeep and C. Subhash, *Tetrahed. Asymm.*, **16**, 717 (2005).
3. R. Mehavar and R.D. Brocks, *J. Pharm. Pharm. Sci*., **4**, 185 (2001).
4. P. Prodeep, H.S. Jagtap and A. Sudalai, *J. Chem.*, **39B**, 950 (2000).
5. W. Hummel and R.M. Kula, *Eur. J. Biochem.*, **184**, 1 (1989).
6. R.A. Sheldon, Chirotechnology, Marcel Dekker, New York, pp.18-53 (1993).
7. J. McConathy and J. M Owens, *J. Clin. Psychiatry.*, **5**, 70 (2003).
8. S. Pedragosa, C. Morisseau, J. Baratti and J. Zylber, *Tetrahedron*, **53**, 9707 (1997).
9. W. Hummel, *Tren. Biotechnol.*, **17**, 487 (1999).
10. K. Nakamura, R. Yamanaka, T. Matsuda and T. Harada, *Tetrahedron. Asymm.*, **14**, 2659 (2003).
11. W. Kroutil, H. Mang and K. Faber, *Curr. Opin. Chem. Biol.*, **8**, 120 (2004).
12. K. Ahmed, S. Mahendra and A.S. Ahmed, *Bioorg. Med. Chem. Lett.*, **14**, 4581 (2004).