EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 2
Year: 2025

NAFITHROMYCIN CHEMISTRY AND MODE OF ACTION

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Article Received on 21/12/2024 **Article Revised on 11/01/2025** **Article Published on 01/02/2025**

ABSTRACT

Ketolides are the fourth generation semisynthetic macrolides derived from erythromycin A, designed to address the limitations of currently available drug treatments including penicillins, macrolides and glycopeptides. Ketolides-the next generation macrolides antibiotics are deliberate to pass the issues of bacterial resistance by introducing keto function at C3 position of macrocycle through the replacement of l-cladinose sugar moiety and brings a broader spectrum of activity. The original research work reported here covers, structure activity relationship based design and synthesis of 34 novel ketolide molecules, are subsequently subjected to desired microbial evaluations. Through this exercise; WCK 4873 (Nafithromycin) has been emerged as clinical candidate among the four different series synthesized. Nafithromycin, consists of novel amidoxime function bearing 2-pyridine-1,3,4-thiadiazole biaryl side chain set apart through a four atom spacer containing a cis double bond and a chiral methyl. This non-flexible spacer possibly aligns the biaryl ring system resulting in a favorable interaction with dual 23S rRNA targets exhibiting better potency to treat gram positive infections. WCK 4873 emerged as preclinical candidate based on in vitro studies, and animal model studies, it had successfully accomplished phase 1 and phase 2 clinical studies in United States, Europe and India. Currently, nafithromycin has acquired the status as phase 3 drug candidate having potential to fulfill the unmet medical needs for treatment of CABP infections.

KEYWORDS: Nafithromycin, WCK 4873, Ketolides, *Streptococcus pneumonia,* Amidoxime, Ribosomal domain II binding, SAR.

INTRODUCTION

Nafithromycin (previously known as WCK 4873) is a novel oral and intravenous lactone-ketolide optimized to provide coverage of multidrug-resistant (MDR) typical and atypical respiratory pathogens, including penicillin- and macrolide-resistant pneumococcal strains. Preclinical studies have shown that nafithromycin has pharma-codynamic action against macrolide- and ketolide-resistant strains of *Streptococcus pneumoniae*, in addition to other important respiratory pathogens such as *Haemophilus influenzae, Moraxella catarrhalis*, methicillin-susceptible *Staphylococcus aureus*, and group A streptococci. An antimicrobial surveillance study involving more than 4,500 clinical isolates collected worldwide during 2013 to 2014 showed nafithromycin MICs for 50% and 90% of isolates (MIC50 and MIC90) of 0.015 mg/liter and 0.06 mg/liter against *S. pneumoniae*, respectively Nafithromycin was 2 to 8 times more potent *in-vitro* than telithromycin, cethromycin, clindamycin, and clarithromycin against *S. pneumoniae*. Against erythromycin- and telithromycin-non-susceptible pneumococci. Nafithromycin is designed to treat both typical and atypical drug-resistant bacteria, making it a crucial tool in addressing the global health crisis of AMR (Anti-microbial Resistance). It boasts superior safety, minimal side effects, and no significant drug interactions. Wockhardt's nafithromycin is India's first FDA-approved antimicrobial that is seen as an alternative to existing antibiotics like azithromycin, which is facing increasing resistance in humans. The antibiotic "Nafithromycin" has been developed with the support of "Biotechnology Industry Research Assistance Council" (BIRAC), a unit of the Department of Biotechnology and has been brought to market under the trade name "Miqnaf" by pharma company "Wolkardt". With the government launching India's first indigenously-developed antibiotic aimed at tackling antimicrobial resistance (AMR), Nafithromycin, there is now hope for treating drug-resistant pneumonia, which is responsible for over two million deaths globally each year. Comment: Nafithromycin (WCK-4873) is a ketolide antibacterial

with Gram-positive activity. Drugmaker Wockhardt on Thursday announced Central Drugs Standard Control Organization (CDSCO) approval for use of its new generation oral antibiotic Miqnaf (Nafithromycin) in the treatment of community-acquired bacterial pneumonia (CABP) in adults.[1-3]

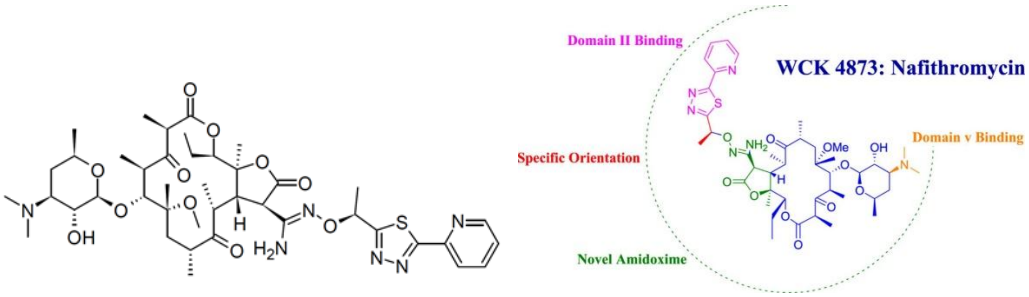


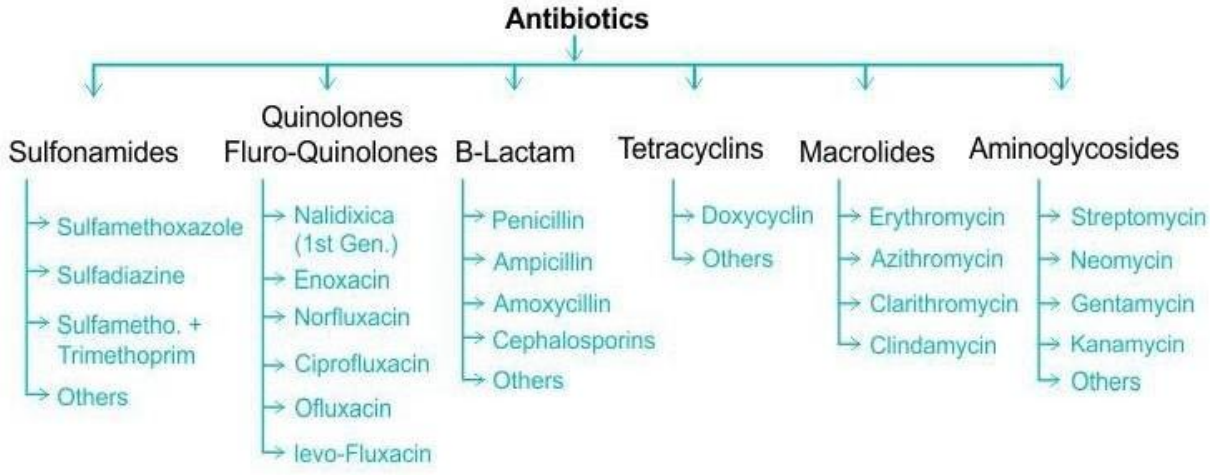
Figure-1: Nafithromycin Structure.

Chemistry: Molecular formula: $C_{42}H_{62}N_6O_{11}S$, Molar mass 859.05 g·mol^{-1} [CAS: 1691240-78-4; IUPAC: 1S,2R,5R,7R,8R,9R,11R,13R,14S,15R)-8-[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-2-ethyl-9-methoxy-1,5,7,9,11,13-hexamethyl-4,6,12,16-tetraoxo-N'-[(1S)-1-(5-pyridin-2-yl-1,3,4-thiadiazol-2-yl)ethoxy]-3,17-dioxabicyclo[12.3.0]heptadecane-15-carboximidamide]. Nafithromycin, consists of novel amidoxime function bearing 2-pyridine-1,3,4-thiadiazole biaryl side chain set apart through a four atom spacer containing a cis double bond and a chiral methyl.

Water Solubility: 0.00705 mg/mL, logP: 4, logS: -5.1, pKa: 9.22, pKa (Strongest Basic): 8.61, Physiological Charge: 1, Hydrogen Acceptor Count: 15, Hydrogen Donor Count: 2, Polar Surface Area: 224.18 Å^2, Rotatable Bond Count: 10, Refractivity: 230.18 m^3·mol^{-1}, Polarizability: 90.19 Å^3, Number of Rings: 5, Bioavailability: 0, Rule of Five: No

Classification of Antibiotics

Table 1: Classification of Antibiotics.



Mechanism action of Nafithromycin

SAR (Structure Activity Relationship): Ketolides are the fourth generation semisynthetic macrolides derived from erythromycin A, designed to address the limitations of currently available drug treatments including penicillins, macrolides and glycopeptides. Ketolides-the next generation macrolides antibiotics are deliberate to pass the issues of bacterial resistance by introducing keto function at C3 position of macrocycle through the replacement of l-cladinose sugar moiety and brings a broader spectrum of activity. The original research work reported here covers, structure activity relationship based design and synthesis of 34 novel ketolide molecules, are subsequently subjected to desired microbial evaluations. Through this exercise; WCK 4873 (Nafithromycin) has been emerged as clinical candidate among the four different series synthesized. Nafithromycin, consists of novel amidoxime function bearing 2-pyridine-1,3,4-thiadiazole biaryl side chain set apart through a four atom spacer containing a cis double bond and a chiral methyl. This non-flexible spacer possibly aligns the biaryl ring system resulting in a favorable interaction with dual 23S rRNA targets exhibiting better potency to treat gram positive infections. WCK 4873 emerged as preclinical candidate based on in vitro studies, and animal model studies, it had successfully accomplished phase 1 and phase 2 clinical studies in United States, Europe and India. Currently, nafithromycin has acquired the status as phase 3 drug candidate having potential to

fulfill the unmet medical needs for treatment of CABP infections.[4-7]

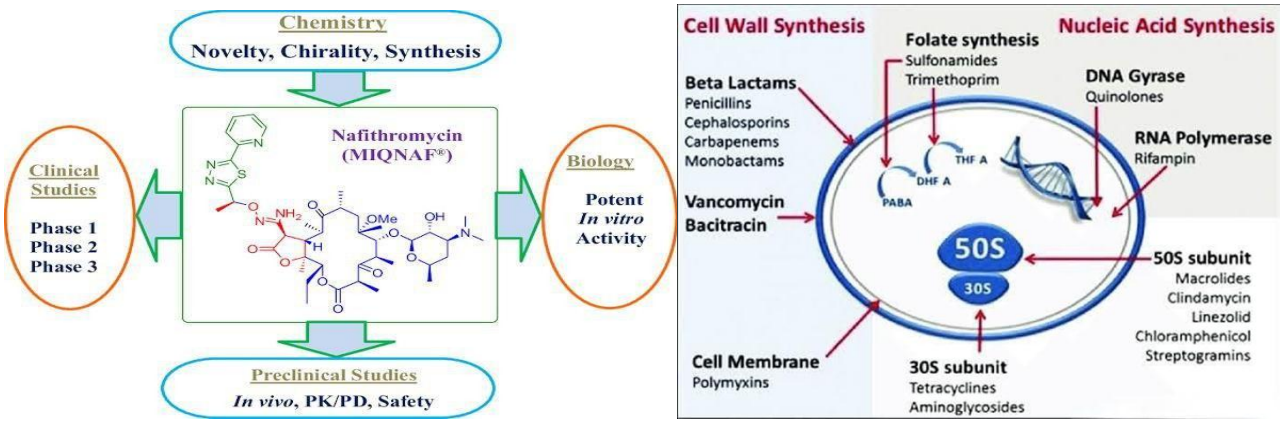


Figure-2: PK/PD studies and mode of action of drug.

Pharmacokinetic: In the SAD/FE study, 55 of 56 subjects were analyzed for nafithromycin PK (1 enrolled subject was excluded upon admission to the clinic). In the MAD study, all 24 enrolled subjects with planned PK collection had analyses done.

(i) Single-dose pharmacokinetics. The mean values for PK parameters of nafithro- mycin in plasma after single doses of nafithromycin are shown. (SAD part), with the mean plasma concentrations plotted (corresponding data for WCK 4978 are presented in Table in the supplemental material). Following treatment with single ascending oral doses of 100 to 1,200 mg nafithromycin under either fasted or fed conditions, drug levels were detected in plasma within 0.5 to 2 h postdose (0.5 h was the first postdose sampling time point). The highest geometric mean plasma concentrations (Tmax) were observed between 1 and 6 h postdose. The mean plasma concentration-time profiles displayed a clear dose-dependent increase. After reaching a maximum, the concentrations decreased rapidly by approximately 12 to 16 h postdose, followed by a slower decline. However, the mean of the combined individual profiles of the 1,200-mg fed treatment had 2 peaks, with the highest peak at approximately 6 h postdose. Consistently, most of the individual profiles for the 1,200-mg treatment showed 2 peaks up to several hours after dosing. Considerable interindividual differences (with about 2-fold to 8-fold differences in plasma concen- trations) in PK profiles across all dose levels were observed. One subject in the 200- mg fasting cohort had low plasma concentrations (maximal concentration - 0.047mg/liter) compared to the other subjects (maximal concentrations, 0.122 to 0.393 mg/liter).The low concentrations seen with this subject may have been due to incomplete absorption of the tablet, since the level of recovery of the drug from feces samples was relatively high (69.2%) in this subject. As for the WCK 4978 metabolite, the dose-dependent increase displayed in its concentration time profiles was similar to that described for the parent (nafithromycin) profiles. Also, the individual and combined individual WCK 4978 concentration time profiles displayed a pattern similar to that described for nafithromycin. For dose proportionality, assessed for the full dose range and by nutritional status, the 95% confidence intervals (CI) and exponents of the power model corresponding to the exposure parameters (slopes of the regression lines) of both nafithromycin and WCK 4978 appeared to be higher than 1 for maximum plasma concentration (Cmax) under fed conditions and for the area under the concentration-time curve from time zero to time t (AUC0 –t) and for AUC from time zero to infinity (AUC0 –inf) under both fasted and fed conditions. Results of the power analyses. Additionally, dose-normalized exposures (AUC0 –inf) versus the dose for nafithromycin are plotted. Overall, plasma exposure to nafithromycin and WCK 4978 ap- peared to increase more than dose proportionally. Across single-dose cohorts, between 10.0% and 19.5% of the administered dose was excreted via urine as unchanged nafithromycin and between 0.9% and 2.6% as the WCK 4978 metabolite. The majority of both analytes was excreted in urine at 48 h postdose, with the process being almost completed at 72 h postdose. The percentage of nafithromycin and WCK 4978 excreted in urine appeared to increase with increasing dose. As for excretion with feces, between 7.4% and 19.0% of the administered nafithromycin dose was recovered and between 9.8% and 13.5% as WCK 4978. The excretion data may have been incomplete at 72 h postdose (the last sampling time point). In contrast to the excretion in urine, the percentage of both analytes excreted in feces appeared to be independent of dose. The mean level of plasma protein binding of nafithromycin across cohorts was 80.3% (3.5%), with a mean plasma albumin level of 4.0 g/dl.[8-10]

(ii) Food effect. The mean values for PK parameters for nafithromycin in plasma after single doses of nafithromycin in crossover fed/fasting cohorts of 400 and 800 mg nafithromycin are shown. (FE part), with the mean plasma concentrations plotted. (Corresponding data for WCK 4978 are presented.

India's Fight against Antimicrobial Resistance: Antimicrobial Resistance (AMR) occurs when bacteria,

viruses, fungi and parasites no longer respond to antimicrobial medicines. As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability and death.

Table 2: PK/PD of drug.

Parameter	Nutritional state	Slope	95% CI	
			Lower limit	Upper limit
Nafithromycin				
C_{max}	Fasted	1.08	0.83	1.32
	Fed	1.26	1.02	1.51
AUC_{0-t}	Fasted	1.50	1.27	1.73
	Fed	1.54	1.29	1.80
AUC_{0-inf}	Fasted	1.42	1.23	1.61
	Fed	1.51	1.27	1.76
WCK 4978				
C_{max}	Fasted	1.14	0.83	1.44
	Fed	1.30	1.03	1.57
AUC_{0-t}	Fasted	1.97	1.51	2.43
	Fed	1.92	1.65	2.20
AUC_{0-inf}	Fasted	1.97	1.20	2.74
	Fed	1.81	1.55	2.07

While AMR is a natural process driven by genetic changes in pathogens its spread is significantly accelerated by human activities, particularly the overuse and misuse of antimicrobial drugs in humans, animals, and plants. Antimicrobial resistance (AMR) has become a major global health issue with around 6 lakh lives lost in India each year due to resistant infections. However, India strides in addressing AMR, particularly through the development of new drugs. Nafithromycin, with ₹8 crore in funding under the Biotechnology Industry Research Assistance Council (BIRAC) Biotech Industry Program for Phase 3 clinical trials, is a key milestone in this effort. Since India carries a large share of the global pneumonia burden, introducing Nafithromycin is especially important, as there have been no new antibiotics in recent years. Nafithromycin offers improved patient compliance and is a vital step in combating AMR.

Nafithromycin: Milestone for Public Health: Nafithromycin, was officially launched on November 20, 2024, by Union Minister Dr. Jitendra Singh. Developed by Wockhardt with support from the Biotechnology Industry Research Assistance Council (BIRAC), Nafithromycin, marketed as "Miqnaf," targets Community-Acquired Bacterial Pneumonia (CABP) caused by drug- resistant bacteria, which disproportionately affects vulnerable populations such as children, the elderly, and those with compromised immune systems. This groundbreaking antibiotic is ten times more effective than current treatments like azithromycin and offers a three- day treatment regimen, significantly shortening the recovery time while improving patient outcomes. Nafithromycin is designed to treat both typical and atypical drug-resistant bacteria, making it a crucial tool in addressing the global health crisis of AMR (Anti-microbial Resistance). It boasts superior safety, minimal side effects, and no significant drug interactions. Nafithromycin's development marks a historic milestone as the first new antibiotic in its class to be introduced globally in over 30 years. The drug, which has undergone extensive clinical trials across the U.S., Europe, and India, has been developed with an investment of ₹500 crores and is now awaiting final approval from the Central Drugs Standard Control Organization (CDSCO). This innovation exemplifies the power of public-private collaboration and underscores India's growing capabilities in biotechnology. Nafithromycin's successful introduction represents a major leap in the fight against AMR, offering hope for treating multi- drug-resistant infections and saving lives worldwide.[11-13]

Figure-3: Combat pneumonia.

Government's Other Initiatives to Combat AMR: Other than developing Nafithromycin, the Government of India has taken significant steps to combat Antimicrobial Resistance (AMR) through a series of strategic initiatives aimed at surveillance, awareness, and collaboration. These efforts focus on enhancing AMR containment, improving infection control, and fostering national and international partnerships.

- **Surveillance and Reporting:** National surveillance networks, including laboratories across the country, have been established to generate annual AMR surveillance reports, with data submitted to the Global AMR Surveillance System (GLASS).
- Awareness and Training: Awareness materials on the judicious use of antimicrobials, hand hygiene, and infection prevention have been developed and shared with stakeholders. National Guidelines on infection prevention have been launched, and a "train-the-trainer" program has been conducted across all states and UTs, with ongoing cascading of training at the state level.[14-16]
- Judicious Use of Antimicrobials: To promote responsible usage, surveillance of antimicrobial use has been initiated at tertiary care hospitals.

CONCLUSION

India's proactive efforts to combat antimicrobial resistance (AMR) through innovative drug development, such as the launch of Nafithromycin, alongside comprehensive national initiatives, underscore the country's leadership in global healthcare. The commitment to surveillance, awareness, and international collaboration highlights India's strategic approach to tackling AMR and improving health outcomes. With continued investments in research, capacity building, and partnerships, India is well-positioned to drive meaningful change in the global fight against antimicrobial resistance and contribute to better health worldwide. Antimicrobial resistance has long been a growing global concern, with pharmaceutical companies striving to develop new medicines to combat it worldwide. Despite years of challenges and relentless effort, a breakthrough has finally emerged. After three decades of research and hard work, India has led the way with the creation of Nafithromycin, the country's first indigenous Macrolide antibiotic. This remarkable achievement marks a pivotal moment in the fight against antimicrobial resistance, showcasing India's growing capabilities in pharmaceutical innovation.

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