International Research Journal of Pharmacy and Medical Sciences
ISSN (Online): 2581-3277

Synthesis and Structural Characterization of Cinacalcet -N-Nitroso Impurity

*Dr. Sudheer Kumar Reddy Gopa[1], Kunja Anusha[2], Vijendra Singh Rawat[3], Rajkumar Sundaram[4]

[1] Research scientist, department of analytical research and development, MANASA Life Sciences, Hyderabad, India. Pin Code 500072

[2]Senior chemist, department of synthetic research and development, MANASA Life Sciences, Hyderabad, India. Pin Code 500072

[3]Research scientist, department of synthetic research and development, MANASA Life Sciences, Hyderabad, India. Pin Code 500072

[4]Science and Humanities-Chemistry Bharath Institute of Higher Education and Research Bharath University (Deemed to be University) Selaiyur, Chennai-600073.Tamilnadu, India.

Email: sales@manasalifesciences.com(1), rksundaram13@gmail.com(4)

Abstract: *Nitrosamines form when secondary amines in cinacalcet react with nitrites or nitrates, especially under acidic conditions that produce nitrosating agents. This study details the synthesis and characterization of N-Nitroso Cinacalcet. The synthesis was carried out by controlled nitration of the Cinacalcet API using tert-Butyl Nitrite at low temperatures. Structural confirmation and purity assessment were performed using, mass spectrometry, NMR, and IR spectroscopy and HPLC. The findings confirm the successful production and thorough characterization of this nitrosamine impurity, emphasizing the necessity of precise analytical techniques to ensure drug safety and compliance with regulatory requirements for mutagenic impurities.*

Keywords: *N-Nitroso Impurity, Cinacalcet, HPLC, LC-MS, NMR.*

I. INTRODUCTION

Cinacalcet is a calcimimetic agent used to treat conditions linked to elevated parathyroid hormone levels[1]. It is mainly prescribed for secondary hyperparathyroidism in dialysis patients with chronic kidney disease, where it reduces excessive PTH secretion[2]. Additionally, it is indicated for primary hyperparathyroidism when surgery is not an option, and for managing severe hypercalcemia in parathyroid carcinoma. Cinacalcet enhances the sensitivity of calcium-sensing receptors in the parathyroid gland, lowering blood levels of PTH, calcium, and phosphorus[3]. It is administered orally as film-coated tablets in doses of 30 mg, 60 mg, or 90 mg, typically taken with or shortly after meals. Treatment usually starts at low doses (e.g.30 mg once or twice daily) and is adjusted based on serum calcium and PTH levels[4]. Fig-1.

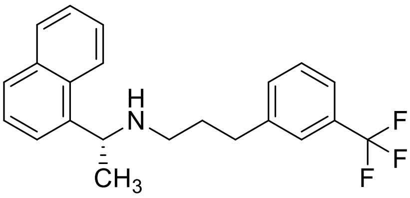

Fig 1: Cinacalcet API

Regulatory bodies including the U.S. FDA, ICH, EU EMA, and British Pharmacopoeia classify nitrosamine impurities as mutagenic carcinogens within the ICH M7 "cohort of concern." These agencies mandate control of nitrosamines at very low levels to maintain negligible lifetime cancer risk[5].

Acceptable Intake (AI) limits are set based on carcinogenicity data commonly ranging from 18 to 96 ng/day for NDMA, 26.5 ng/day for NDEA, and category-based AIs for nitrosamine drug substance-related impurities (NDSRIs)[6]. These limits align with or are more stringent than the ICH M7 threshold of 1.5 µg/day for mutagenic impurities. The regulations require risk assessment, confirmatory testing, and process controls for affected APIs and drug products, with specified timelines and mandatory reporting for variations. Substances listed in the British Pharmacopoeia must comply with pharmacopoeia standards. "Available literature suggests that no synthetic method has been documented for the production of cinacalcet's N-nitroso impurity.

II. MATERIAL AND METHODS

A. Chemical and reagents

Cinacalcet API used in starting material Cinacalcet API Cas no 226256-56-0;

IUPAC name (R)-N-(1-(naphthalen-1-yl)ethyl)-3-(3-(trifluoromethyl)phenyl)propan-1-amine,

Molecular formula $C_{22}H_{22}F_3N$, Molecular weight 357.42 g/mol. Ammonium formate, Acetonitrile, Methanol used in mobile phase, Tert Butyl Nitrite and Acetonitrile chemicales used in synthesis

B. HPLC Instrument Conditions

Chromatographic separation was conducted using a Waters Alliance HPLC system, which included a gradient

Dr. Sudheer Kumar Reddy Gopa, Dr. Usenireddy Mallu, and Dr. Venkateswarlu Korthikunta, "Synthesis and Structural Characterization of Cinacalcet -N-Nitroso Impurity," *International Research Journal of Pharmacy and Medical Sciences (IRJPMS)*, Volume 9, Issue 4, pp. 30-32, 2026.

pump, column oven temparature 25°C, autosampler temperature 5C°, and photodiode array (PDA) detector. The separation was achieved using a Kromasil C18 column with dimensions of 150 mm x 4.6 mm and a particle size of 5 µm. The system flow rate was maintained at 1.0 µL/min, and the injection volume was set at 10 µL. The gradient program was as follows: from 0.0 to 5.0 minutes, mobile phase A (MP-A) was 80% and mobile phase B (MP-B) was 20%; at 12.0 minutes, MP-A was 35% and MP-B was 65%; at 22.0 minutes, MP-A was 20% and MP-B was 80%; at 28.0 minutes, MP-A was 10% and MP-B was 90%; at 28.1 minutes, MP-A was 80% and MP-B was 20%; and at 35 minutes, MP-A was 80% and MP-B was 20%. The detector wavelength was set at 220 nm. For the preparation of mobile phase A, 0.63 g of ammonium formate was accurately weighed and dissolved in 1000 mL of Milli-Q water, followed by sonication to ensure complete dissolution. Mobile phase B consisted of 100% acetonitrile. Methanol at 100% concentration was used as the diluent.

C. Mass Spectrometer Instrument Conditions:

The UPLC system was interfaced with a Waters Xevo TQ-Absolute mass spectrometer (USA), optimized for LC MS/MS analysis. Electrospray ionization (ESI) was conducted in positive ion mode, utilizing multiple reaction monitoring (MRM). The capillary voltage was set at 3.40 kV. The source and desolvation temperatures were maintained at 550 °C and 400 °C, respectively. Desolvation and cone gas flows were regulated at 800 L/h and 150 L/h, respectively. To reduce ion source contamination, the initial eluent was diverted to waste using a switching valve. Data acquisition and processing were executed using Waters Empower software.

D. NMR Instrument Details:

The NMR instrument used was an Agilent, Model No. 400 MR, from the US, operated with Vnmrj 4.2 software. A sample of 5 mg was dissolved in DMSO solvent for analysis.

E. NMR Instrument Details:

A Bruker ATR IR instrument was employed. A sample ranging from 3 mg to 5 mg was placed on an ATR crystal and analyzed within the range of 4000-550 cm^{-1}.

F. Other Instrument Details:

Additional instruments included a Sartorius micro balance, a Sartorius analytical balance, a Remi centrifuge instrument, a PCI Solicitor, and a magnetic stirrer with a hot plate.

III. EXPERIMENTAL PROCEDURE FOR THE SYNTHESIS OF N-NITROSO CINACALCET

One gram of Cinacalcet API was accurately weighed and transferred into a cleaned, dried 100 ml borosilicate round-bottom flask. Approximately 10 ml of acetonitrile was added to the flask, and the mixture was sonicated to ensure complete dissolved of the starting material. The reaction setup was arranged with a magnetic stirrer under controlled cooling conditions, maintaining the temperature between 0°C and 5°C. The reaction mass was stirred for 30 minutes to achieve homogeneity before the slow addition of tert-Butyl Nitrite (TBN) under continuous cooling. The reaction mixture was then maintained at the cooled temperature and stirred for one hour to facilitate the nitration process. After stirring for two hours, the completion of the reaction was confirmed by thin-layer chromatography (TLC) using a mobile phase composed of 50% ethyl acetate (EA) and hexane (HXN). The resulting compound was a brown-colored liquid, identified as N-Nitroso Cinacalcet, chemically named (R)-N-(1-(naphthalen-1-yl)ethyl)-N-(3-(3-(trifluoromethyl)phenyl)propyl)nitrous amide. Fig-2

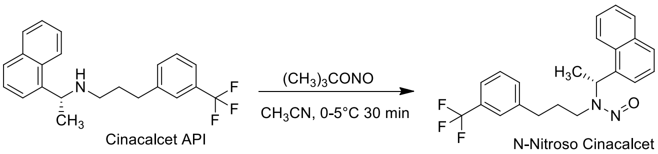

Fig. 2. Synthesis of N-Nitroso Cinacalcet

This synthesis highlights the careful control of reaction conditions and purification steps necessary to obtain the target nitroso derivative of Cinacalcet.

IV. RESULT AND DISCUSSION

A. Mass Spectroscopy Results:

The mass spectrum of N-Nitroso-Cinacalcet exhibited a prominent and well-defined peak at *m/z* 387.28, corresponding to the protonated molecular ion ([M+H]^+). This value is in close agreement with the theoretical molecular weight of 386.4 g/mol, thereby confirming the successful formation of the nitrosated product. The slight difference of one mass unit is expected under positive electrospray ionization conditions due to proton addition during ionization. The presence of an intense molecular ion peak indicates efficient ionization of the compound under ES+ conditions and demonstrates its stability within the ion source. The peak at *m/z* 387.28 was observed as the base peak, indicating that the protonated parent ion is the most abundant and stable species in the spectrum. This strongly supports the molecular identity of N-Nitroso-Cinacalcet and is consistent with the conformed chemical structure.

B. NMR Results:

One CH_3 group attached in methane group, 6H (three CH_2 attached Nitrogen and methane) eleven CH Stored in benzene reing. ^{1}H NMR spectrum of the given compound is expected to show multiple distinct proton environments corresponding to its aromatic, heteroaromatic, and aliphatic regions. The aromatic protons appear as complex multiplets in the range of δ 6.5–8.5 ppm due to substituted benzene rings, while protons near nitrogen-containing groups or imine functionalities resonate further downfield (δ 7.5–9.0 ppm). The methylene protons adjacent to aromatic rings and nitrogen atoms are observed as multiplets or triplets in the δ 2.5–3.8 ppm region, reflecting deshielding effects from nearby electronegative atoms. Aliphatic chain methylene groups appear as multiplets between δ 1.5–2.5 ppm.

Additionally, protons attached to fluorinated carbons may resonate in the δ 4.5–6.5 ppm region and exhibit characteristic splitting due to ^{1}H–^{19}F coupling. If present, NH protons typically appear as broad signals in the δ 7–10 ppm range due to exchange effects. Overall, the spectrum is characterized by dominant aromatic signals, heteroatom-induced downfield shifts, and complex splitting patterns.

C. IR Results:

The IR spectrum of the given compound shows characteristic absorption bands corresponding to its functional groups. The peaks observed around 3053 cm^{-1} indicate aromatic C–H stretching, while the bands near 2980 cm^{-1} correspond to aliphatic C–H stretching vibrations. A strong absorption band at approximately 1671 cm^{-1} is attributed to the C=N stretching of the imine group, along with possible conjugated C=O/N functionalities. The absorption at 1491–1446 cm^{-1} further supports the presence of aromatic rings and CH bending. A strong band around 1326 cm^{-1} indicates C–N stretching vibrations, while peaks in the 1270–1040 cm^{-1} region correspond to C–F stretching and C–N/C–O vibrations. Additionally, bands observed near 900–700 cm^{-1} (such as 802, 779, 723 cm^{-1}) are characteristic of aromatic C–H out-of-plane bending. Overall, the IR spectrum confirms the presence of aromatic rings, imine functionality, aliphatic chains, and fluorinated substituents in the structure.

D. HPLC Results:

Inject the blank, Cinacalcet API, N-Nitroso Cinacalcet impurity, the purity obtained N-Nitroso Cinacalcet impurity 99.69%

V. CONCLUSION

The study successfully developed a simple, controlled synthesis of N-nitroso-cinacalcet using tert-butyl nitrite on cinacalcet API in acetonitrile under cooling, yielding a high-purity (99.69% by HPLC) brown liquid reference standard. Comprehensive characterization via LC-MS (m/z 387.28 $[M+H]^+$), NMR, and IR confirmed the structure and stability, addressing nitrosamine regulatory concerns for cinacalcet quality control. This impurity standard enables precise detection and control strategies to meet FDA/EMA limits (~0.03 ppm).

REFERENCES

[1] Locatelli F, Pontoriero G, Limardo M, Tentori F. Cinacalcet hydrochloride: calcimimetic for the treatment of hyperparathyroidism. Expert Review of Endocrinology & Metabolism. 2006 Mar 1;1(2):167-79.

[2] Rodzoń-Norwicz M, Norwicz S, Sowa-Kućma M,Gala-Błądzińska A. Secondary hyperparathyro idism in chronic kidney disease: pathomechanism and current treatment possibilities. Endokrynologia Polska. 2023;74(5):490-8.

[3] Rothe HM, Liangos O, Biggar P, Petermann A, Ketteler M. Cinacalcet treatment of primary hyperparathyroidism. International journal of endocrinology. 2011;2011(1):415719.

[4] Balfour JA, Scott LJ. Cinacalcet hydrochloride. Drugs. 2005 Feb;65(2):271-81

[5] ICH Harmonised Guideline: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk M7(R2). Geneva: ICH; 2023 Apr 03 [cited 2026 Apr 11]. Available from: ich.org

[6] U.S. Food and Drug Administration. CDER nitrosamine impurity acceptable intake limits [Internet]. Silver Spring (MD): FDA; 2023 Aug 4 [updated 2026 Mar 19; cited 2026 Apr 11].

[7] World Health Organization. Information note: Nitrosamine impurities in medications. Geneva: WHO; 2019.

[8] Medicines Agency. Nitrosamine impurities in human medicinal products [Internet]. Amsterdam: EMA; 2020 [cited 2026 Apr 3]