

Comprehensive Investigation of Esmolol-Related Organic Impurities: Synthesis, Purification, Characterization, and In Silico ADME-Toxicity Evaluation

Shejal Wakade

PES's, Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India

Amit Tapkir

amittapkir.8@gmail.com

PES's, Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India

Ashok Pathak

Anasyn Lab Private Limited, Tathawade, Pune-411033, Maharashtra, India.

Sneha Rashinkar

PES's, Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India

Samarth Kore

PES's, Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India

Ankita Pomaji

PES's, Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India

Research Article

Keywords: Esmolol hydrochloride, Impurity profiling, Structural characterization, In silico ADME, ProTox-3.0, Pharmaceutical quality control

Posted Date: October 23rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11127275/v1>

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Additional Declarations: No competing interests reported.

“Comprehensive Investigation of Esmolol-Related Organic Impurities: Synthesis, Purification, Characterization, and In Silico ADME-Toxicity Evaluation”

Shejal Kailas Wakade¹, Amit Suryakant Tapkir^{1*}, Ashok Bhau Pathak², Sneha Nandkumar Rashinkar¹, Samarth Shankar Kore¹, Ankita Gundappa Pomaji³.

¹Department of Pharmaceutical Chemistry, PES's, Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India.

²Department of Synthetic Chemistry, Anasyn Lab Private Limited, Tathawade, Pune-411033, Maharashtra, India.

³Department of Pharmaceutical Quality Assurance, PES's, Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra, India.

*Correspondence:

Dr. Amit Suryakant Tapkir

Associate Professor,

Department of Pharmaceutical Chemistry,

PES's, Modern College of Pharmacy,

Nigdi, Pune-411044, Maharashtra, INDIA.

Email: amittapkir.8@gmail.com

Tel: +91 9022890289

ABSTRACT

Background: Esmolol hydrochloride is a short-acting, β_1 -selective adrenergic blocker commonly used in acute cardiovascular care. However, information on the preparation and characterization of several Esmolol-related impurities is still limited. **Objective:** The study was undertaken to prepare, purify, characterize, and assess selected Esmolol-related impurities using experimental and in silico approaches. **Materials and Methods:** Esmolol Impurities 7, 9, 10, 11, and 12, N-Ethyl Esmolol, Esmolol Isopropyl Amide (USP), and Esmolol Dimer (USP) were prepared through appropriate organic transformations. The products were purified by recrystallisation and column chromatography using suitable solvent systems. Structural confirmation was performed using FTIR, ^1H NMR, and mass spectrometry. SwissADME was used to examine physicochemical and ADME characteristics, while ProTox-3.0 was used for toxicity prediction. **Results:** The investigated impurities were obtained, and their structures were confirmed by spectroscopic and mass-spectrometric techniques. Most compounds showed acceptable physicochemical characteristics and satisfied Lipinski's rule of five, while Esmolol Dimer did not fully meet these criteria. Predicted oral LD_{50} values ranged from 500 to 8140 mg/kg, corresponding to toxicity classes IV-VI. No predicted hepatotoxic, neurotoxic, or cardiotoxic effects were observed. Esmolol Isopropyl Amide and Esmolol Dimer were isolated in yields of 15% and 20%, respectively. **Conclusion:** The study establishes practical methods for obtaining selected Esmolol-related impurities and provides their preliminary physicochemical and toxicity profiles, which may be useful for impurity profiling, analytical method development, stability studies, and regulatory assessment.

KEYWORDS: Esmolol hydrochloride; Impurity profiling; Structural characterization; In silico ADME; ProTox-3.0; Pharmaceutical quality control.

INTRODUCTION

Hypertension is a widespread health problem worldwide and is considered one of the major contributors to cardiovascular diseases and related deaths.^{1,2} Esmolol hydrochloride is a cardio-selective β_1 -adrenergic receptor blocker widely prescribed for rapid management of supraventricular tachyarrhythmias, perioperative hypertension, and other acute cardiovascular disorders because of its ultra-short duration of action.³ Structurally, Esmolol belongs to the aryloxypropanolamine class of β -blockers, in which the aryloxypropanolamine moiety is responsible for β -adrenergic receptor binding and selectivity (See Figure 1a and 1b).⁴ The presence of an ester linkage enables rapid hydrolysis by red blood cell esterases, resulting in a short elimination half-life and precise dose titration. Esmolol lowers cardiovascular activity by selectively blocking β_1 -adrenergic receptors in the heart. This action decreases heart rate and myocardial contractility, ultimately reducing cardiac output, as depicted in Figure 2.⁵

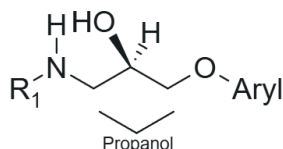


Fig 1. a: General Structure of
Aryloxypropanolamine

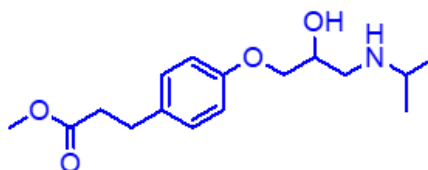


Fig 1. b: Esmolol API Structure

Organic impurities formed during drug synthesis, purification, storage, or degradation may affect the quality, safety, and stability of pharmaceutical products. Therefore, the International Council for Harmonization (ICH) guidelines Q3A(R2) and Q3B(R2) require the identification, characterization, and control of impurities present above the specified reporting thresholds.^{6,7} Well-characterized impurity reference standards are essential for impurity profiling, analytical method validation, stability studies, and regulatory compliance.

Although analytical methods for Esmolol have been reported, systematic studies on the synthesis and characterization of Esmolol-related organic impurities remain limited. In addition, information on their predicted ADME and toxicity profiles is scarce. Therefore, the present study describes the synthesis, purification, and Characterization of eight Esmolol-related organic impurities using FTIR, ^1H NMR, and mass spectrometry. SwissADME and ProTox-3.0 were employed to assess the physicochemical properties, drug-likeness, pharmacokinetic characteristics, and predicted toxicity profiles of the synthesized Esmolol-related impurities. The generated data provide reference standards and computational safety information to support impurity profiling, pharmaceutical quality control, and regulatory evaluation of Esmolol formulations.

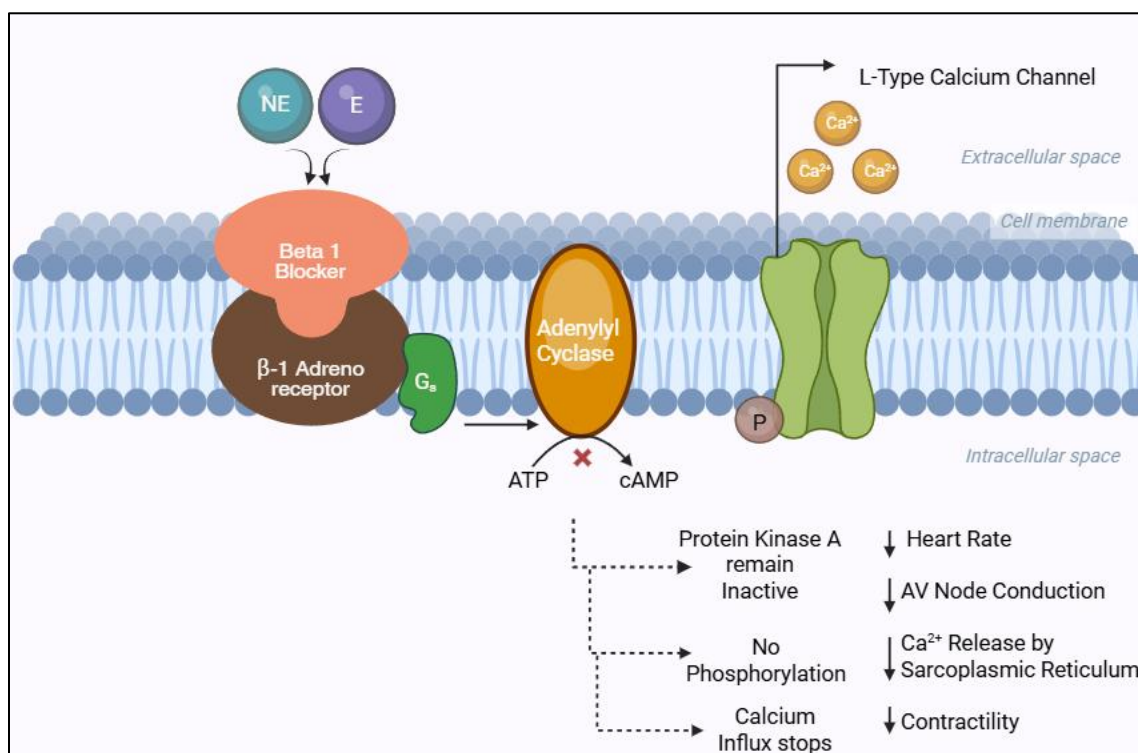


Fig 2: Mechanism of action of Beta 1 Blocker on β_1 -Adrenoreceptor

MATERIALS AND METHODS

Chemicals, Reagents, and Solvents

All chemicals and reagents used throughout the study were of analytical grade and were procured from reliable commercial sources without additional purification unless

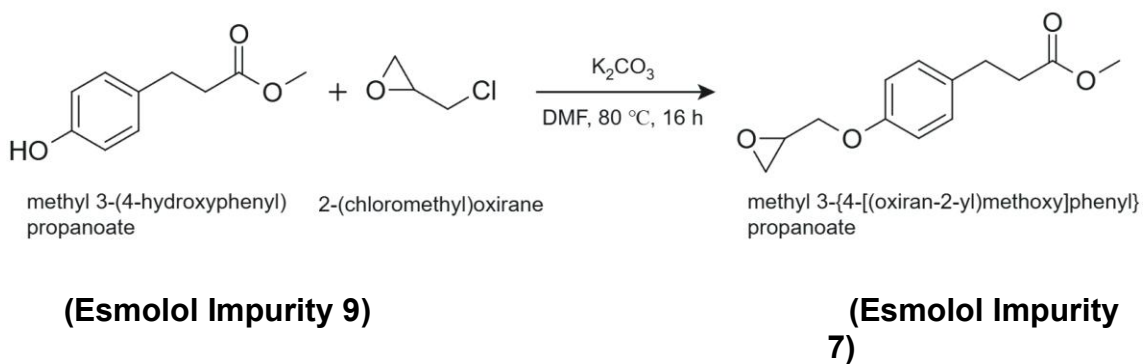
otherwise specified. Solvents were procured from Advent Chembio Pvt. Ltd. (Mumbai, India), Spectrochem Pvt. Ltd. (Mumbai, India), BLD Pharmatech Pvt. Ltd. (Hyderabad and Mumbai, India), TCI Chemicals (India) Pvt. Ltd. (Chennai, India), Merck Specialities Pvt. Ltd. (Mumbai, India), and Fisher Scientific Pvt. Ltd. (Mumbai, India). Ultrapure water required for the study was obtained through purification with a Milli-Q® water system (Millipore, USA).

Synthetic Protocol

In this investigation, esmolol (API) and eight impurities were synthesized as detailed in the following section and scheme illustrated in Figure 3:

Synthesis of methyl 3-{4-[(oxiran-2-yl)methoxy]phenyl}propanoate (Esmolol Impurity 7)-

Methyl 3-(4-hydroxyphenyl)propanoate (Esmolol Impurity 9) (20 g, 0.11 mol) was transferred to a 250 mL round-bottom flask containing dry DMF (60 mL) and stirred at room temperature for 5 min. Potassium carbonate (15.3 g, 1 equivalent) was then added, followed by the gradual addition of epichlorohydrin (10.4 mL, 1.2 equivalents). The reaction mixture was heated at 80 °C with continuous stirring for 16 h. Reaction progress was monitored by TLC using ethyl acetate:n-hexane (10:90, v/v). After completion, the mixture was cooled to room temperature and carefully quenched with cold water (40-50 mL), followed by stirring for an additional 5 min. The reaction mixture was extracted with ethyl acetate, and the aqueous phase was re-extracted with the same solvent. The combined organic extracts were washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Esmolol Impurity 7 was obtained as a brown oily liquid in 86% yield.



Synthesis of methyl 3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)propanoate (Esmolol API)-

Step I: Synthesis of 3-(4-hydroxyphenyl)propanoic acid (Esmolol Impurity 10)-

(As discussed earlier)

Step II: Synthesis of methyl 3-(4-hydroxyphenyl)propanoate (Esmolol Impurity 9)-

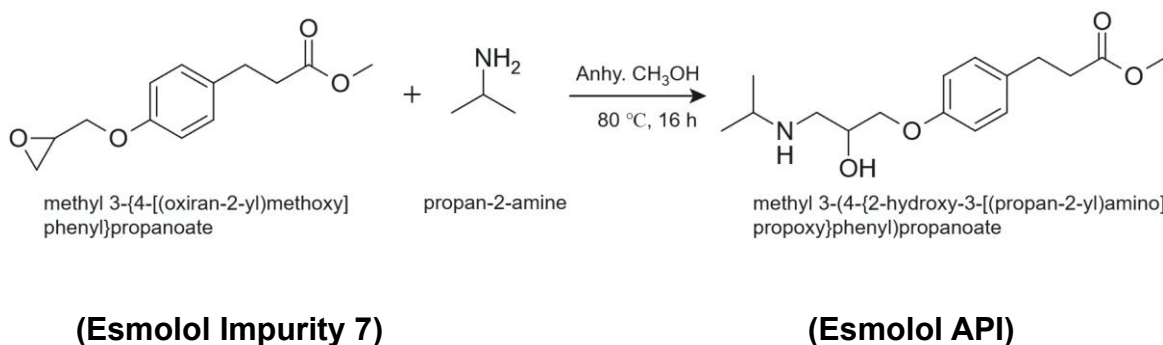
(As discussed earlier)

Step III: Synthesis of methyl 3-{4-[(oxiran-2-yl)methoxy]phenyl}propanoate (Esmolol Impurity 7)-

(As discussed earlier)

Step IV: Synthesis of methyl 3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)propanoate- (Esmolol API)

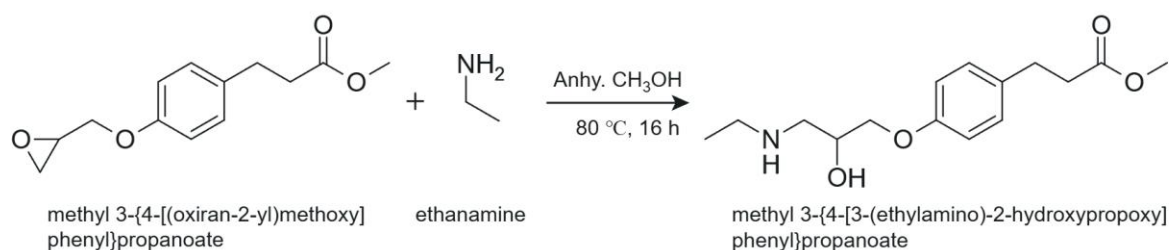
Methyl 3-{4-[(oxiran-2-yl)methoxy]phenyl}propanoate (Esmolol Impurity 7) (20 g, 0.084 mol) was dissolved in anhydrous methanol (60 mL) with stirring. The resulting solution was cooled to 0 °C using an ice-water bath, and isopropylamine (10.9 mL, 1.5 equivalents) was added gradually with continuous stirring. The reaction mixture was subsequently heated under reflux at 80 °C for 16 h. Reaction completion was monitored by TLC using methanol:dichloromethane (5:95, v/v) as the mobile phase. After completion, the mixture was allowed to cool to room temperature and quenched with cold water (40–50 mL), followed by stirring for 5 min. Methanol and excess isopropylamine were removed under reduced pressure using a rotary evaporator. The resulting crude Esmolol was obtained as a brown viscous oil in 46.5% yield.



Synthesis of methyl 3-{4-[3-(ethylamino)-2-hydroxypropoxy]phenyl}propanoate (N-Ethyl Esmolol)-

Methyl 3-{4-[(oxiran-2-yl)methoxy]phenyl}propanoate (Esmolol Impurity 7) (13 g, 0.055 mol) was dissolved in anhydrous methanol (40 mL) and cooled to 0 °C. A 70% aqueous

solution of N-ethylamine (6.5 mL, 1.5 equivalents) was added slowly with continuous stirring while maintaining the temperature at 0 °C. The reaction mixture was then heated to 80 °C and stirred overnight. Reaction progress was monitored by TLC using methanol:dichloromethane (5:95, v/v) as the mobile phase. Upon completion, the reaction mixture was cooled to room temperature and quenched with cold water (approximately 40 mL). Volatile components were removed under reduced pressure, and the remaining residue was extracted with ethyl acetate. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. N-Ethyl Esmolol was obtained as a yellow viscous oil in 38.18% yield.

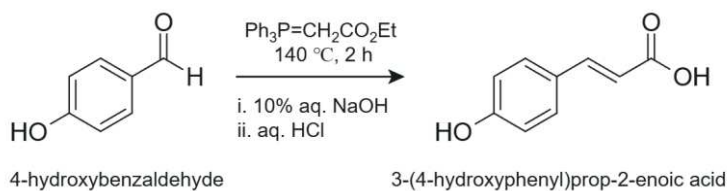


(Esmolol Impurity 7)

(N-Ethyl Esmolol)

Synthesis of 3-(4-hydroxyphenyl)prop-2-enoic acid (Esmolol Impurity 11)-

The preparation was carried out based on a reported Wittig olefination approach,⁸ with modifications to the reaction conditions and product isolation. 4-Hydroxybenzaldehyde (1.22 g, 0.01 mol) was combined with ethyl (triphenylphosphoranylidene)acetate (3.48 g, 1 equivalent) in a 100 mL round-bottom flask. The mixture was heated at 140 °C for 90 min in the absence of a solvent. Reaction completion was assessed by TLC using ethyl acetate:n-hexane (30:70, v/v) as the mobile phase. The reaction mixture was then cooled to room temperature, followed by the addition of 10% aqueous sodium hydroxide (20 mL), and stirred for 30 min. The insoluble triphenylphosphine oxide was removed by filtration. The filtrate was acidified to pH 2 with 6 M hydrochloric acid, and the separated solid was collected and washed with cold water. After drying under vacuum, Esmolol Impurity 11 was obtained as a pale-yellow solid in 75% yield.



(Starting Compound)

(Esmolol Impurity 11)

Synthesis of methyl 3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)prop-2-enoate (Esmolol Impurity 12)-

Step I: Synthesis of 3-(4-hydroxyphenyl)prop-2-enoic acid (Esmolol Impurity 11)-

(As discussed earlier)

Step II: Synthesis of methyl 3-(4-hydroxyphenyl)prop-2-enoate (Intermediate 1)

3-(4-Hydroxyphenyl)prop-2-enoic acid (Impurity 11) (5.0 g, 0.03 mol) was dissolved in anhydrous methanol (30 mL). Concentrated sulfuric acid (2.43 mL, 1.5 equivalents) was added carefully while maintaining the reaction mixture under cooling. The mixture was then heated under reflux at 65 °C, and the reaction progress was monitored by TLC using methanol:DCM (5:95, v/v) as the mobile phase. After completion, the reaction mixture was allowed to cool and transferred into ice-cold water to induce precipitation. The separated solid was collected by filtration, washed thoroughly with water, and dried under reduced pressure. Intermediate 1 was obtained as a white crystalline solid in 88% yield.



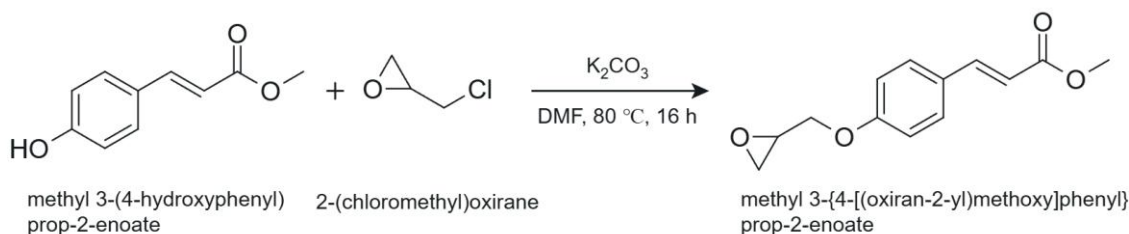
(Esmolol Impurity 11)

(Intermediate 1)

Step III: Synthesis of methyl 3-{4-[(oxiran-2-yl)methoxy]phenyl}prop-2-enoate(Intermediate 2)

Methyl 3-(4-hydroxyphenyl)prop-2-enoate (Intermediate 1) (4.72 g, 0.026 mol) was dissolved in DMF (30 mL), and potassium carbonate (3.66 g, 1 equivalent) was added. Epichlorohydrin (2.49 mL, 1.2 equivalents) was then introduced gradually with continuous

stirring. The reaction mixture was heated at 80 °C, and the reaction progress was monitored by TLC using methanol:dichloromethane (5:95, v/v) as the mobile phase. Upon completion, the mixture was cooled to room temperature and poured into ice-cold water. The resulting precipitate was collected by filtration, washed with water, and dried under vacuum to afford Intermediate 2 as a light-pink crystalline powder in 98% yield.

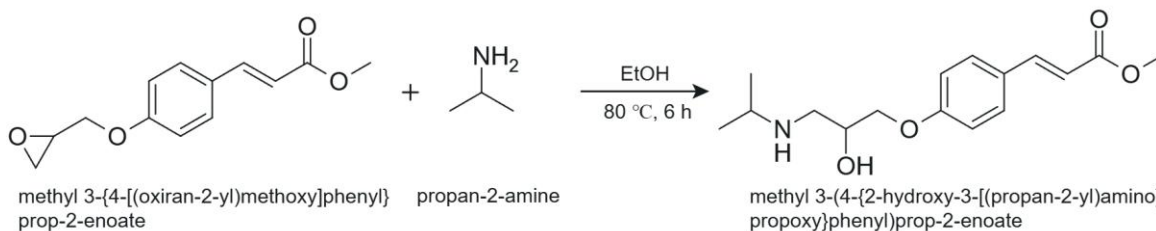


(Intermediate 1)

(Intermediate 2)

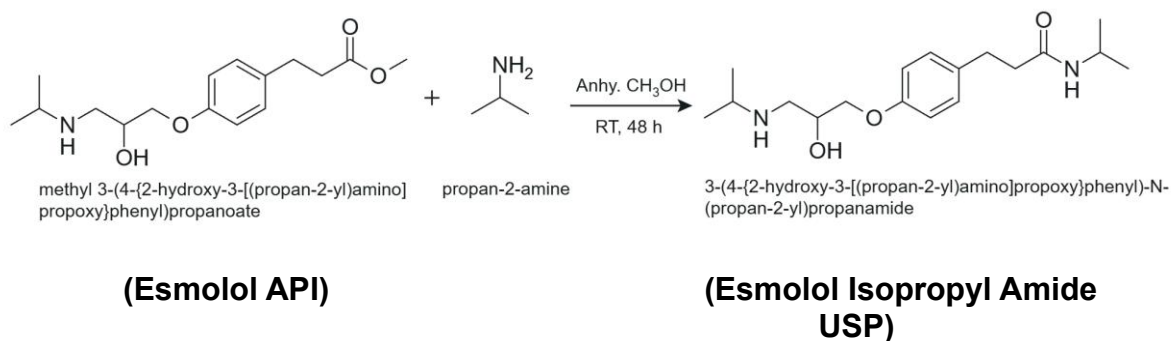
Step IV: Synthesis of methyl 3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)prop-2-enoate (Esmolol Impurity 12)

Intermediate 2, methyl 3-{4-[(oxiran-2-yl)methoxy]phenyl}prop-2-enoate (6.2 g, 0.026 mol), was dissolved in absolute ethanol (30 mL). Isopropylamine (2.7 mL, 1.2 equivalents) was added slowly at 0 °C with continuous stirring. The reaction mixture was subsequently heated under reflux at 80 °C for 6 h, and the reaction progress was monitored by TLC using methanol:DCM (5:95, v/v) as the mobile phase. After completion, the solvent was removed under reduced pressure, and the residue was diluted with water. The aqueous mixture was extracted with ethyl acetate (3 × 25 mL), and the combined organic layers were washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Esmolol Impurity 12 was obtained as a white crystalline powder in 77% yield.



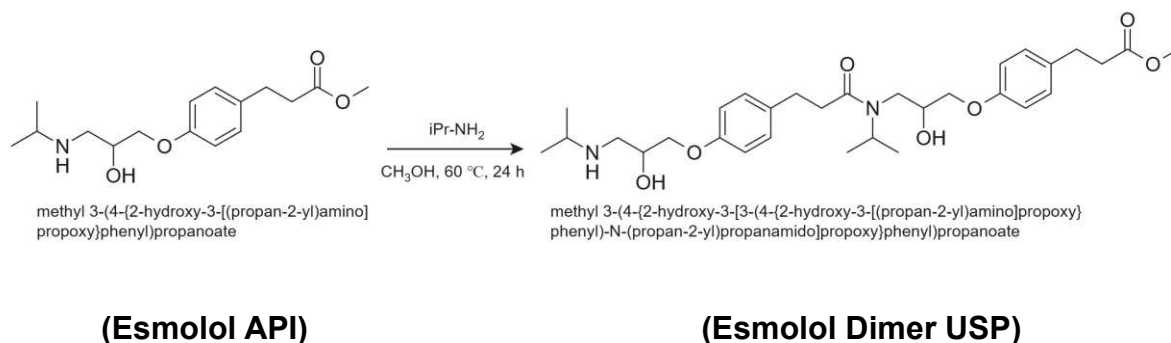
(Intermediate 2)**(Esmolol Impurity 12)****Synthesis of 3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)-N-(propan-2-yl)propanamide (Esmolol Isopropyl Amide USP)-**

Esmolol API (2.2 g, 0.007 mol) was dissolved in anhydrous methanol (10 mL). Isopropylamine (1.9 mL, 3 equiv.) was added slowly to the solution under cooling with continuous stirring. The reaction mixture was then allowed to reach room temperature and stirred for 48 h. Reaction completion was monitored by TLC using ethyl acetate/n-hexane (20:80, v/v) as the mobile phase. Upon completion, methanol and the excess isopropylamine were removed under reduced pressure. The resulting residue was further dried under vacuum to obtain Esmolol isopropyl amide (USP) as a yellow oily liquid in 15% yield.

**Synthesis of methyl 3-(4-{2-hydroxy-3-[3-(4-{2-hydroxy-3-[(propan-2-yl)amino]Propoxy}phenyl)-N-(propan-2-yl)propanamido]propoxy}phenyl)propanoate (Esmolol Dimer USP)-**

Esmolol API (1.0 g, 0.003 mol) was dissolved in anhydrous methanol (20 mL) in a 50 mL round-bottom flask. The solution was cooled to 0 °C, and isopropylamine (0.434 mL, 1.5 equiv.) was added slowly with stirring. The reaction mixture was subsequently heated at 60 °C and maintained at this temperature for 24 h. Reaction progress was monitored by

TLC using methanol/dichloromethane (5:95, v/v) as the mobile phase. After completion, the reaction mixture was concentrated under reduced pressure, and the resulting residue was dried under vacuum to obtain Esmolol dimer (USP) as a white, sticky solid in 20% yield.



TLC Monitoring and Purification by Column Chromatography

TLC analysis was carried out using silica gel 60 F254 precoated aluminium plates (Merck, Germany), and the separated spots were visualized under UV illumination at 254 nm. Purification of the synthesized compounds was performed by column chromatography using silica gel (mesh size 70-230). The solvent system comprised methanol and DCM in varying ratios, selected based on the polarity of the target molecules as determined by preliminary TLC analyses. TLC was carefully monitored to track the elution progress to ensure effective separation of the desired compounds from impurities.

Structural Characterization

FTIR Analyses

FTIR spectra were recorded using a JASCO M-4100 FTIR spectrophotometer (JASCO, Tokyo, Japan). Samples were prepared as KBr pellets and analysed under standard operating conditions. For liquid samples, FTIR spectra were recorded by placing a thin film of the sample between two sodium chloride (NaCl) plates. Absorption bands are reported as ν_{max} (cm^{-1}), corresponding to characteristic vibrational transitions that confirm the presence of specific functional groups. Representative FTIR data provided in the Supplementary Material.

NMR Analyses

The ^1H NMR spectra were acquired using a Bruker Avance 400 MHz spectrometer (Bruker, Rheinstetten, Germany) fitted with a broadband observe (BBO) probe. Chemical shifts (δ) are presented in ppm, referenced to tetramethylsilane (TMS) or the residual solvent peak, while coupling constants (J) are given in Hz. Representative Proton NMR spectra are provided in the Supplementary Materials.

Mass Analyses

Mass spectrometric characterization was carried out using an Agilent 6320 Ion Trap mass spectrometer (Agilent Technologies, USA) fitted with an electrospray ionization (ESI) source and operated in both +ve and -ve ion modes. Corresponding spectra are included in the Supplementary Materials.

In Silico ADME Prediction (SwissADME)-

The physicochemical properties, drug-likeness, and pharmacokinetic profiles of Esmolol API and the synthesized impurities were predicted using SwissADME. ChemDraw-generated SMILES were used as input for the SwissADME web server to assess molecular descriptors, Lipinski's rule-of-five compliance, and bioavailability scores.

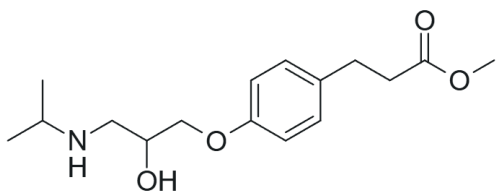
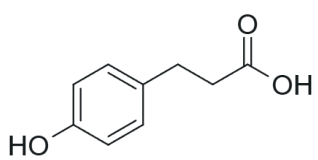
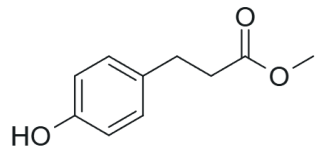
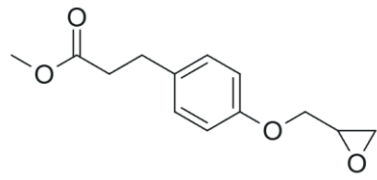
In Silico Toxicity Prediction (ProTox-3.0)-

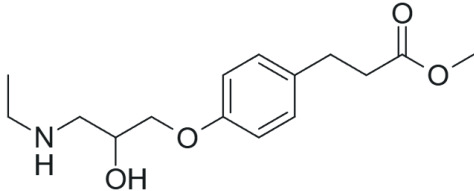
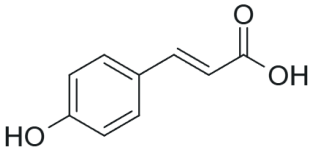
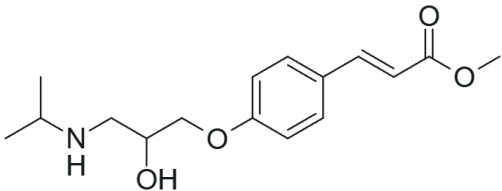
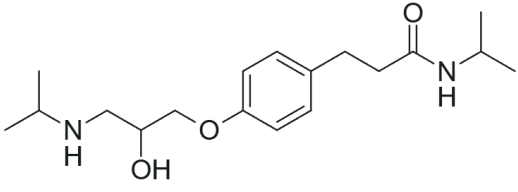
The toxicity profiles of the Esmolol API and the investigated compounds were predicted using the ProTox-3.0 platform. SMILES generated in ChemDraw were submitted to the server to predict oral LD_{50} , toxicity class, and organ toxicity endpoints for preliminary safety assessment.

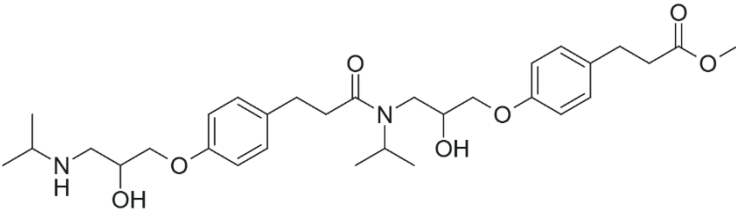
RESULTS

Esmolol API and its related organic impurities were successfully synthesized and purified. The synthesized compounds were characterized based on their key physicochemical properties, which are summarized in Table 1.

Table No. 1: Summary of Esmolol API and Related Impurities

Sr. No.	Common Name	Structure and IUPAC Name	Appearance, % Yield	M. P./ B. P.
1	Esmolol API	 methyl 3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)propanoate	Brown oily viscous liquid, 46.5 %	432-434 °C
2	Esmolol Impurity 10	 3-(4-hydroxyphenyl)propanoic acid	Off-white solid, 83 %	126-128 °C
3	Esmolol Impurity 9	 methyl 3-(4-hydroxyphenyl)propanoate	Yellowish oily liquid, 100 %	106-108 °C
4	Esmolol Impurity 7	 methyl 3-{4-[(oxiran-2-yl)methoxy]phenyl}propanoate	Brown oily liquid, 86 %	348-350 °C

5	N-Ethyl Esmolol	 methyl 3-{4-[3-(ethylamino)-2-hydroxypropoxy]phenyl}propanoate	Yellow oily viscous liquid, 38.18 %	593- 595 °C
6	Esmolol Impurity 11	 3-(4-hydroxyphenyl)prop-2-enoic acid	Pale yellow Solid, 75 %	208- 210 °C
7	Esmolol Impurity 12	 methyl-3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)prop-2-enoate	White crystalline solid, 77 %	62- 64 °C
8	Esmolol Isopropyl Amide USP	 3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)-N-(propan-2-yl)propanamide	Yellow oily liquid, 15 %	498- 500 °C

9	Esmolol Dimer USP	 methlyl 3-(4-{2-hydroxy-3-[3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)-N-(propan-2-yl)propanamido]propoxy}phenyl)propanoate	White sticky solid, 20 %	710- 712 °C
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Spectral Characterization

(Refer to Figures S1-S23 For Spectroscopic Analysis Data)

Spectral data of 3-(4-hydroxyphenyl)propanoic acid (Esmolol Impurity 10)-

FTIR Spectra: 3401.82 (O-H carboxylic acid), 3050 (C-H aromatic), 2955 (C-H alkane), 1706.69 (C=O carboxylic acid), 1598.7 (C=C aromatic), 1217.83 (C-O phenol).

¹H NMR Spectra: δ 12.04 (s, 1H, COOH), 9.13 (s, 1H, Ar-OH), 7.00 (d, 2H, Ar-CH₂), 6.65 (d, 2H, Ar-CH₂), 2.69 (t, 2H, O-C-CH₂), 2.44 (t, 2H, O-C-CH₂-CH₂).

Mass Spectr: m/z 165.5 [M-H]⁺ (C₉H₁₀O₃, M. W. = 166.18 g. mol⁻¹).

Spectral data of methyl 3-(4-hydroxyphenyl)propanoate (Esmolol Impurity 9)-

FTIR Spectra: 3404.71 (O-H, phenol), 2953.45 (C-H, aliphatic), 1715.37 (C=O, ester), 1615.09, 1516.74 (C=C, aromatic ring), 1135.87 (C-O, ester & phenol).

¹H NMR Spectra: δ 7.04 (d, 2H, Ar-CH₂), 6.74 (d, 2H, Ar-CH₂), 5.28 (s, 1H, Ar-OH), 3.65 (s, 3H, COOCH₃), 2.86 (t, 2H, -OOC-CH₂-CH₂), 2.58 (t, 2H, -OOC-CH₂).

Mass spectra: m/z 181.3 [M+H]⁺ (C₁₀H₁₂O₃, M. W.= 180.2 g. mol⁻¹).

Spectral data of methyl 3-{4-[(oxiran-2-yl)methoxy]phenyl}propanoate (Esmolol Impurity 7)-

FTIR Spectra: 3033.48 (C-H, aromatic), 2950.55 (C-H, aliphatic), 1735.62 (C=O, ester), 1614.13, 1584.24 (C=C, aromatic), 1141.65 (C-O, ester & ether), 957.48 (C-O-C, epoxide, aromatic).

^1H NMR Spectra: δ 7.10 (d, 2H, Ar-CH₂), 6.83 (dd, 2H, Ar-CH₂), 4.12 (m, 3H, -OCHCH₂O), 3.93 (m, 2H, -OCH₂CH), 3.65 (s, 3H, COOCH₃), 2.87 (t, 2H, -OOCCH₂CH₂), 2.58 (t, 2H, -OOCCH₂).

Mass spectra: m/z 236.8 [M+H]⁺ (C₁₃H₁₆O₄, M. W.= 236.27 g. mol⁻¹).

Spectral data of methyl 3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)propanoate (API)-

FTIR Spectra: 3610.09, 3553.2 (O-H, phenol), 3300.57 (N-H, secondary amine), 2954.41 (C-H, aromatic), 2870.52 (C-H, aliphatic), 1736.58 (C=O, ester), 1614.13, 1581 (C=C, aromatic), 1437.67, 1365.35 (CH₃, Symmetric bending, isopropyl), 1154.19 (C-O, ester).

^1H NMR Spectra: δ 7.12 (d, 2H, Ar-CH₂), 6.84 (d, 2H, Ar-CH₂), 5.01 (s, 1H, COH), 3.89 (m, 1H, -OCH(CH₂)₂), 3.82 (d, 2H, -OCH₂), 3.56 (s, 3H, COOCH₃), 2.72-2.78 (m, 3H, CH₂NHCH), 2.68-2.69 (t, 2H, -OOCCH₂CH₂), 2.55-2.59 (t, 2H, -OOCCH₂), 0.98 (dd, 6H, -NHCH(CH₃)₂).

Mass spectra: m/z 296.6 [M+H]⁺ (C₁₆H₂₅NO₄, M. W.= 295.38 g. mol⁻¹).

Spectral data of methyl 3-{4-[3-(ethylamino)-2-hydroxypropoxy]phenyl}propanoate (N-Ethyl Esmolol)-

FTIR Spectra: 3610.09, 3555.13 (O-H, phenol), 3359.39 (N-H, secondary amine), 3008 (C-H, aromatic), 2930 (C-H, aliphatic), 1734.66 (C=O, ester), 1617.98, 1512.88 (C=C, aromatic), 1438.64 (CH₂/CH₃ bending), 1098.26 (C-O, ester/ether).

^1H NMR Spectra: δ 7.12 (d, 2H, Ar-CH₂), 6.83 (d, 2H, Ar-CH₂), 5.12 (s, 1H, COH), 3.79-3.90 (m, 4H, OCH₂CHOH), 3.56 (s, 3H, COOCH₃), 2.65-2.78 (m, 4H, CH₂NHCH₂), 2.55-2.59 (m, 4H, OOCCH₂CH₂), 1.00 (t, 3H, NCH₂CH₃).

Mass spectra: m/z 282.5 [M+H]⁺ (C₁₅H₂₃NO₄, M. W.= 281.35 g. mol⁻¹).

Spectral data of 3-(4-hydroxyphenyl)prop-2-enoic acid (Esmolol Impurity 11)-

FTIR Spectra: 3385.42 (O-H, phenol & Carboxylic acid), 3028.66 (C-H, aromatic), 1672.95 (C=O, carboxylic acid), 1627.63 (C=C, Alkene), 1511.92 (C=C, aromatic ring), 1245.79 (C-O, phenol).

^1H NMR Spectra: δ 12.11 (s, 1H, COOH), 9.95 (s, 1H, Ar-OH), 7.46-7.50 (m, 3H, OOC-CH=CH, Ar-CH₂), 6.79 (d, 2H, Ar-CH₂), 6.30 (d, 1H, OOC-CH=CH).

Mass spectra: m/z 163.7 $[\text{M}-\text{H}]^+$ (C₉H₈O₃, M. W.= 164.16 g. mol⁻¹).

Spectral data of methyl-3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)prop-2-enoate (Esmolol Impurity 12)-

FTIR Spectra: 3352.64 (O-H, phenol; N-H, secondary amine), 3175.22 (C-H, aromatic), 2989.12 (C-H, aliphatic), 1698.98 (C=O, ester), 1629.55 (C=C, conjugated), 1602.56, 1510.95 (C=C, aromatic), 1394.28 (CH₃/CH₂, isopropyl), 1259.29, 1207.22, 1108.87, 1078.98 (C-O, ester/ether).

^1H NMR Spectra: δ 7.62 (d, 1H, -OOCCH=CH), 7.43 (d, 2H, Ar-CH₂), 6.88 (d, 2H, Ar-CH₂), 6.30 (d, 1H, -OOCCH=CH), 4.60 (m, 1H, -OCH(CH₂)₂), 4.07 (d, 1H, Ar-OCH₂), 3.78 (s, 3H, COOCH₃), 3.40 (m, 1H, -NHCH), 3.26 (m, 2H, -NHCH₂), 1.46 (d, 6H, -NHCH(CH₃)₂).

Mass spectra: m/z 294.1 $[\text{M}+\text{H}]^+$ (C₁₆H₂₃NO₄, M. W.= 293.36 g. mol⁻¹).

Spectral data of 3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)-N-(propan-2-yl)propanamide (Esmolol Isopropyl Amide USP)-

Mass spectr: m/z 323.4 $[\text{M}+\text{H}]^+$ (C₁₈H₃₀N₂O₃, M. W.= 322.4 g. mol⁻¹).

Spectral data of methyl 3-(4-{2-hydroxy-3-[3-(4-{2-hydroxy-3-[(propan-2-yl)amino]propoxy}phenyl)-N-(propan-2-yl)propanamido]propoxy}phenyl)propanoate (Esmolol Dimer USP)-

Mass spectra: m/z 559.1 $[\text{M}+\text{H}]^+$ (C₃₁H₄₆N₂O₇, M. W.= 558.7 g. mol⁻¹).

ADME Prediction

Lipinski's rule and bioavailability were checked using the SwissADME software (Refer to Table 2).

Table 2: SwissADME data for Esmolol API and related impurities

Compound	MW (g/mol)	TPS A (Å²)	H A	H B D	Rotatable Bonds	Consensus Log P	ESOL Log S	Solubility Class	Lipinski Rule	Bioavailability Score
Esmolol API	295.3	67.7	5	2	10	2.24	-2.39	Soluble	Pass (0 violation)	0.55
Impurity 10	166.1	57.5	3	2	3	1.31	-1.77	Very soluble	Pass (0 violation)	0.85
Impurity 9	180.2	46.5	3	1	4	1.80	-2.27	Soluble	Pass (0 violation)	0.55
Impurity 7	236.2	48.6	4	0	7	2.11	-2.16	Soluble	Pass (0 violation)	0.55
N-Ethyl Esmolol	281.3	67.7	5	2	10	1.88	-1.97	Very soluble	Pass (0 violation)	0.55
Impurity 11	164.1	57.5	3	2	2	1.26	-2.02	Soluble	Pass (0 violation)	0.85
Impurity 12	293.3	67.7	5	2	9	2.09	-2.30	Soluble	Pass (0 violation)	0.55
Esmolol Isopropyl amide (USP)	322.4	70.5	4	3	11	2.45	-2.62	Soluble	Pass (0 violation)	0.55

Esmolol Dimer (USP)	558.7	117.5	8	3	20	3.71	-4.27	Modera tely soluble	Fail (1 violation)	0.55
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Organ Toxicity Prediction

The potential organ toxicity of the synthesized Esmolol impurities was evaluated using ProTox-3.0 (Refer to Table 3).

Table 3: Organ Toxicity data derived from ProTox 3.0

Compound Name	Predicted LD₅₀ (mg/kg)	Toxicity class	Hepatotoxicity	Neurotoxicity	Nephrotoxicity	Respiratory Toxicity	Cardiotoxicity
Esmolol API	2000	IV	Inactive	Inactive	Active	Active	Inactive
Impurity 10	2000	IV	Inactive	Inactive	Active	Inactive	Inactive
Impurity 9	2000	IV	Inactive	Inactive	Active	Inactive	Inactive
Impurity 7	8140	VI	Inactive	Inactive	Active	Inactive	Inactive
N-Ethyl Esmolol	2000	IV	Inactive	Inactive	Active	Active	Inactive
Impurity 11	2850	V	Inactive	Inactive	Active	Inactive	Inactive
Impurity 12	978	IV	Inactive	Inactive	Active	Active	Inactive
Esmolol Isopropyl amide (USP)	2000	IV	Inactive	Inactive	Active	Active	Inactive
Esmolol Dimer (USP)	500	IV	Inactive	Inactive	Active	Active	Inactive

** Toxicity Class (ProTox-3.0) based on predicted oral LD₅₀ values: Class I (≤ 5 mg/kg, fatal if swallowed); Class II (> 5 – 50 mg/kg, fatal if swallowed); Class III (> 50 – 300 mg/kg, toxic if swallowed); Class IV (> 300 – 2000 mg/kg, harmful if swallowed); Class V (> 2000 – 5000 mg/kg, may be harmful if swallowed); and Class VI (> 5000 mg/kg, non-toxic).*

DISCUSSION

The present study demonstrates an efficient and reproducible synthetic strategy for the preparation of Esmolol-related organic impurities using readily available starting materials and conventional synthetic transformations. The sequential application of the Wittig reaction, catalytic hydrogenation, esterification, glycidylation, and nucleophilic epoxide ring-opening enabled the synthesis of both process-related and pharmacopeial impurities with satisfactory yields.⁹ The successful conversion of the common epoxide intermediate (Impurity 7) into Esmolol API and structurally related derivatives highlights the synthetic utility of this intermediate for generating β -amino alcohol analogues.

Structural elucidation using complementary spectroscopic techniques verified the molecular identity of all synthesized impurities, enabling their use as authenticated reference standards for impurity profiling and analytical validation.

SwissADME analysis indicated that most synthesized impurities possess favourable physicochemical properties and acceptable drug-likeness, with compliance to Lipinski's rule of five. Esmolol Dimer (USP) exhibited a single Lipinski violation due to its higher molecular weight, a predictable consequence of molecular dimerization rather than an indication of poor molecular quality. Overall, the predicted ADME characteristics suggest that structural modifications introduced during impurity formation do not markedly alter the fundamental physicochemical behaviour of the Esmolol scaffold.

Toxicological assessment using ProTox-3.0 predicted oral LD₅₀ values corresponding to toxicity classes IV-VI,¹⁰ indicating comparatively low acute toxicity risk. None of the synthesized impurities demonstrated predicted hepatotoxicity, cardiotoxicity, or neurotoxicity, whereas nephrotoxicity was predicted for all compounds and respiratory toxicity was predicted for selected impurities. Although these predictions require

experimental confirmation, they provide an initial toxicological profile that may support impurity qualification and risk assessment during pharmaceutical development.¹¹

Collectively, the developed synthetic methodology, comprehensive structural characterization, and in silico ADME-toxicity evaluation offer a structured framework for the preparation and assessment of Esmolol-related organic impurities. The generated data are expected to facilitate impurity identification, analytical method development, stability evaluation, and regulatory assessment of Esmolol drug substances and pharmaceutical formulations.

CONCLUSION

This research successfully integrates synthetic routes for the preparation of selected Esmolol-related organic impurities, followed by purification and structural confirmation using FTIR, ¹H NMR, and mass spectrometry. The synthesized impurities serve as well-characterized reference standards for impurity profiling, analytical method validation, and stability studies. In silico assessment using SwissADME and ProTox-3.0 predicted favourable physicochemical and pharmacokinetic properties, with low to moderate acute toxicity and no predicted hepatotoxicity, cardiotoxicity, or neurotoxicity for the investigated impurities. These findings provide scientifically validated data to support impurity identification, quality control, and regulatory compliance in accordance with ICH guidelines. The availability of authenticated Esmolol impurity standards will facilitate pharmaceutical quality assurance and strengthen the regulatory evaluation of Esmolol drug substances and formulations. The study confirmed the feasibility of preparing Esmolol isopropyl amide and dimer, although their isolation in limited yields indicates that the respective reaction conditions require further refinement. The unsuccessful formation of the nitroso impurity suggests that the selected synthetic approach was unsuitable for this transformation. Further investigation of reaction conditions and synthetic strategies may help improve impurity formation, yield, and isolation, thereby supporting a more comprehensive impurity profile of Esmolol. Further studies integrating molecular docking, molecular dynamics simulations, and experimental toxicological evaluation may provide additional insights into the biological significance of these impurities.

SUMMARY

This study accomplished the synthesis, purification, and structural characterization of Esmolol-related organic impurities through a simple and efficient synthetic approach employing classical organic transformations. The synthesized compounds were successfully characterized by FTIR, ¹H NMR, and mass spectrometry, confirming their chemical structures. The developed synthetic route afforded satisfactory yields and demonstrated good scope for structural modification of the Esmolol scaffold. Esmolol isopropyl amide and Esmolol dimer were obtained successfully under the selected experimental conditions, giving isolated yields of 15% and 20%, respectively. An additional attempt was made to generate a nitroso-related impurity of Esmolol, but the desired compound was not formed under the conditions employed. Computational evaluation using SwissADME indicated that most compounds possessed favourable physicochemical properties, acceptable pharmacokinetic characteristics, and met the Lipinski rule-of-five criteria, except Esmolol Dimer USP, which showed a marginal molecular weight violation. Toxicity prediction using ProTox-3.0 suggested low to moderate acute toxicity (LD₅₀: 500-8140 mg/kg) with no predicted hepatotoxicity, cardiotoxicity, or neurotoxicity. Overall, the study establishes a reliable approach for the preparation and characterization of Esmolol-related compounds and provides preliminary ADME and toxicity data useful for pharmaceutical quality assessment and future biological investigations.

Abbreviations

API: Active Pharmaceutical Ingredient; **β₁-AR:** Beta-1 Adrenergic Receptor; **DALY:** Disability Adjusted Life Year; **RT:** Room Temperature; **Min:** Minute; **RBF:** Round Bottom Flask; **DCM:** Dichloromethane; **DMF:** Dimethylformamide; **EtOAc:** Ethyl Acetate; **PKA:** Protein Kinase A; **RPM:** Revolutions Per Minute; **FTIR:** Fourier Transform Infrared Spectroscopy; **MS:** Mass Spectrometry; **NMR:** Nuclear Magnetic Resonance; **TLC:** Thin-Layer Chromatography; **UV:** Ultraviolet; **LD₅₀:** Median Lethal Dose; **ADME:** Absorption,

Distribution, Metabolism, Excretion; **MW**: Molecular Weight; **TPSA**: Topological Polar Surface Area; **HBA**: Hydrogen Bond Acceptor; **HBD**: Hydrogen Bond Donor; **Log P**: Octanol/Water Partition Coefficient; **ESOL Log S**: Predicted Aqueous Solubility.

Declarations

- **Acknowledgment**

We sincerely acknowledge Anasyn Lab Pvt. Ltd., Pune, Maharashtra, India for providing the essential synthetic materials and analytical support required for this study. Their valuable assistance and cooperation were instrumental in the successful completion of this work.

- **Funding statement**

This Research work (Project) was not funded by any funding agency.

- **Author Contributions**

Conceptualization: S. K. Wakade, Dr. A. S. Tapkir, S. N. Rashinkar, Dr. A. B. Pathak, S.M. Bhagat, S. S. Kore, A. G. Pomaji.

Methodology:

1. Design: Dr. A. S. Tapkir, Dr. A. B. Pathak

2. Synthesis: S. K. Wakade, S.M. Bhagat.

3.Characterization: Dr. A. S. Tapkir, Dr. A. B. Pathak, S. K. Wakade

Writing -Original Draft: S. K. Wakade

Formal Analysis: Dr. A. S. Tapkir, S. N. Rashinkar, Dr. P. D. Choudhari,

Data Curation: Dr. A. S. Tapkir,

Investigation: Dr. A. S. Tapkir,

Writing -Review & Editing: Writing: S. K. Wakade,

Review & Editing: Dr. A. S. Tapkir, S. S. Kore, A. G. Pomaji

- **Conflict of Interest**

The authors declare they have no conflicts of interest.

- **Ethics Approval and Consent**

Ethics approval and consent was not required.

- **Consent to Participate**

Not Applicable

- **Consent to Publish**

Not Applicable

Data availability

Data will make available on request to corresponding author.

References

1. Chong B, Jayabaskaran J, Jauhari SM, Chan SP, Goh R, Kueh MT, et al. Global burden of cardiovascular diseases: projections from 2025 to 2050. *European journal of preventive cardiology*. 2024 Sep 13;zwae281. <https://doi.org/10.1093/eurjpc/zwae281>
2. Kario K, Okura A, Hoshida S, Mogi M. The WHO Global report 2023 on hypertension warning the emerging hypertension burden in globe and its treatment strategy. *Hypertension Research*. 2024 May;47(5):1099-102. <https://doi.org/10.1038/s41440-024-01622-w>
3. Wiest DB, Haney JS. Clinical pharmacokinetics and therapeutic efficacy of esmolol. *Clinical pharmacokinetics*. 2012 Jun;51(6):347-56. <https://doi.org/10.2165/11631590-000000000-00000>
4. Konkar AA, Zhu Z, Granneman JG. Aryloxypropanolamine and catecholamine ligand interactions with the β 1-adrenergic receptor: evidence for interaction with distinct conformations of β 1-adrenergic receptors. *The Journal of pharmacology and experimental therapeutics*. 2000 Sep 1;294(3):923-32. [https://doi.org/10.1016/S0022-3565\(24\)39154-2](https://doi.org/10.1016/S0022-3565(24)39154-2)
5. Tucker WD, Sankar P, Kariyanna PT. Selective beta-1 blockers. In: StatPearls [Internet]. 2023 Jan 30. StatPearls Publishing. <https://pubmed.ncbi.nlm.nih.gov/29763157/>
6. Finotti Cordeiro C, Lopardi Franco L, Teixeira Carvalho D, Bonfilio R. Impurities in active pharmaceutical ingredients and drug products: A critical review. *Critical Reviews in Analytical Chemistry*. 2026 Jan 2;56(1):55-75. <https://doi.org/10.1080/10408347.2024.2384046>
7. Snodin DJ, McCrossen SD. Guidelines and pharmacopoeial standards for pharmaceutical impurities: overview and critical assessment. *Regulatory Toxicology and Pharmacology*. 2012 Jul 1;63(2):298-312. <https://doi.org/10.1016/j.yrtph.2012.03.016>
8. Heravi MM, Zadsirjan V, Daraie M, Ghanbarian M. Applications of Wittig reaction in the total synthesis of natural macrolides. *ChemistrySelect*. 2020 Aug 21;5(31):9654-90. <https://doi.org/10.1002/slct.202002192>

9. Waghmare, P. S., Tapkir, A. S., Chaudhari, P. D., et al. Synthesis, Purification and Characterization of Some Impurities of Propranolol. Indian Journal of Pharmaceutical Education and Research. 2025-12-06;60(1s):s280-s286. <https://doi.org/10.5530/ijper.20265308>
10. Banerjee P, Kemmler E, Dunkel M, Preissner R. ProTox 3.0: a webserver for the prediction of toxicity of chemicals. Nucleic acids research. 2024 Jul 5;52(W1):W513-20. <https://doi.org/10.1093/nar/gkae303>
11. Lutfor M. Strategic impurity control in next-generation pharmaceuticals: Analytical technologies, toxicological assessment, and regulatory integration. Journal of Angiotherapy. 2025 Jul 16;9(1):1-5. <https://doi.org/10.25163/biomedical.9110292>

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