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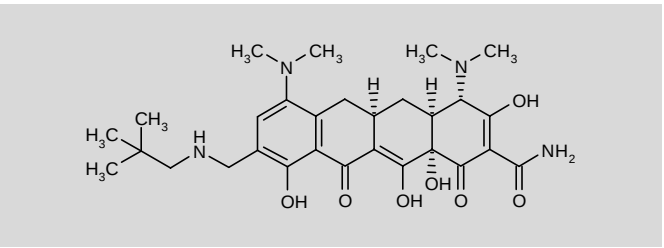
AMADACYCLINE

Tetracycline Antibiotic

PTK-0796

(4*S*,4a*S*,5a*R*,12a*S*)-4,7-Bis(dimethylamino)-9-(2,2-dimethylpropylaminomethyl)-3,10,12,12a-tetrahydroxy-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide

InChI=1/C29H40N4O7/c1-28(2,3)12-31-11-14-10-17(32(4)5)15-8-13-9-16-21(33(6)7)24(36)20(27(30)39)26(38)29(16,40)25(37)18(13)23(35)19(15)22(14)34/h10,13,16,21,31,34,36-37,40H,8-9,11-12H2,1-7H3,(H2,30,39)/t13-,16-,21-,29-/m0/s1



$C_{29}H_{40}N_4O_7$
Mol wt: 556.6505
CAS: 389139-89-3
EN: 327251

ABSTRACT

Amadacycline (PTK-0796) is an aminomethylcycline antibiotic with a broad spectrum of antibacterial activity. The agent showed potent in vitro activity against multidrug-resistant and -susceptible Gram-positive, Gram-negative, anaerobic and atypical bacteria, including methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus faecium and penicillin-resistant Streptococcus pneumoniae. Amadacycline demonstrated superior in vivo activity to other antibiotics, including vancomycin, linezolid and minocycline, against infections caused by various bacterial pathogens and its activity was not affected by the presence of other antibiotics. In patients with complicated skin and skin structure infections, amadacycline and linezolid showed comparable safety and efficacy profiles. No discontinuations due to adverse events and no serious drug-related adverse events were reported in the amadacycline group. Clinical trials of both oral and intravenous formulations of amadacycline have been completed.

SYNTHESIS*

Amadacycline is prepared from the tetracycline antibiotic minocycline (I) by chemical modification via three alternative methods:

1) Condensation of minocycline (I) with *N*-(hydroxymethyl)phthalimide (II) by means of trifluoromethanesulfonic acid and trifluoromethanesulfonic anhydride gives the 2-*N*,9-bis(phthalimidomethyl) derivative (III) along with minor amounts of the tris(phthalimidomethyl) compound (IV), which are deprotected with methylamine in EtOH, yielding a mixture of primary amines (V) and (VI). Finally, amines (V)/(VI) are reductively alkylated with pivalaldehyde (VII), with concomitant de-aminomethylation of (VI) in the presence of H_2 and Pd/C (1) or $NaBH(OAc)_3$ (2). Scheme 1.

2) Reaction of neopentylamine (VIII) with paraformaldehyde in hot hexane gives the triazine derivative (IX), which upon treatment with chloroacetic anhydride (X) in CH_2Cl_2 gives the *N*-(acyloxymethyl)-amide (XI). Subsequent Tscherniac–Einhorn reaction of (XI) with minocycline (I) in the presence of trifluoromethanesulfonic acid yields the protected compound (XII), which is submitted to acidic hydrolysis of the chloroacetyl and hydroxymethyl groups in 3 N HCl at 70 °C, followed by reequilibration of the partly epimerized C4 center with NaOH or ethanolamine (3). Scheme 1.

3) Iodination of minocycline (I) by treatment with *N*-iodosuccinimide in methanesulfonic acid gives compound (XIII) (4), which is converted to aldehyde (XIV) by palladium-catalyzed carbonylation with carbon monoxide gas in the presence of phosphine ligands and triethylsilane. Finally, aldehyde (XIV) is reductively aminated with neopentylamine (VIII) under catalytic hydrogenation conditions (5). Scheme 2.

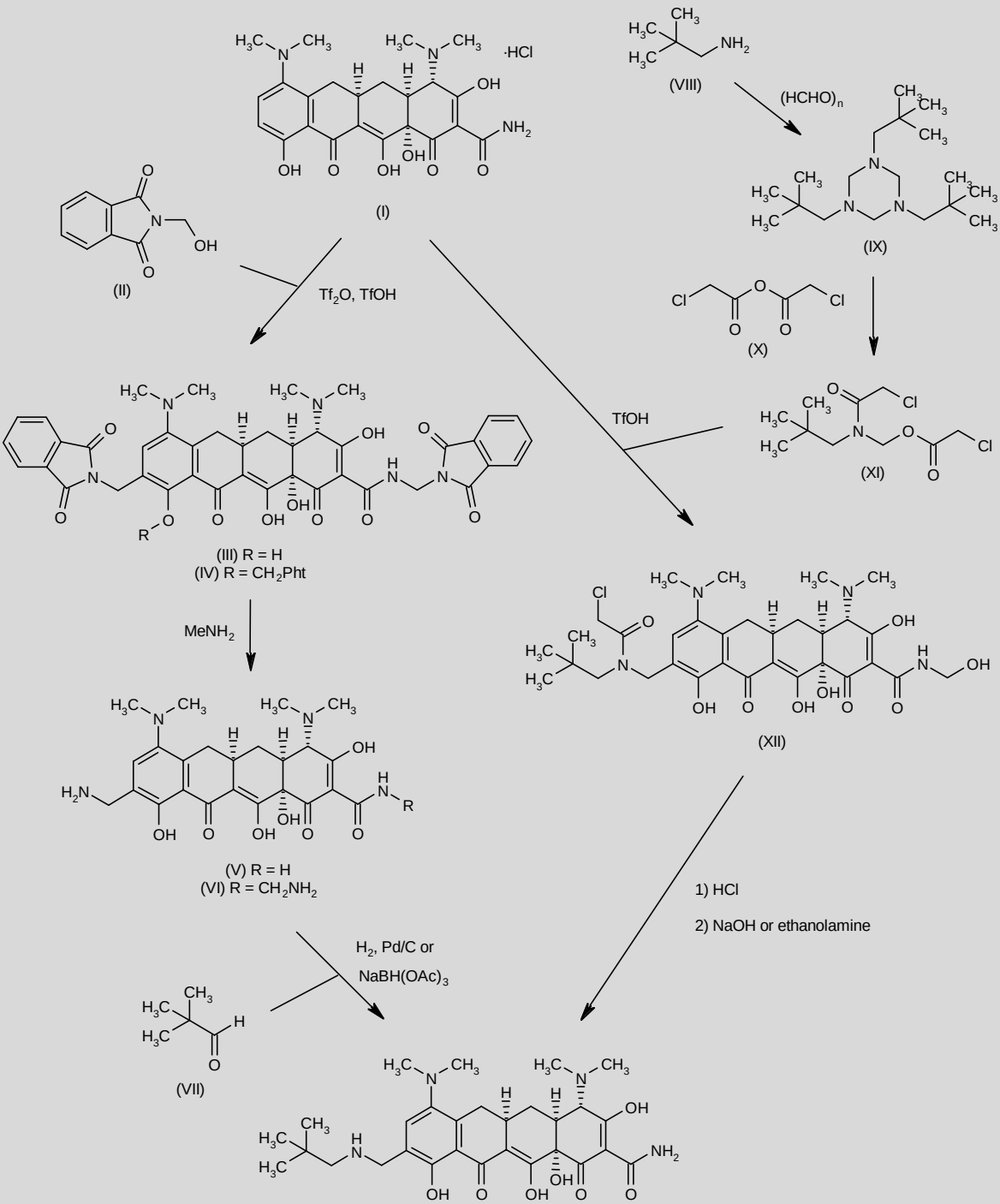
BACKGROUND

Antibiotic resistance has become a huge public health concern. During the past decade a number of new antibiotics have been approved by drug regulatory authorities for the treatment of bacterial infections. However, there is still an urgent need for new antibiotics to treat infections caused by drug-resistant pathogens. New agents with novel antimicrobial mechanisms and new approaches to increase the efficacy of existing antibiotics are especially desirable (6, 7).

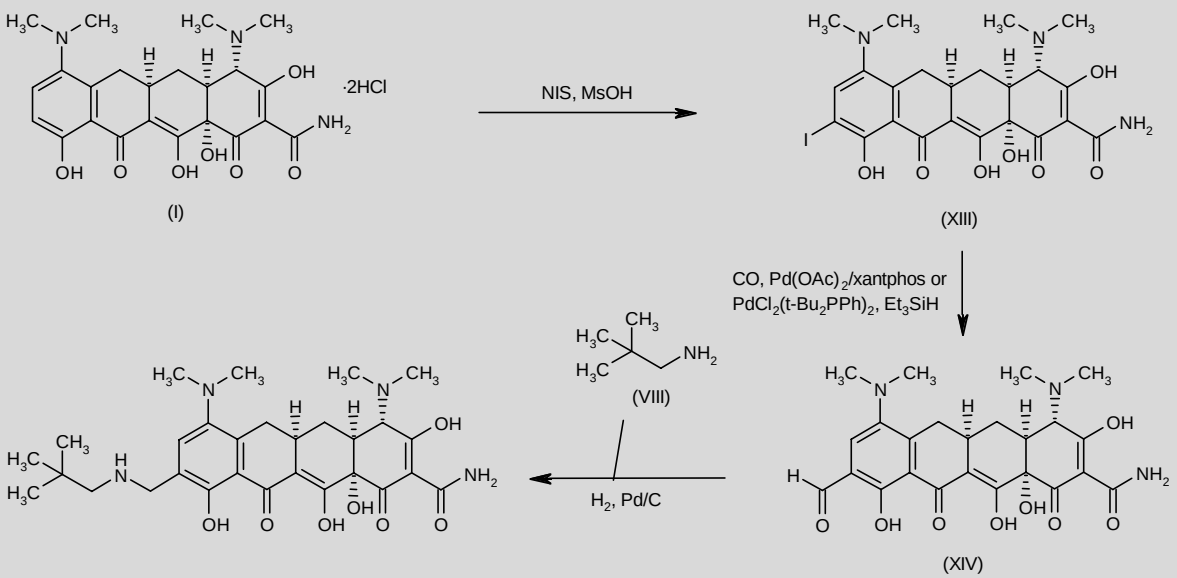
Amadacycline (PTK-0796; formerly referred to as MK-2764, BAY-73-6944 and BAY-73-7388) is a first-in-class aminomethylcycline antibiotic developed by Paratek Pharmaceuticals. Evolved from

Y. Wang. 8106 Runnymeade Dr., Frederick, MD 21702, USA. *Synthesis prepared by R. Castañer, J. Bolós, C. Estivill. Prous Science, Provenza 388, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Amadacycline



Scheme 2. Alternative Synthesis of Amadacycline



tetracycline, amadacycline is expected to be unaffected by the known tetracycline resistance mechanisms, while retaining the safety and tolerability profile of the tetracycline family. Because there is no cross-resistance between the tetracycline family and other antibiotics, amadacycline is expected to be active against isolates resistant to all other currently available antibiotics (8).

Targeted indications for amadacycline encompass serious acute bacterial infections, including complicated skin and skin structure infection (cSSSIs), diabetic foot infections, community- and hospital-acquired pneumonia, and intraabdominal infection. Both intravenous (i.v.) and oral formulations of amadacycline are currently in clinical development (8-10).

PRECLINICAL PHARMACOLOGY

The in vitro activity of amadacycline against more than 200 strains of Gram-positive and Gram-negative clinical isolates was evaluated and compared with that of other antibiotics, including vancomycin, tetracycline and ciprofloxacin. Amadacyline demonstrated high activity against a broad spectrum of pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA; MIC_{90} = 0.5 µg/ml), vancomycin-resistant *Enterococcus faecium* (VRE; MIC_{90} = 0.5 µg/ml) and penicillin-resistant *Streptococcus pneumoniae* (PRSP; MIC_{90} = 0.06 µg/ml or less). Amadacycline exhibited potent activity, particularly against Gram-positive pathogens. Amadacycline also showed in vitro activity against Gram-negative pathogens, including *Escherichia coli* (MIC_{90} = 2.0 µg/ml), *Klebsiella pneumoniae* (MIC_{90} = 4.0 µg/ml) and *Haemophilus influenzae* (MIC_{90} = 2.0 µg/ml) (11).

To evaluate potential microbiological interactions between amadacycline and other antibiotics, the in vitro activity was evaluated in the presence of other antibacterial agents, including tetracycline, using checkerboard MIC methods. No effect on the antibacterial activity of amadacycline or the other agents was seen and the activity of amadacycline was not affected by tetracycline resistance (12).

The susceptibility of 112 strains of *S. aureus*, including 25 methicillin-susceptible (MSSA) and 87 MRSA strains, to amadacycline was compared with that of vancomycin, linezolid, daptomycin, clindamycin, azithromycin, ceftriaxone and levofloxacin. Amadacycline exhibited potent activity against all the strains tested, with MICs ranging from 0.06 to 2 µg/ml, and its activity was not affected by resistance to other antibiotics (13).

To explore the potential use of amadacycline for the treatment of pneumonia, its in vitro activity was tested against 45 clinical isolates of *Legionella* spp. obtained from respiratory tract and environmental sources. The activity of amadacycline was compared with that of doxycycline, telithromycin, azithromycin, erythromycin, moxifloxacin and gatifloxacin. Results of the study indicated that amadacycline was effective against all *Legionella* spp. tested, being more active (MIC_{90} = 0.25 mg/l) than erythromycin (MIC_{90} = 1.0 mg/l), azithromycin (MIC_{90} = 0.5 mg/l) and doxycycline (MIC_{90} = 1.0 mg/l), but less active than moxifloxacin (MIC_{90} = 0.03 mg/l or less), gatifloxacin (MIC_{90} = 0.03 mg/l or less) and telithromycin (MIC_{90} = 0.06 mg/l) against *L. pneumophila*. For other species of *Legionella*, the activity of amadacycline (MIC_{90} < 0.03-0.25 mg/l) was similar to that of azithromycin (MIC_{90} < 0.03-0.25 mg/l) and telithromycin (MIC_{90} < 0.03-0.12 mg/l) and superior to doxycycline (MIC_{90} < 0.03-1.0 mg/l) and erythromycin (MIC_{90} < 0.03-1.0 mg/l) (14).

The effect of environmental variations on the activity of amadacycline was also examined. Minor variations in pH, divalent cation concen-

tration, incubation in CO_2 and the presence of blood in the test medium showed little effect on the in vitro activity of amadacycline. However, MICs increased up to 4-fold with increasing inoculum size (from 10^3 to 10^7 cfu/ml) for *S. aureus* and *E. faecalis,* but not for *E. coli* (15).

To determine the mechanism of the antimicrobial activity of amadacycline, its effect on protein, DNA, RNA and peptidoglycan synthesis was studied. The effect on ribosome binding was also assessed. Amadacycline was found to specifically inhibit protein synthesis, with an IC_{50} of < 0.03 μg/ml. The IC_{50} values for RNA, DNA and peptidoglycan synthesis were > 32, > 32 and 11.5 μg/ml, respectively (16).

The in vivo activity of amadacycline against both Gram-positive and Gram-negative infections was evaluated in both immunocompromised and immunocompetent mice. The efficacy of amadacycline was compared with that of minocycline, vancomycin and linezolid, all administered i.v. For systemic *S. aureus* infection, amadacycline (PD_{50} = 0.4 mg/kg) was as effective as vancomycin (PD_{50} = 0.4 mg/kg) and more effective than minocycline (PD_{50} = 1.0 mg/kg) and linezolid (PD_{50} = 3.5 mg/kg). For thigh infections caused by MRSA, amadacycline showed superior efficacy to vancomycin, linezolid and minocycline, with a > 5-fold decrease in ED_{50}. For systemic *E. faecalis* infection, the efficacy of amadacycline (ED_{50} = 4.5 mg/kg) was again superior to that of linezolid (ED_{50} = 14.3 mg/kg), vancomycin (ED_{50} = 70.3 mg/kg) and minocycline (ED_{50} = 71.0 mg/kg). Amadacycline also showed potent activity against urinary tract infection caused by *E. coli* (17).

The in vivo antimicrobial activity of amadacycline against a wide spectrum of organisms, including MSSA, *S. pneumoniae*, *E. coli* and *K. pneumoniae*, was further evaluated in neutropenic and normal mice with thigh infections. Amadacycline demonstrated potent activity against all the pathogens tested. The extent of killing of *S. pneumoniae* was moderate and similar over the first 2 h after s.c. administration of amadacycline at single doses of 0.312, 1.25 and 5 mg/kg. However, organism regrowth began soon after the total concentration of amadacycline fell below the corresponding MIC. Single doses of 1.25 and 5 mg/kg also showed modest activity against *S. aureus* ATCC 29213 in the first 2 h following administration, but a single dose of 0.125 mg/kg showed less potent activity. Compared with tigecycline, amadacycline showed higher potency against *E. coli* and *K. pneumoniae,* with 4-fold higher MICs. Enhancement of the activity of amadacycline against *S. pneumoniae* (6-fold) and *K. pneumoniae* (2-fold) in the presence of neutrophils was also observed in the study. The 24-h AUC/MIC was the pharmacokinetic (PK)/pharmacodynamic (PD) index that best correlated with efficacy (18).

The efficacy of i.v. amadacycline against multidrug-resistant staphylococcal infection was compared with that of vancomycin and linezolid in murine sepsis model. Amadacycline (100% survival at 0.3 mg/kg) was more potent than vancomycin (100% survival at 10 mg/kg) and linezolid (100% survival at 10 mg/kg) against infections caused by MSSA, quinolone-resistant MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE). In neutropenic mice, amadacycline at 50 mg/kg led to 100% survival, whereas vancomycin and linezolid at 50 mg/kg (highest dose tested) were associated with zero survival (19).

The efficacy of amadacycline against systemic infections caused by tetracycline-resistant *E. faecalis* or VRE was also compared with that of vancomycin, linezolid, imipenem and metronidazole. The potency of amadacycline (100% survival at 1 mg/kg i.v.) was superior to vancomycin (100% survival at 10 mg/kg i.v.) and linezolid (100% survival at 3 mg/kg i.v.) against *E. faecalis* or VRE. For the *E. faecium* septicemia model, a dose of 15 mg/kg i.v. amadacyline led to 100% survival, whereas neither vancomycin nor linezolid provided 100% survival at the highest dose tested (50 mg/kg i.v.). In the pouch infection model caused by *Bacteroides fragilis*, treatment with 25 mg/kg i.v. amadacycline led to a greater reduction in colony-forming units (cfu) than metronidazole at a similar dose level. Treatment of intraabdominal infections and postoperative polymicrobial peritonitis with amadacyline (2 × 10 mg/kg i.v.) was associated with better survival (80%) than imipenem (70%) and linezolid (30%) (20).

Amadacycline also demonstrated good activity in murine models of pneumococcal pneumonia. At doses of 1, 3 and 10 mg/kg i.v. b.i.d. both amadacycline and vancomycin provided 100% survival compared to 0%, 40% and 80% survival, respectively, for the same doses of linezolid. Mice administered a dose of 1 mg/kg amadacycline experienced a reduction of > 6 log cfu. No reduction in cfu was observed in mice treated with the same doses of linezolid (21).

PHARMACOKINETICS AND METABOLISM

The protein binding of amadacycline in mouse, rat and monkey plasma was assessed using an equilibrium dialysis-based method. Compared with doxycycline and minocycline, amadacycline was found to be substantially unbound in plasma. To evaluate the metabolic stability of amadacycline to cytochrome P-450 enzymes, the antibiotic was incubated with liver microsomes derived from mice, rats and monkeys for up to 60 min. In the presence of liver microsomes derived from all three species, amadacycline appeared to be metabolically stable, with no detectable loss of the compound for more than 60 min (22).

The pharmacokinetics of i.v. amadacycline were evaluated in rats (5-40 mg/kg), monkeys (25 mg/kg) and mice (10 mg/kg). Amadacycline plasma levels exhibited biexponential decline, with a rapid distribution phase and a prolonged elimination phase. In rats, $AUC_{0\text{-}24}$ increased linearly with dose. At similar doses of amadacyline mice and rats showed similar $AUC_{0\text{-}24}$ values. In rats, the kidney concentrations of amadacycline were over 6-fold higher than the corresponding plasma concentrations, and the lung concentrations were 10- to 20-fold higher than the corresponding plasma concentrations. Time for plasma concentrations above 1 mg/ml was 15 min in mice, 40 min to > 9 h in rats and at least 24 h in monkeys. The plasma $t_{1/2}$ of amadacycline was 3.5, 6.5 and 10-11 h, respectively, in mice, rats and monkeys (23).

The pharmacokinetics of single s.c. doses of amadacycline (0.5-10 mg/kg) were also evaluated in neutropenic mice with pneumonia. C_{max} and $AUC_{0\text{-}24}$ increased in a dose-dependent manner. The concentrations of amadacycline in lung tissue were higher than those in epithelial lining fluid (24).

CLINICAL STUDIES

The safety and efficacy of amadacycline for the treatment of cSSSI were evaluated in a randomized, investigator-blinded phase II clinical trial. Patients were randomized to receive either amadacycline (n = 111; 100 mg i.v. once daily initially, followed by 200 mg p.o. once daily) or linezolid (n = 108; 600 mg i.v. b.i.d. initially, followed by 600

mg p.o. b.i.d.). Amadacycline showed comparable efficacy to linezolid. In the intent-to-treat population, the clinical success rates for amadacycline and linezolid were 88.3% and 75.9%, respectively, in the clinically evaluable population, the clinical success rates were 98% and 93.2%, respectively, and in the microbiologically evaluable patients, 2 failures were observed in the amadacycline group and 4 failures in the linezolid group. None of the failures in the amadacycline group were associated with MRSA, whereas 2 failures in the linezolid group were associated with MRSA. Amadacycline and linezolid showed similar clinical efficacy for MSSA. In both treatment groups, the incidence and pattern of adverse events (AEs) were similar. The most common drug-related AEs were gastrointestinal (12 on amadacycline and 13 on linezolid). Mild gastrointestinal events associated with amadacycline were observed almost entirely during oral therapy. No discontinuations due to AEs were reported in the amadacycline group, whereas 2 patients discontinued due to AEs in the linezolid group. No drug-related serious AEs were reported in either group. Similar hematology or serum chemistry parameters were observed in both treatment groups (9).

SOURCE

Paratek Pharmaceuticals, Inc. (US).

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