J. Pharm. Res. 2019;18(4):17–20

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

RESEARCH ARTICLE

Design and Optimization of a Process for the Manufacture of (S)-5,6-Dihydro-6-Methylthieno [2,3-B]Thiopyran-4-One: an Intermediate for Dorzolamide

Lovely Rajan[1], M D Karvekar[1,*]

[1] *Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India*

ARTICLE INFO

Article history:
Revised 12.10.2019
Accepted 18.11.2019
Published 20.12.2019

[*] *Corresponding author.*
M D Karvekar
drmdkarvekar@gamil.com

https://doi.org/10.18579/jopcr/v18.4.rajan

ABSTRACT

(S)-5,6-Dihydro-6-methylthieno [2,3-b] thiopyran-4-one is a valuable intermediate in Dorzolamide HCl production, a medication that is prevalent in the management of glaucoma. Productive manufacturing of the intermediate ensures its production on an industrial scale with high yield and purity. The synthesis of the important intermediate should be optimized through this research to enhance the yield, purity, and production at large scales. The synthesis process involved several steps, beginning with the reaction of methyl (R)-3-hydroxybutyrate with p-toluenesulfonyl chloride, followed by the formation of a lithiated thiophene intermediate, esterification, hydrolysis, and cyclization. The reactions were monitored using Thin-Layer Chromatography (TLC), Infrared (IR) Spectroscopy, Proton Nuclear Magnetic Resonance (1H NMR) Spectroscopy, and Gas Chromatography-Mass Spectrometry (GC-MS) to monitor the reaction progress and establish product purity. The major reagents used were n-butyllithium, oxalyl chloride, and DMF. Reaction conditions such as solvent selection, temperature, and stirring time were also maximized to improve the yield of the end product. Route optimization greatly enhanced the yield of (S)-5,6-dihydro-6-methylthieno[2,3-b]thiopyran-4-one from 40% to 60%. TLC, IR, NMR, and GC-MS characterization established that the purity and structure of the final product were very high. The process was more efficient in every step of the reaction, especially the cyclization and esterification steps, with better yields and lower byproducts. This study optimized the synthesis of (S)-5,6-dihydro-6-methylthieno[2,3-b]thiopyran-4-one, which greatly enhanced the purity and yield and provided a scalable and efficient process for large-scale production of the Dorzolamide HCl intermediate.

Keywords: Synthesis optimization; Dorzolamide; Pharmaceutical intermediates

INTRODUCTION

Dorzolamide hydrochloride is a selective and potent inhibitor of human carbonic anhydrase isoenzymes II and IV, which are responsible for maintaining the balance between electrolytes and fluids in different physiological systems, including the eye. The enzyme inhibitory activity of dorzolamide is particularly high, with IC50 values of 0.18 nM for carbonic anhydrase II and 6.9 nM for carbonic anhydrase IV.[1] These values are indicative of the potency and specificity of dorzolamide, and it is thus a potent treatment for ocular diseases, such as glaucoma. It has been extensively approved for use in many countries to treat open-angle glaucoma and ocular hypertension, mainly because it can reduce the intraocular pressure (IOP) by inhibiting the production of aqueous humor. By suppressing the action of carbonic anhydrase, dorzolamide reduces aqueous humor secretion, thus reducing IOP and providing relief to patients with these conditions.[1]

The development of dorzolamide has resulted from extensive research spanning years in the medicinal chemistry of the thienothiopyransulfonamide class.[2] The initial structure-activity relationship was directed towards the improvement of solubility in water without loss of efficacy as an inhibitor of carbonic anhydrase. Such a pursuit was necessary because the compound had to be efficacious as well as stable in an aqueous setting, something that is required for ocular delivery.[3] The success of dorzolamide in the clinic is not only due to its pharmacologically potent properties, but also to the fact that it has a good pharmacokinetic profile that

KRUPANIDHI COLLEGE OF PHARMACY

makes it suitable for topical application to the eye.

Recent studies have also explored the concept of designing single-molecule, multi-targeted drugs for glaucoma treatment. This strategy is intended to design drugs that can target several pathways involved in the pathophysiology of glaucoma, which may enhance therapeutic outcomes. Benzenesulfonamide derivatives containing 2-hydroxypropylamine moieties have been reported to exhibit dual activity by acting on both carbonic anhydrase and β-adrenergic receptors. Such compounds may enhance the reduction of intraocular pressure with respect to the combination therapy of timolol and dorzolamide, routinely prescribed for the treatment of patients with glaucoma.[4] This multireceptor modulation is a likely future direction for glaucoma therapy, where such compounds would result in even more effective medicines with fewer undesirable effects.

The manufacturing of dorzolamide involves the synthesis of a key intermediate, (S)-5,6-dihydro-6-methylthieno[2,3-b]thiopyran-4-one, which is an essential precursor of the final drug molecule. The synthesis and optimization of this intermediate are essential for cost-effective and efficient manufacture of dorzolamide. As glaucoma drugs are in demand, the optimization of the manufacturing process for this intermediate can enhance the scalability and availability of dorzolamide, making it more accessible to patients globally. By optimizing the synthesis of this intermediate, drug companies can lower production costs and maintain a steady supply of the drug, making it more accessible to patients with glaucoma and ocular hypertension. This research seeks to maximize the yield of the intermediate "(S)-5,6-Dihydro-6-methylthieno[2,3-b]thiopyran-4-one," a critical intermediate in the synthesis of dorzolamide.

MATERIALS AND METHODS

The chemicals and reagents used in this project were of AR and LR quality and were acquired from credible sources such as Lancaster, Sigma, and Merck. The pure crystalline compounds were characterized and identified using various analytical methods. The melting points were obtained using Thiel's tube method to determine the purity, where pure crystalline compounds gave sharp melting points. Thin-layer chromatography (TLC) was used to determine the Rf values of the organic compounds, track the reaction progress, and test product purity. The mobile phase was a hexane-ethyl acetate mixture (4:1 or 9:1), and spots were detected under UV light. Infrared (IR) spectroscopy was used to determine the functional groups, with spectra recorded between 4000-400 cm-1 using a Fourier Transform IR spectrophotometer. Proton Nuclear Magnetic Resonance (1H NMR) spectroscopy was used to determine the molecular structure and spectra were recorded on a Bruker spectrospin-400 NMR spectrophotometer using deuterated chloroform (CDCl3) as the solvent. Mass spectrometry (MS) has yielded molecular weight and structural data, which were analyzed using a GC-MS-QP5050 system.[5–7]

The preparation of (S)-5,6-dihydro-6-methylthieno[2,3-b]thiopyran-4-one required several steps. Methyl (R)-3-(p-toluenesulfonyloxy)butyrate was first prepared by the reaction of methyl (R)-3-hydroxybutyrate with p-toluenesulfonyl chloride and pyridine at temperatures below -5°C. The reaction mixture was stirred at 0-5°C for 24 hours, after which the product was isolated by filtration and washed with hexane and water. In step 2, methyl (S)-3-(2-thienylthio)butyrate was synthesized by reacting methyl (R)-3-(p-toluenesulfonyloxy)butyrate with n-butyllithium, followed by treatment with formamide at room temperature to produce a thienylthio derivative. In step 3, the hydrolysis of the methyl ester gave (S)-3-(2-thienylthio)butyric acid in an acidic medium. Finally, (S)-5,6-dihydro-6-methylthieno[2,3-b]thiopyran-4-one was obtained by treating (S)-3-(2-thienylthio)butyric acid with oxalyl chloride and DMF as catalysts, followed by the addition of stannic chloride at -10°C. The isolated product was characterized and obtained using the analytical procedures described above. The yield and purity of each step were closely monitored and determined.

RESULTS

The products of six batches of intermediates (Tosylate, Ester, Acid, and Ketone) were prepared, and the minute observations are tabulated in Table 1. The different steps of the synthesis were recognized and characterized by employing various analytical methods to ascertain the purity and structure of the compounds. Thin Layer Chromatography (TLC) was conducted with a mobile phase of Hexane and Ethyl Acetate in the ratio of 9:1. This method is especially effective for tracking the development of reactions and evaluating the purity of the compounds formed. Infrared (IR) spectra were obtained on a SHIMADZU FTIR 8400S spectrometer using the KBr pellet technique at Lake Chemicals Pvt. Ltd., Bangalore. The IR analysis identified the functional groups in the synthesized intermediates. Proton Nuclear Magnetic Resonance (1H-NMR) spectroscopy was carried out on a Bruker spectrospin-200 NMR spectrophotometer at AstraZeneca Pharma India Ltd. using CDCl3 as the solvent. This technique provides in-depth information about the molecular structures of the compounds, especially in identifying the presence of the desired protons in the intermediates. Mass spectrometry (MS) of the final ketone product was also carried out on a GC-MS-QP5050, GC-17A system from SHIMADZU at IISC, Bangalore. The GC-MS analysis provided useful data on the molecular weight and integrity of the ketone structure. From the batches synthesized, it was noted that the yield of the intermediate ketone improved from 40% in the earlier batches to 60% in the subsequent batches (Table 2), reflecting improved optimization of the reaction conditions and efficiency of the synthesis process.

Table 1: Synthesis Data for Methyl (R)-3-hydroxybutyrate (MHB) to Ketone, including Yields and Purity Levels

SL No	MHB	TOSYLATE		ESTER	ACID	KETONE	% YIELD
Molecular Formula	C5H10O3	C12H16O5S		C9H12O2S2	C8H10O2S2	$C_8H_8OS_2$	
Molecular Weight	118.13	272.32		216.32	202.29	184.28	
Melting Point		45 °C- 47 °C					
Description	Clear colorless liquid	White Crystalline powder		Brown Liquid	Light brown liquid	Light brown liquid	
		CRUDE	PURE				
B 1	250 g	500 g	415 g	306 g	220 g	180 g	72%
% Purity				88.61%	85.28%	87.60%	
B 2	250 g	500 g	460 g	330 g	230 g	350 g	
% Purity				75.55%	96.24%	75.50%	
B 3	250 g	500 g	460 g	325 g	220 g		70%
% Purity				78.77%	96.32%		
B 4	500 g	1.15 Kg	880 g	656 g	473 g	360 g	72%
% Purity				86.41%	98%	84.17%	
B 5	500 g	1.15 Kg	860 g	600 g	360 g	260 g	52%
% Purity				87.17%	99%	88.40%	
B 6	600 g	1.2 Kg	980 g	670 g	330 g	260 g	43. 3 %
% Purity				78.09%	99.70%	99.40%	

Table 2: Synthesis of Methyl (R)-3-hydroxybutyrate (MHB) to Ketone with Yield Percentage

Input	Output	% Yield
Methyl (R)-3-hydroxybutyrate [MHB]	Ketone	
2.350 Kg	1.410Kg	60%

DISCUSSION

The synthesis of (S)-5,6-dihydro-6-methylthieno[2,3-b]thiopyran-4-one, a crucial intermediate for the synthesis of Dorzolamide HCl, was successfully carried out through a sequence of optimized reactions. The reaction commenced with the reaction of methyl (R)-3-hydroxybutyrate with tosyl chloride, using pyridine and 4-dimethylaminopyridine (DMAP) as catalysts at room temperature, resulting in the production of methyl (R)-3-(p-toluenesulfonyloxy)butyrate. This reaction was closely followed by Thin Layer Chromatography (TLC), Infrared Spectroscopy (IR), and Nuclear Magnetic Resonance (NMR) spectroscopy, which validated the high purity of the desired product. The TLC results, employing hexane and ethyl acetate mixtures as solvents, showed clean products, whereas IR and NMR established the structure of the sulfonylated compound. n-Butyllithium was employed in the second step to create a lithiated thiophene intermediate in dry tetrahydrofuran (THF) with powdered sulphur. This intermediate then reacts with methyl (R)-3-(p-toluenesulfonyloxy)butyrate to give methyl (S)-3-(2-thienylthio)butyrate ester. The reaction was assisted by formamide, which promoted esterification. NMR and IR spectroscopy confirmed the successful introduction of the thienylthio group, with n-butyllithium being crucial for the formation of the reactive thiophene intermediate. The third step consists of acid-catalyzed hydrolysis of the ester with hydrochloric acid (HCl) and water to give (S)-3-(2-thienylthio)butyric acid in almost quantitative yield. The hydrolysis reaction was conducted under careful control to avoid ester reformation to obtain high-purity carboxylic acid products. This is an important step in the final cyclization reaction. The cyclization step includes the reaction of (S)-3-(2-thienylthio)butyric acid with oxalyl chloride, dimethylformamide (DMF), and stannic chloride, resulting in the production of (S)-5,6-dihydro-6-methylthieno[2,3-b]thiopyran-4-one. The reaction was also improved by increasing the stirring time in both Steps III and IV, which improved the ketone yield from 40% to 60%. Dry dichloromethane (DCM) and sodium sulphate were used to enhance the extraction process and final yield. The product was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), which established the identity and purity of the intermediate. The optimized synthesis path led to greater and more concentrated yields, indicating that the process is efficient and can be scaled. The strict management of reaction time, solvents, and purifications ensured that reproducible manufacture of the valuable pharmaceutical intermediate was possible, and thus, the process qualifies for scale-up. The study also emphasized the role of reagents and conditions, including the application of oxalyl chloride in the final cyclization step, which was in line

with earlier reports on heterocyclic compound synthesis[8] and the established procedure of employing n-butyllithium to generate reactive intermediates in heterocyclic chemistry. The final cyclization step was the most important step for improving the yield and purity of the product. These findings show the significance of optimized synthetic pathways in the pharmaceutical industry, where scalability and purity are paramount for commercial bulk production. The synthesis of (S)-5,6-dihydro-6-methylthieno[2,3-b]thiopyran-4-one proved the significance of optimizing the reaction conditions to enhance the yield and purity, which is a key requirement for pharmaceutical advancement. Optimization of this synthesis pathway may have the potential to provide more cost-effective and efficient manufacturing of Dorzolamide HCl, an important glaucoma treatment drug used extensively worldwide, with far-reaching implications for both scale-up and high-quality pharmaceutical intermediate production. Some methods used to improve the yield included increasing the stirring time during some steps, raising the yield from 60% to 40%, and keeping dry dichloromethane (DCM) and sodium sulfate available to achieve dryness upon extraction. Additional research has emphasized other procedures for improving efficiency and yield, such as limiting the reaction time but maintaining high enantiomeric purity.[9]

CONCLUSION

The process design and optimization of (S)-5,6-dihydro-6-methylthieno[2,3-b]thiopyran-4-one synthesis, an important intermediate for Dorzolamide HCl, effectively enhanced the overall yield and purity of the product. Optimization of the process was achieved through precise modification of the reaction times, solvents, and purification methods, and the yield was increased from 40% to 60%. The application of analytical tools such as TLC, IR, NMR, and GC-MS validated the intermediate structure and purity. This optimized procedure illustrates efficiency and scalability on a large pharmaceutical scale and provides a consistent pathway for the synthesis of high-quality intermediates for producing Dorzolamide HCl.

REFERENCES

1. Sugrue MF. Review the preclinical pharmacology of dorzolamide hydrochloride, a topical carbonic anhydrase inhibitor. *Journal of Ocular Pharmacology and Therapeutics*. 2009;12(3):363–376. Available from: https://doi.org/10.1089/jop.1996.12.363.
2. Balfour JA, Wilde MI. Dorzolamide: a review of its pharmacology and therapeutic potential in the management of glaucoma and ocular hypertension. *Drugs & aging*. 1997;10(5):384–403. Available from: https://doi.org/10.2165/00002512-199710050-00006.
3. Ponticello GS, Freedman MB, Habecker CN, Lyle PA, Schwam H, Varga SL, et al. Thienothiopyran-2-sulfonamides: a novel class of water-soluble carbonic anhydrase inhibitors. *Journal of Medicinal Chemistry*. 1987;30(4):591–597. Available from: https://doi.org/10.1021/jm00387a002.
4. Nocentini A, Ceruso M, Bua S, Lomelino CL, Andring JT, Mckenna R, et al. Discovery of β-adrenergic receptors blocker-carbonic anhydrase inhibitor hybrids for multi-targeted antiglaucoma therapy. *Journal of Medicinal Chemistry*. 2018;61(12):5380–5394. Available from: https://doi.org/10.1021/acs.jmedchem.8b00625.
5. Bele AA, Khale A. An overview on thin layer chromatography. *International Journal of Pharmaceutical Sciences and Research*. 2011;2(2):256–267. Available from: http://dx.doi.org/10.13040/IJPSR.0975-8232.2(2).256-67.
6. Judge K, Brown CW, Hamel L. Sensitivity of infrared spectra to chemical functional groups. *Analytical chemistry*. 2008;80(11):4186–4192. Available from: https://doi.org/10.1021/ac8000429.
7. Lundquist K. Proton (1H) NMR spectroscopy. In: Methods in Lignin Chemistry . Springer Series in Wood Science;Berlin, Heidelberg. Springer. 1992;p. 242–249. Available from: https://doi.org/10.1007/978-3-642-74065-7_17.
8. Alvarez-Builla J, Vaquero JJ, Barluenga J. Modern Heterocyclic Chemistry, 4 Volumes. John Wiley & Sons. 2011. Available from: https://onlinelibrary.wiley.com/doi/book/10.1002/9783527637737.
9. Mathre DJ, Sohar P, Brown LM. Inventors; Merck and Co Inc, Assignee. Synthesis of an intermediate for the preparation of 5, 6-dihydro-(s)-4-(ethylamino)-(s)-6-methyl-4h-thieno [2, 3-b] thiopyran-2-sulfonamide 7, 7-dioxide intermediates and related compounds. United States patent US 5,574,176. 1996. Available from: https://patents.google.com/patent/US5574176.