Research Progress on the Extraction, Separation, Total Synthesis, and Pharmacological Activity of Nitidine Chloride (Postprint)

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Abstract

Zanthoxylum nitidum is one of the traditional Chinese medicines commonly used in folk medicine, and formulations prepared with it as the principal drug or in combination with related medicinal herbs are clinically applicable to multiple conditions. Modern research has demonstrated that Zanthoxylum nitidum exhibits diverse biological activities, including anti-inflammatory, antibacterial, antioxidant, anti-HIV, and anti-tumor effects, along with certain cardioprotective efficacy. Nitidine chloride, isolated from the roots, constitutes the primary active component responsible for the plant' s various biological activities. In vitro studies have confirmed that nitidine chloride and its structurally analogous benzo[c]phenanthridine alkaloids can inhibit proliferation of multiple tumor cell types—including hepatic, lung, gastric, renal, and breast cancers—induce apoptosis, and suppress tumor metastasis and invasion, thereby garnering increasing attention from medicinal chemists as a novel potential anticancer agent. Currently, direct extraction and isolation from plants suffers from low yields and high costs, while nitidine chloride itself exhibits poor water solubility and low bioavailability. Consequently, increasing efforts are being devoted to structural modification for enhanced activity and utilization, as well as the development of efficient and economical total synthetic routes to meet research and production demands. This review, based on relevant domestic and international literature, comprehensively summarizes research progress on the extraction, separation, and purification of nitidine chloride, total synthetic routes to structural analogues, and pharmacological mechanisms of action, identifies existing research deficiencies, and proposes future research directions, aiming to provide a theoretical basis for in-depth investigations into activity modification of nitidine and its derivatives, mechanistic elucidation, and development of innovative drug molecules based on this scaffold.

Full Text

Preamble

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Abstract

Zanthoxylum nitidum (Roxb.) DC., locally known as "Liangmianzhen," is a traditional Chinese medicine widely used in folk medicine. Modern research has demonstrated that this herb exhibits diverse biological activities, including anti-inflammatory, antibacterial, antioxidant, anti-HIV, and antitumor effects, along with cardiovascular protective properties. Nitidine chloride, extracted from the roots, represents the principal active constituent responsible for these bioactivities. In vitro studies have confirmed that nitidine chloride and its structurally related benzo[c]phenanthridine alkaloids can inhibit proliferation of various cancer cell types—including liver, lung, gastric, renal, and breast cancers—while inducing apoptosis and suppressing tumor metastasis and invasion. Consequently, these compounds have attracted increasing attention from medicinal chemists as novel potential anticancer agents. However, direct extraction from plants suffers from low yield, high cost, poor water solubility, and low bioavailability. To address these limitations, chemists have focused on structural modification to enhance activity and utilization, while developing efficient and economical total synthetic routes to meet research and production demands. This review summarizes recent advances in the extraction, separation, purification, total synthesis, and pharmacological mechanisms of nitidine chloride based on domestic and international literature. We discuss current research deficiencies and future directions, aiming to provide a theoretical foundation for further studies on activity modification, mechanism elucidation, and development of innovative drug candidates based on this scaffold.

Keywords: Nitidine chloride; Extraction; Total synthesis; Pharmacological activity

1. Physicochemical Properties and Chemical Structure of Nitidine Chloride

Nitidine chloride has the molecular formula $C_{21}H_{18}ClNO_4$, with a melting point of 275–276 °C. It appears as light yellow needle-like crystals, soluble in methanol and ethanol but only slightly soluble in water. Its structural formula is shown in Figure 1 [Figure 1: see original paper].

2. Extraction of Nitidine Chloride

As a benzo[c]phenanthridine alkaloid, nitidine chloride can be extracted using standard alkaloid extraction methods, primarily including reflux extraction, enzymatic extraction, and ultrasonic-assisted extraction.

2.1 Reflux Extraction

Reflux extraction is the most widely employed method for nitidine chloride, comprising two main approaches: ethanol reflux and acidified ethanol reflux. Lei et al. (2011) optimized the extraction process using 80% ethanol reflux with orthogonal experimental design. Subsequently, Huang et al. (2011) compared these two methods, demonstrating that acidified ethanol reflux significantly improved extraction efficiency, representing a major advancement in nitidine chloride extraction technology. Zhao et al. (2015) employed 8-fold volume of 80% ethanol with hydrochloric acid to adjust pH to 5, achieving an average total alkaloid extraction yield of 12.8% with nitidine chloride content reaching 41 $mg \cdot g^{-1}$.

While acidified ethanol reflux enhances extraction yield, it suffers from prolonged extraction times and severe equipment corrosion. Zeng et al. (2013; 2014) improved this process using an internal ebullition method, which reduced extraction temperature and time while decreasing total solvent consumption and corrosion.

2.2 Enzymatic Extraction

Since most alkaloids are extracted from plant materials, enzymatic pretreatment with cellulase to disrupt plant cell walls represents an effective strategy for releasing active constituents (Liu and Liao, 2007). Lu et al. (2013a) treated Zanthoxylum nitidum with a cellulase/pectinase mixture before acidified ethanol extraction at room temperature, achieving an 85.96% extraction yield for nitidine chloride. This enzymatic pretreatment approach reduces extraction cycles and solvent consumption, meeting environmental protection and energy-saving requirements.

2.3 Ultrasonic-Assisted Extraction

Ultrasonic extraction utilizes cavitation, thermal effects, and mechanical vibration to rupture plant cell walls, accelerating organic solvent penetration and

alkaloid dissolution (Guo, 1999). Currently, ultrasonic extraction serves primarily as an auxiliary method in natural product isolation. Lu et al. (2013b) combined enzymatic pretreatment with ultrasonic extraction to increase nitidine chloride yield to 90.26%. Their subsequent work (Lu et al., 2014) demonstrated that ultrasonic-enzyme assisted semi-bionic extraction significantly improved dry extract yield, making it suitable for large-scale production. Li et al. (2015) employed a semi-bionic-ultrasonic method using citric acid-triethylamine in 60% ethanol after composite enzyme pretreatment, achieving a nitidine chloride yield of 0.252%. This approach overcomes the poor water solubility of pure aqueous buffers and promotes higher extraction efficiency.

Acidified ethanol reflux offers simple operation and high extraction rates, but equipment corrosion remains a significant concern for industrial scale-up. Enzymatic and ultrasonic-assisted methods maintain high extraction efficiency while eliminating acid use, substantially reducing solvent consumption and solving corrosion problems in an environmentally friendly manner.

3. Separation and Purification of Nitidine Chloride

Zanthoxylum nitidum contains numerous alkaloids, including chelerythrine, skimmianine, dihydrochelerythrine, liriodenine, nitidine chloride, and oxynitidine. Further purification is required to obtain high-purity nitidine chloride from crude extracts, though research reports on this topic remain scarce.

3.1 Crystallization

Huang and Li (1980) and Wang (1980) purified nitidine chloride by allowing crystals to form from methanolic extracts. However, this method requires chloroform, posing significant environmental concerns.

3.2 Resin Adsorption

Lu et al. (2010) employed Ls006 cation exchange resin for nitidine chloride enrichment via static adsorption-desorption, achieving over 90% purity. Zeng et al. (2014) used 732 cation exchange resin to adsorb extracts from acidified internal ebullition, followed by ultrasonic-assisted desorption, achieving desorption rates above 99% and nitidine chloride purity of 94.5%. Resin purification demonstrates excellent performance, simple operation, and low cost, offering significant industrial application potential.

3.3 Chromatography

Huang et al. (2013) purified nitidine chloride by repeatedly grinding the root extract with chloroform, dissolving the precipitate in methanol for recrystallization, and subjecting the crude product to secondary recrystallization followed by HPLC purification using a C-18 column, yielding nitidine chloride with purity exceeding 98% suitable for quality control and research reference standards.

4. Total Synthesis of Nitidine Chloride

Current commercial nitidine chloride is primarily obtained through plant extraction, which suffers from low yields and high costs, limiting systematic research. To overcome these challenges, synthetic chemists have pursued efficient total synthetic routes, with the most critical step being construction of the B or C ring. This review categorizes synthetic approaches by the chemical methodology employed.

4.1 Friedel-Crafts Reaction

In 1978, Cushman and Cheng developed a pioneering total synthesis route utilizing intramolecular Friedel-Crafts reaction to construct the C ring (Figure 2a [Figure 2: see original paper]). Intermediate (1) underwent Arndt-Eistert homologation and Wolff rearrangement, followed by Friedel-Crafts cyclization to afford key intermediate (4), which was then transformed through lithium aluminum hydride reduction and dehydrogenation to complete C ring formation. Luo et al. (2006) also employed intramolecular Friedel-Crafts reaction in their synthesis of nornitidine (Figure 2b). Nickel- or palladium-catalyzed annulation constructed the B ring to give intermediate (7), which after Swern oxidation afforded intermediate (8). Final Friedel-Crafts cyclization under acidic conditions yielded nornitidine (9). Compared to Cushman's route, this approach uses readily available starting materials, avoids hazardous diazomethane, features fewer steps, and offers significantly improved yields.

4.2 Benzyne Method

The benzyne approach was applied early in nitidine synthesis. Kessar et al. (1988) constructed the B ring via benzyne cyclization (Figure 3 [Figure 3: see original paper]). Intermediate (10) generated a benzyne species under strong base KNH_2/NH_3 conditions, which underwent cycloaddition and oxidation to afford nornitidine, albeit in extremely low yield (~10%). Subsequent use of LDA/THF at -78 °C improved yields but required strict anaerobic conditions; otherwise, yields remained very low or the reaction failed to proceed.

Martín et al. (1993) developed an intermolecular benzyne cycloaddition approach (Figure 4a [Figure 4: see original paper]). Compound (12) served as an aza-diene equivalent, undergoing [4+2] cycloaddition with benzyne to construct the isoquinoline skeleton. While selective, this route suffered from low yields. Perez et al. (1993) employed intermolecular Diels-Alder reaction between pyranone derivatives and benzyne to construct the C ring; product (19) could be converted to nitidine after reduction and dehydrogenation (Figure 4b).

In 2015, Castillo et al. developed a tandem approach for rapid preparation of functionalized isoquinoline scaffolds applicable to nitidine synthesis (Figure 5 [Figure 5: see original paper]). This route features readily prepared starting materials, mild reaction conditions, and moderate yields.

4.3 Radical Reactions

Nakanishi and Suzuki (1999) constructed the B ring through radical-mediated intramolecular aryl-aryl coupling (Figure 6 [Figure 6: see original paper]). Under initiation by tin reagents and AMBN, intermediate (22) underwent aryl coupling to form cyclized product (23), which was further transformed into the anticancer compound NK 109.

In 2001, Moreno et al. utilized hypervalent iodine reagent (PIFA) to initiate radical coupling for B ring construction (Figure 7 [Figure 7: see original paper]). This method features short steps, mild conditions, and good yields, though substituent effects on reactivity limit its generality.

In 2013, De et al. developed an intramolecular radical coupling approach promoted by potassium tert-butoxide and organic small molecules for benzo[c]phenanthridine alkaloid synthesis (Figure 8 [Figure 8: see original paper]). When the nitrogen substituent was alkyl, dihydrobenzo[c]phenanthridine structures were obtained; changing to an aroyl substituent enabled a one-pot aryl coupling and oxidative deprotection to directly afford aromatized products. This method offers short routes and environmentally friendly catalysts applicable to diverse alkaloid syntheses.

4.4 Bischler-Napieralski Reaction

The Bischler-Napieralski cyclization represents a common method for B ring construction. Geen et al. (1998) employed $POCl_3$ to catalyze cyclization of key intermediates (33) or (34), affording nornitidine and nitidine chloride with B-N cyclization yields exceeding 90% (Figure 9a [Figure 9: see original paper]). Saito et al. (2001) replaced $POCl_3$ with triphosgene, reducing reaction temperature and time while simplifying workup (Figure 9b).

4.5 Metal Catalysis

Metal-catalyzed synthesis has emerged as a popular approach for benzo[c]phenanthridine alkaloids. Harayama (2005) first developed a palladium-catalyzed intramolecular aryl-aryl coupling to directly construct oxynitidine (Figure 10a [Figure 10: see original paper]). Xu et al. (2015) improved upon Suzuki' s route by implementing palladium acetate-catalyzed intramolecular Heck coupling, completing nitidine chloride synthesis in five steps (Figure 10b).

In 2010, Enomoto et al. reported a gold(I)-catalyzed intramolecular hydroamination cascade reaction that efficiently constructed both B and C rings of nitidine in high yield (Figure 11 [Figure 11: see original paper]).

In 2011, Lv et al. developed a palladium-catalyzed tandem reaction for oxobenzo[c]phenanthridine synthesis (Figure 12 [Figure 12: see original paper]). Methyl o-iodobenzoate derivative (46) and N-heterobicyclic intermediate (47) underwent a ring-opening coupling-cyclization reaction under combined

palladium(II) and zinc catalysis to afford product (48) in high yield. This method features a concise route and high efficiency.

Blanchot et al. (2011) employed aryl triflates instead of aryl iodides (Figure 13 [Figure 13: see original paper]), which underwent palladium-catalyzed tandem direct arylation/N-arylation with N-silyl imine (52) to prepare benzo[c]phenanthridine derivatives, enabling efficient synthesis of nitidine and NK 109 with moderate overall yields.

In 2015, Jiang et al. developed a novel visible-light-induced approach where acyl oximes generate iminyl radicals at room temperature, which undergo intramolecular aromatic substitution to form nitrogen heterocycles, enabling efficient synthesis of diverse functionalized benzo[c]phenanthridine derivatives (Figure 14 [Figure 14: see original paper]).

In recent years, increasingly effective synthetic methods for nitidine have been explored, greatly facilitating studies on total synthesis, structure-activity relationships, and biological activities. However, among these routes, some suffer from excessive length and low overall yields, while others, though concise with high-yielding key steps, involve complex starting material preparation or expensive reagents. Therefore, developing efficient and economical total synthetic routes to nitidine and its derivatives remains both a challenge and an active research area.

5. Pharmacological Activities of Nitidine Chloride

Nitidine chloride exhibits remarkable bioactivity, demonstrating significant effects in antitumor, analgesic and anti-inflammatory, antimalarial, antibacterial, anti-HIV, and cardiovascular protection applications.

5.1 Antitumor Activity

Extensive research has investigated nitidine chloride' s antitumor effects and mechanisms against liver cancer, lung cancer, ovarian cancer, breast cancer, and other malignancies. While mechanisms remain debated, proposed pathways include inhibition of DNA topoisomerase I, induction of apoptosis, cell cycle arrest, and suppression of DNA ligase activity through modulation of multiple signaling pathways (e.g., ERK, JAK/STAT3, AKT).

Fang et al. (1993) and Wang et al. (1993) demonstrated that nitidine chloride and related benzo[c]phenanthridine compounds function as topoisomerase I inhibitors, stabilizing the covalent topoisomerase I-DNA binary complex at concentrations of 0.15–0.3 $M \cdot L^{-1}$. Huang et al. (2013) found that nitidine chloride significantly reduced E2F and RB gene mRNA and E2F protein expression in SMMC-7721 hepatocellular carcinoma cells, blocking G1-to-S phase transition, inhibiting proliferation ($IC_{50} = 1.05 \pm 0.12$ $mg \cdot mL^{-1}$), and inducing apoptosis. Sun et al. (2014) showed that nitidine chloride upregulated Bax/Bcl-2 ratios and induced G2/M arrest to promote breast cancer cell apoptosis. Liu et

al. (2007) demonstrated concentration-dependent effects on KB cells: low concentrations caused G2/M arrest, moderate concentrations induced apoptosis, and high concentrations led to necrosis.

Lin et al. (2014) administered nitidine chloride to hepatocellular carcinoma-bearing mice, observing significant tumor volume and weight reduction without notable body weight changes, confirming antitumor efficacy. Their studies revealed that nitidine chloride suppresses tumor cell proliferation and promotes apoptosis through inhibition of STAT3, ERK, and SHH signaling pathways. Zhai et al. (2016) and Sun et al. (2016) demonstrated that nitidine chloride inhibits proliferation and migration of colorectal and ovarian cancer cells by modulating ERK signaling pathway expression. Cheng et al. (2016) showed that nitidine chloride at concentrations exceeding 5 M significantly suppressed proliferation, migration, and invasion of U2OS osteosarcoma cells via the AKT/GSK-3β/Snail signaling pathway.

Li et al. (2017) developed a supramolecular formulation NC@CB[7] that dramatically reduced toxicity to normal human liver LO2 cells while increasing anticancer activity against MCF-7 breast cancer cells, opening new avenues for supramolecular drug formulations.

5.2 Antimalarial Activity

According to WHO statistics, 3.2 billion people remain at risk of malaria annually, with approximately 2 million deaths from *Plasmodium falciparum*. Novel, efficient, and economical antimalarial agents are urgently needed. Jullian et al. (2006) isolated multiple benzo[c]phenanthridine alkaloids from *Zanthoxylum rhoifolium*, identifying nitidine as the most potent against *P. falciparum* ($IC_{50} < 0.27$ $M \cdot L^{-1}$). Bouquet et al. (2012) found nitidine chloride equally active against chloroquine-sensitive and resistant strains, demonstrating formation of 1:1 complexes with heme that inhibit β-hematin formation. Nyangulu et al. (2005) synthesized nitidine chloride derivatives with even stronger antimalarial activity than the parent compound.

5.3 Antimicrobial Activity

Nitidine chloride demonstrates potent inhibitory and bactericidal effects against various bacteria and fungi. Poeta et al. (1999) showed that nitidine chloride effectively inhibits fungal infections in vitro, with activity comparable to camptothecin, representing a promising antifungal agent. Ye et al. (2013) evaluated 11 compounds from *Zanthoxylum nitidum* roots, finding nitidine chloride particularly active against methicillin-resistant *Staphylococcus aureus* (MRSA) resistant to six major antibiotic classes. Zhang et al. (2014) isolated nitidine chloride from *Zanthoxylum bungeanum* leaves, demonstrating >50% activity against five pathogenic fungi (*B. cinerea*, *P. oryzae*, *P. piricola*, *G. cingulata*, and *V. pyrina*), with strongest activity against *G. cingulata*. Tavares et al. (2014) confirmed strong antifungal activity against yeasts (MIC 6.25–25 $g \cdot mL^{-1}$). Cesari et

al. (2015) reported significant bactericidal and inhibitory effects against *Bacillus* ATCC 3584 and *Streptococcus pyogenes* ATCC 19615 (MIC 0.19 M and 3.64 M, respectively).

5.4 Anti-inflammatory and Analgesic Effects

Traditionally used for trauma, inflammation, and pain relief, *Zanthoxylum nitidum* has been formulated into oral care products based on these properties. Liu et al. (2005) evaluated analgesic and anti-inflammatory effects using hot-plate and writhing tests (analgesia) and xylene-induced ear edema and dye exudation methods (inflammation), demonstrating significant efficacy in mouse models.

Wang et al. (2012) investigated anti-inflammatory mechanisms in LPS-induced Raw264.7 macrophages, showing that nitidine chloride significantly reduced pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) at both RNA and protein levels by inhibiting MAPK phosphorylation and p65 transcription, offering new therapeutic prospects for inflammatory diseases. Wang et al. (2016) demonstrated that nitidine chloride protects dopaminergic neurons in Parkinson' s disease models by inhibiting Jak2–Stat3 signaling and enhancing $\alpha\beta$-crystallin binding to p-Stat3, thereby suppressing microglia-mediated inflammation.

5.5 Cardiovascular Effects

Wei et al. (2006) showed that nitidine chloride reduces myocardial enzyme release, attenuates oxygen free radical damage, decreases arrhythmia incidence, delays onset time, and shortens duration in myocardial ischemia-reperfusion rat models, with dose-dependent efficacy.

5.6 Other Activities

Beyond the aforementioned effects, nitidine chloride demonstrates promising activity against leukemia, HIV, and liver injury. Liu et al. (2015) found that nitidine chloride accelerates c-Myc degradation through increased Thr58 phosphorylation and downregulates c-Myc-activated miRNAs, exerting cytotoxic effects against K562 and primary CML cells. Wang et al. (2015) demonstrated that nitidine chloride modulates HIV-1 promoter expression and stabilizes G-quadruplex structures, increasing the Tm value from 56.6 °C to 63.2 °C, providing new insights for anti-HIV-1 drug development. Pang et al. (2006) showed that *Zanthoxylum nitidum* extracts protect against carbon tetrachloride-induced liver injury by reducing serum ALT, AST, and hepatic MDA levels while increasing SOD activity in a dose-dependent manner.

Conclusion and Future Perspectives

Nitidine chloride exhibits excellent antibacterial, anti-inflammatory, and anti-tumor properties with broad development potential. However, its low natural abundance and extraction yield cannot meet research and production de-

mands, making artificial synthesis the preferred approach. Although numerous synthetic routes have been developed, structural derivative synthesis remains limited due to route diversity and starting material variability, particularly regarding site-specific substituted derivatives. The structure-activity relationships concerning oxygen atoms, substituent positions/types/steric effects, methylene groups, and other structural features remain unclear, severely limiting compound development. Therefore, developing novel chemical reactions and optimized synthetic routes for economical and efficient preparation of target compounds and derivatives, followed by systematic SAR studies, represents a key future research direction. Additionally, as a benzo[c]phenanthridine alkaloid, nitidine chloride may demonstrate high in vitro activity but suboptimal in vivo performance due to metabolic or stability issues. Future derivative design must consider not only activity enhancement but also stability and bioavailability improvement.

Current controversies regarding nitidine chloride' s antitumor mechanisms require further investigation. Elucidating the antitumor mechanisms of nitidine chloride and its derivatives will clarify their effects on tumor cells, confirm the material basis for antitumor activity, and guide structural optimization to identify lead compounds with improved activity and drug-like properties, laying the foundation for innovative drug development.

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Note: Figure translations are in progress. See original paper for figures.