J. Pharm. Res. 2020;19(2):1–5

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

Journal of Pharmaceutical Research

RESEARCH ARTICLE

Synthesis and Characterization of Structurally Related Compounds of Lorazepam and Temazepam

Sanket Sanjayrao Kulkarni[1], M D Karvekar[1,*]

[1]*Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, 560035, Karnataka, India*

ARTICLE INFO

Article history:
Received 18.12.2019
Accepted 22.3.2019
Published 12.4.2019

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

https://doi.org/
10.18579/jopcr/v19.2.sanket

ABSTRACT

Benzodiazepines are widely used for their anxiolytic, sedative, and hypnotic properties, and understanding their structure-activity relationships is crucial for the development of new therapeutic agents with improved efficacy and safety profiles. The study explores the synthesis and characterization of compounds structurally related to Lorazepam and Temazepam, two benzodiazepine derivatives with significant pharmacological activity. The synthesis of Lorazepam (LZP) derivatives started with the preparation of LZP 1. This was achieved by reacting (2-amino-5-chlorophenyl)(2-chlorophenyl)methanone with chloroacetyl chloride, followed by treatment with hydroxylamine hydrochloride. LZP 1 was subsequently transformed into LZP 2 through Polonovski rearrangement. Dehydration of LZP 2 yielded LZP 3, which was further processed to produce LZP 4 and LZP 5. For the Temazepam (TZP) series, TZP 1 was synthesized by reacting (2-amino-5-chlorophenyl)(phenyl)methanone with hydroxylamine hydrochloride and chloroacetyl chloride. This intermediate was then treated with sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH). TZP 1 underwent Polonovski rearrangement to form TZP 2, which was then subjected to treatment with potassium hydroxide (KOH). Finally, TZP 3 was synthesized through chlorination followed by methanol treatment. The structurally related compounds of Lorazepam and Temazepam were successfully synthesized and identified. Their structures and purity were confirmed through characterization using a range of techniques, including melting point determination, Thin Layer Chromatography (TLC), Infrared Spectroscopy (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry. The research introduces new synthetic pathways and intermediates for lorazepam and temazepam derivatives, contributing to the development of novel anti-depressant agents. This work emphasizes the importance of impurity monitoring in ensuring the quality of synthesized compounds.

Keywords: Lorazepam; Temazepam; Thin Layer Chromatography; Infrared Spectroscopy; Nuclear Magnetic Resonance; Mass Spectral analysis

INTRODUCTION

Lorazepam and Temazepam are short-acting benzodiazepines from the 3-hydroxy-1,4-benzodiazepine class, renowned for their efficacy as central nervous system depressants[1]. Chemically, Lorazepam is identified as 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2H-1,4-benzodiazepin-2-one, while Temazepam is 7-chloro-1,3-dihydro-3-hydroxy-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one[2]. Both drugs are commonly utilized for their anxiolytic, anticonvulsant, hypnotic, and sedative properties, derived from Oxazepam. Lorazepam is valued for its potent anxiolytic effects and is a well-tolerated preanesthetic sedative with notable amnesic properties. It offers rapid metabolism and effective sleep induction, although its low lipid solubility reduces oral availability.[3] Conversely, Temazepam is favored for its ability to induce and maintain sleep with minimal residual effects, making it a preferable hypnotic over longer-acting benzodiazepines. It also possesses anxiolytic, anticonvulsant, and skeletal muscle relaxant properties but is contraindicated in hepatic and renal deficiencies and other conditions[4]. This study aims to synthesize and characterize novel derivatives of Lorazepam and Temazepam using advanced analytical techniques to develop new, potent compounds with enhanced therapeutic potential for depression, while meticulously profiling impurities to ensure drug quality and safety.

METHODOLOGY

Structurally related compounds of lorazepam

Synthesis of LZP 1

Step 1: Synthesis of 2-chloro-N-[4-chloro-2-(phenyl carbonyl)phenyl]acetamide

A 1-liter round-bottom flask was equipped with a mechanical stirrer, reflux condenser, thermometer, and addition funnel. The flask was charged with 50 g (0.188 moles) of (2-amino-5-chlorophenyl)(2-chlorophenyl)methanone in 250 ml toluene and heated to 60°C. 19.47 ml (0.244 moles) of chloroacetyl chloride was added dropwise. The reaction was maintained at 50°C for 6 hours, then cooled, filtered, washed with toluene and methanol, and dried at 60-70°C for 4 hours. Yield: 84.42%.

Step 2: Synthesis of 7-chloro-5-(2-chlorophenyl)-4-hydroxy-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-4-ium (LZP 1)

A 1-liter four-necked round-bottom flask was set up with a mechanical stirrer, reflux condenser, thermometer, and addition funnel. 50 g (0.1466 moles) of 2-chloro-N-[4-chloro-2-(phenylcarbonyl)phenyl]acetamide, 250 ml isopropyl alcohol, 50 g (0.8334 moles) of hydroxylamine hydrochloride, 50 g (0.2873 moles) of NS5, and 11 g (0.0733 moles) of sodium iodide were added. The mixture was heated to 50°C, and 50 g (0.3623 moles) of potassium carbonate was added slowly. The reaction was maintained at 70-75°C for 12 hours, then cooled, acidified with 50% sulfuric acid to pH 2, reheated, filtered, washed with water, and dried at 70°C. Yield: 66.49%.

Characterization: LZP 1 is a yellowish-white solid with a molecular formula of $C_{15}H_{10}Cl_2N_2O_2$ and a molecular weight of 321.15. Melting point: 210-213°C. IR spectrum: 1693.50 cm^{-1} (C=O), 3354.21 cm^{-1} (N-H), 1643.35 cm^{-1} (C-N), 758.02 cm^{-1} (C-Cl). ^1H NMR ($CDCl_3$): δ 4.76 (s, 1H, amine), δ 7.26-7.58 (m, 7H, Ar-H).

Synthesis of LZP 2

Step 1: Synthesis of 7-chloro-5-(2-chlorophenyl)-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl acetate

A 500 ml four-necked round-bottom flask was equipped with a mechanical stirrer, reflux condenser, thermometer, and addition funnel. 100 g (0.3125 moles) of 7-chloro-5-(2-chlorophenyl)-4-hydroxy-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-4-ium and 117.75 g (1.9625 moles) of acetic acid were added. The mixture was heated to 75°C, and 188.38 g (1.8468 moles) of acetic anhydride was added dropwise at 70-80°C for 2 hours. The residue was filtered, washed with methanol, and dried at 60°C for 6 hours. Yield: 67.89%.

Step 2: Synthesis of 7-chloro-5-(2-chlorophenyl)-4,5-dihydro-1H-1,4-benzodiazepine-2,3-dione (LZP 2)

A 500 ml four-necked round-bottom flask was equipped with a mechanical stirrer, reflux condenser, thermometer, and addition funnel. 25 g (0.0692 moles) of 7-chloro-5-(2-chlorophenyl)-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl acetate, 150 ml methanol, and 4 g (0.0289 moles) of potassium carbonate were added. The mixture was heated at 60°C for 4 hours, then cooled to room temperature. Methanol was distilled off, and the mixture was washed with 500 ml water, filtered, refluxed with acetone (three times), filtered, and dried at 70°C for 6 hours. Yield: 55.22%.

Characterization: LZP 2 is an orange-coloured amorphous solid with a molecular formula of $C_{15}H_{10}Cl_2N_2O_2$ and a molecular weight of 321.15. Melting point: 245-247°C. IR spectrum: 1681.93 cm^{-1} (C=O), 3228.84 cm^{-1} (N-H). ^1H NMR ($CDCl_3$): δ 4.76 (s, 1H, amine), δ 7.26-8.43 (m, 7H, Ar-H).

Synthesis of LZP 3

Synthesis of 6-chloro-4-(2-chlorophenyl)quinazoline-2-carbaldehyde

A 500 ml four-necked round-bottom flask was set up with a mechanical stirrer, Y-bend, thermometer, and addition funnel (azeotropic assembly). 50 g (0.1557 moles) of 7-chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2H-1,4-benzodiazepin-2-one- and 200-ml toluene were added. The mixture was refluxed for 12 hours, cooled, and the toluene was distilled off. The residue was filtered and dried at 60-70°C for 6 hours. Yield: 80.52%.

Characterization: LZP 3 is a whitish-orange solid with a molecular formula of $C_{15}HCl_2N_2O$ and a molecular weight of 303.14. Melting point: 156-159°C. Soluble in chloroform, insoluble in water. IR spectrum: 1720.50 cm^{-1} (aldehyde C=O), 2858.51 cm^{-1} and 2777.50 cm^{-1} (aldehyde C-H), 1633.71 cm^{-1} (C=N), 752.23 cm^{-1} (C-Cl). ^1H NMR ($CDCl_3$): δ 7.27-8.33 (m, 7H, Ar-H), δ 10.31 (s, 1H, aldehyde). Mass spectrum: m/z 302.

Synthesis of LZP 4

Synthesis of [6-chloro-4-(2-chlorophenyl)quinazolin-2-yl]methanol

A 500 ml four-necked round-bottom flask was equipped with a mechanical stirrer, bent tube, thermometer, and addition funnel. 25 g (0.0825 moles) of 6-chloro-4-(2-chlorophenyl)quinazoline-2-carbaldehyde (LZP 3) and 125 ml methanol were added. 1.56 g (0.0412 moles) of sodium borohydride ($NaBH_4$) was added slowly. The mixture was stirred for 12 hours, then a small amount of dilute acetic acid was added. Methanol was distilled off, and the product was extracted with dichloromethane and purified by column chromatography. Yield: 32.19%.

Characterization: LZP 4 is a white crystalline solid with a molecular formula of $C_{15}HCl_2N_2O$ and a molecular weight of 305.15. Melting point: 102-104°C. Soluble in chloroform. IR spectrum: 3244.27 cm^{-1} (O-H), 1593.20 cm^{-1} (aromatic C=C), 3101.54 cm^{-1} (aromatic C-H).

Structurally Related Compounds of Temazepam

Synthesis of TZP 1

Step 1: Synthesis of 4-chloro-2-[(hydroxyimino)(phenyl)methylaniline]

In a 1-litre round-bottom flask, 300 g of (2-amino-5-chlorophenyl)(phenyl)methanone was mixed with 300 ml of methanol. The mixture was heated to 50°C, and 132 g of hydroxylamine hydrochloride was added. The reaction was maintained at 65-70°C for 12 hours, then cooled, and the pH was adjusted to 8 with sodium hydroxide. The product was filtered, washed with water, and dried at 70°C. The yield obtained was 99.53%.

Step 2: Synthesis of 2-chloro-N-{4-chloro-2-[(hydroxyimino) (phenyl)methyl] phenyl} acetamide

In a 3-litre flask, 300 g of 4-chloro-2-[(hydroxyimino)(phenyl)methylaniline] was dissolved in 484 ml of toluene. Sodium bicarbonate (84.36 g) was added, followed by the dropwise addition of 69.9 ml of chloroacetyl chloride at 50-55°C for 8 hours. After cooling, 90 ml of 5% ammonia was added in ice. The mixture was filtered and dried at 70°C. The yield was 71.08%.

Step 3: Synthesis of 6-chloro-2-(chloromethyl)-4-phenylquinazoline 3-oxide

In a 2-litre flask, 174.34 ml of sulfuric acid was combined with 275 ml of dichloromethane at 10°C. Slowly, 275 g of 2-chloro-N-{4-chloro-2-[(hydroxyimino) (phenyl) methyl]phenyl} acetamide was added. The mixture was stirred, then extracted with dichloromethane. The product was recrystallized from isopropanol (IPA) in a 1:1.5 ratio and dried at 60-70°C. The yield was 69.39%.

Step 4: Synthesis of 7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one 4-oxide

To a 1-litre flask, 10 g of sodium hydroxide was mixed with 35 ml of water and 229 ml of methanol. Subsequently, 25 g of 6-chloro-2-(chloromethyl)-4-phenylquinazoline 3-oxide was added, and the mixture was stirred for 1 hour. The reaction was neutralized with acetic acid, and the product was filtered and dried at 65-70°C. The mother liquor was concentrated, and the solid was dried. The yield was 47.48%.

Characterization: 7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one 4-oxide (TZP-1) is a white crystalline solid with a molecular formula of $C_{15}H_{11}ClN_2O_2$ and a molecular weight of 286.71. It is chemically named 7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one 4-oxide. The substance is soluble in chloroform and has a retention factor (Rf) value of 0.31. The percentage yield of the compound is 47.48%, and it has a melting point in the range of 225-227°C. Infrared (IR) spectral data show characteristic peaks at 3394.72 cm^{-1} for N-H stretching and 1707.00 cm^{-1} for the C=O group. Proton nuclear magnetic resonance (^{1}H NMR) in $CDCl_3$ reveals a δ (4.68, s, 1H of Amine), δ [7.07-7.64, m, 8H of Ar. H].

Synthesis of TZP 2

Step 1: Synthesis of 7-chloro-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl acetate

A 250 ml four-necked round-bottom flask equipped with a mechanical stirrer, bent tube, thermometer, and addition funnel was assembled. 6 g (0.0209 moles) of 7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one 4-oxide and 8.28 ml (0.1317 moles) of acetic acid were added to the flask, and the reaction mixture was heated to 75°C. 13.59 ml (0.1235 moles) of acetic anhydride was added dropwise while maintaining the temperature at 75°C. The heating was continued for 2 hours, after which the mixture was cooled, filtered, washed with a small amount of methanol, and dried at 65-70°C. Yield: 78.26%.

Step 2: Synthesis of 7-chloro-5-phenyl-4,5-dihydro-1H-1,4-benzodiazepine-2,3-dione

A 250 ml four-necked round-bottom flask with a mechanical stirrer, reflux condenser, thermometer, and addition funnel were set up. 3 g (0.0091 moles) of 7-chloro-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl acetate and 25 ml of methanol were added. The reaction mixture was heated to reflux for 5 hours. Methanol was then distilled off, and the mixture was neutralized with approximately 30 ml of water and a small amount of acetic acid. The residue was filtered, washed with water, and dried at 65-70°C for 6 hours. Yield: 77.22%.

Characterization of TZP 2: TZP 2 is a white crystalline solid with a molecular formula of $C_{15}H_{11}ClN_2O_2$ and a molecular weight of 286.71. It is soluble in chloroform and has a retention factor (Rf) of 0.22. The melting point ranges from 250-255°C. The IR spectrum shows peaks at 1697.36 cm^{-1} (C=O stretching), 1658.78 cm^{-1} (C=O stretching), and 3238.48 cm^{-1} (N-H stretching). The ^{1}H NMR spectrum in $CDCl_3$ displays signals at δ 4.99 (s, 1H, amine), δ 5.04 (s, 1H, amine), and δ 7.25-8.12 (m, 8H, Ar-H). The mass spectrum reveals a molecular ion peak at m/z 286.

Synthesis of TZP 3

Synthesis of 7-chloro-3-methoxy-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one

A 250 ml four-necked round-bottom flask with a mechanical stirrer, thermometer, and addition funnel was assembled. 15 ml of tetrahydrofuran and 2.58 ml (0.0332 moles) of thionyl chloride were added to the flask, followed by 10 g (0.332 moles) of 7-chloro-3-hydroxy-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one. The mixture was stirred at room temperature for 3 hours. The reaction mixture was then added dropwise to 10 ml of methanol with continuous stirring for 1 hour. Methanol was distilled off, and the compound was extracted with 100 ml of dichloromethane. Dichloromethane was distilled off, and the compound was purified by column chromatography. Yield: 11.49%.

Characterization of TZP 3: TZP 3 is a yellowish crystalline solid with a molecular formula of $C_1H_{15}ClN_2O_2$ and a molecular weight of 314.76. It is soluble in chloroform and has an Rf value of 0.90. The melting point ranges from 176-179°C. The IR spectrum displays peaks at 1689.64 cm^{-1} (C=O stretching), 1670.35 cm^{-1} (C=O stretching), 2900.94 cm^{-1} (C-H stretching), and 1600.92 cm^{-1} (C=N stretching). The ^1H NMR spectrum in $CDCl_3$ shows signals at δ 2.88 (s, 1H, OCH_3) and δ 7.07-7.64 (m, 8H, Ar-H).

RESULTS AND DISCUSSION

The synthesis and characterization of structurally related compounds to Lorazepam and Temazepam were successfully conducted using various analytical techniques. The melting points of the synthesized compounds were determined using Thiel's melting point tube (capillary tube method), providing an initial assessment of purity and identity. Thin Layer Chromatography (TLC) was employed to assess the purity and monitor the progress of the synthesis, utilizing ethyl acetate and petroleum ether in the ratio 1:1 and 2:8. Infrared (IR) spectroscopy was performed using KBr pellets in range of 4000-400cm^{-1} on a Fourier Transform IR Spectrophotometer to identify functional groups and confirm the chemical structure of the compounds (model Shimadzu 8700, R. L Fine Chemicals, Yelahanka, Bangalore). Nuclear Magnetic Resonance (NMR) spectroscopy was conducted at 400 MHz in deuterated chloroform, with the analysis carried out on an Amx – 200 liquid state NMR spectrometer (Astra Zeneca, Bangalore), revealing detailed structural information. High-Performance Liquid Chromatography (HPLC) was used to analyze the chromatograms of the synthesized compounds on a Shimadzu SPD-10A UV-Visible detector at Ray Chemicals Pvt. Ltd., Yelahanka, Bangalore, ensuring the purity and stability of the compounds. Mass Spectroscopy (MS) was performed using an Electron Spray Ionization (ESI) spectroscope at IISc, Bangalore, providing molecular weight and structural insights. The Tables 1 and 2 provides detailed characterization data for a series of compounds that are structurally related to Lorazepam and Temazepam.

In this study, we synthesized and characterized several structurally related compounds of Lorazepam and Temazepam, aiming to address known impurities in these pharmaceuticals and enhance our understanding of their chemical properties. The synthesized compounds were analyzed using a variety of qualitative analytical methods, including melting point determination, thin-layer chromatography (TLC), infrared (IR) spectroscopy, proton nuclear magnetic resonance (^1H NMR) spectroscopy, high-performance liquid chromatography (HPLC), and mass spectrometry (MS).

The melting points of the synthesized compounds, such as LZP 3, which exhibited a melting range of 156-159°C, provided initial indications of their purity and structural integrity. A consistent melting point often suggests a well-purified product with minimal impurities.

TLC was employed to identify and assess the presence of impurities. This method proved useful in distinguishing between the target compounds and any by-products or unreacted starting materials. By comparing the Rf values of the synthesized compounds with those of known standards, the purity of each compound was evaluated.

IR spectroscopy was used to confirm the presence of functional groups within the synthesized compounds. For instance, the IR spectrum of LZP 3 displayed characteristic peaks at 1720.50 cm^{-1} for the carbonyl group (C=O) in aldehydes, 2858.51 cm^{-1} and 2777.50 cm^{-1} for the C-H stretch in aldehydes, 1633.71 cm^{-1} for the C=N stretch, and 752.23 cm^{-1} for the C-Cl bond. These peaks corroborated the expected functional groups and confirmed the chemical structure of LZP 3.

^1H NMR spectroscopy provided detailed information on the hydrogen environment in the compounds. The spectrum of LZP 3 revealed a multiplet at δ 7.27-8.33, corresponding to the 7 hydrogen atoms of the aromatic ring (Ar-H), and a singlet at δ 10.31 for the aldehyde group's hydrogen. These spectral features validated the compound's structure and confirmed the substitution pattern around the aromatic ring.

HPLC analysis offered additional evidence of the synthesized compounds' purity. The chromatograms showed distinct peaks corresponding to the target compounds, with minimal evidence of impurities, thus supporting the consistency and reliability of the synthesis process.

Mass spectrometry provided precise molecular weight measurements. For LZP 3, the molecular ion peak at m/z 302 confirmed the expected molecular weight, reinforcing the compound's identity and purity.

Overall, the synthesis and characterization of these structurally related compounds have demonstrated their chemical purity and structural fidelity. The use of various analytical techniques has enabled a comprehensive assessment of the compounds, supporting the successful synthesis of novel derivatives related to Lorazepam and Temazepam. This thorough characterization contributes valuable insights into the chemical properties and potential applications of these compounds in pharmaceutical research and development.

CONCLUSION

This study successfully synthesized and characterized several structurally related compounds of Lorazepam and Temazepam. By employing infrared (IR) spectroscopy, proton nuclear magnetic resonance (^1H NMR) spectroscopy, and mass spectrometry (MS), we confirmed the structural integrity and purity of these compounds. The results not only validate the synthesis but also provide essential data for future research and development in pharmaceutical chemistry.

Table 1: Characterization Data for Structurally Related Compounds of Lorazepam

Compound code	Molecular formula	Mol weight	Melting point (°C)	Yield (%)	Rf Value
LZP 1	$C_{15}H_{10}Cl_2N_2O_2$	321.15	210-213	66.49	0.83
LZP 2	$C_{15}H_{10}Cl_2N_2O_2$	321.15	245 - 247	55.22	0.32
LZP 3	$C_{15}H_8Cl_2N_2O$	303.14	156 – 159	80.52	0.61
LZP 4	$C_{15}H_8Cl_2N_2O$	305.15	102 – 104	32.19	0.54
LZP 5	$C_{15}H_8Cl_2N_2O_2$	319.14	246- 248	40.10	0.16

Table 2: Characterization Data for Structurally Related Compounds of Temazepam

Compound code	Molecular formula	Mol weight	Melting point (°C)	Yield (%)	Rf Value
TZP 1	$C_{15}H_{11}ClN_2O_2$	286.71	225- 227	47.48	0.31
TZP 2	$C_{15}H_{11}ClN_2O_2$	286.71	250-255	77.22	0.22
TZP 3	$C_{17}H_{15}ClN_2O_2$	314.76	176-179	11.49	0.90

This work enhances our understanding of benzodiazepine derivatives and supports their further exploration and optimization for drug development and quality control.

REFERENCES

1. Griffin CE, Kaye AM, Bueno FR, Kaye AD. Benzodiazepine pharmacology and central nervous system-mediated effects. *Ochsner Journal*. 2013;13(2):214–223. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3684331/.
2. Chananont P, Hamor TA, Martin IL. The structures of 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one and 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one. *Acta Crystallographica Section B Structural Crystallography and Crystal Chemistry*. 1981;37(7):1371–1375. Available from: https://dx.doi.org/10.1107/s0567740881005967.
3. Baldwin DS, Aitchison K, Bateson A, Curran HV, Davies S, Leonard B, et al. Benzodiazepines: Risks and benefits. A reconsideration. *Journal of Psychopharmacology*. 2013;27(11):967–971. Available from: https://dx.doi.org/10.1177/0269881113503509.
4. Lader M. Benzodiazepines revisited—will we ever learn? *Addiction*. 2011;106(12):2086–2109. Available from: https://dx.doi.org/10.1111/j.1360-0443.2011.03563.x.