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Ataluren

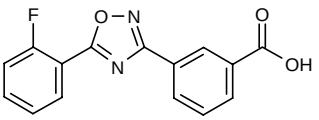
USAN

Nonsense Mutation Suppressor
Treatment of Cystic Fibrosis
Treatment of Muscular Dystrophy

PTC-124

3-[5-(2-Fluorophenyl)-1,2,4-oxadiazol-3-yl]benzoic acid

InChI=1/C15H9FN2O3/c16-12-7-2-1-6-11(12)14-17-13(18-21-14)9-4-3-5-10(8-9)15(19)20/h1-8H,(H,19,20)



$C_{15}H_9FN_2O_3$
Mol wt: 284.242

CAS: 775304-57-9

CAS: 775304-59-1 (sodium salt)

EN: 387029

Abstract

Genetic disorders such as cystic fibrosis (CF) and Duchenne muscular dystrophy (DMD) can be caused by nonsense mutations, or single point mutations that yield a premature stop codon and result in a truncated, often malfunctioning protein. Ataluren (PTC-124) is a small molecule from PTC Therapeutics designed to selectively induce readthrough of, or ignore, premature stop codons to increase the expression of proteins affected by nonsense mutations in CF and DMD, *i.e.*, cystic fibrosis transmembrane conductance regulator (CFTR) and dystrophin, respectively. Phase I and II clinical studies to date have shown that ataluren is well tolerated upon oral administration as a liquid suspension, has a good safety profile and demonstrates efficacy in DMD patients via elevated dystrophin expression. Further phase II studies are currently ongoing in CF and DMD patients.

Synthesis

Ataluren can be synthesized as follows: 3-Cyanobenzoic acid (Ia) is condensed with NH_2OH in *t*-BuOH to yield oxime (IIa). Without isolation, compound (IIa) couples with 2-fluorobenzoyl chloride (III) in the presence of triethylamine to give oxime ester (IV), which is readily cyclized under reflux conditions (about 80 °C) (1).

Ataluren can also be obtained from its methyl ester precursor (V) by saponification with NaOH in *t*-BuOH/H_2O (1) or THF/H_2O (2) and subsequent acidification with H_2SO_4 (1) or HCl (2). Ataluren methyl ester (V) can be synthesized from methyl 3-cyanobenzoate (Ib) by subjecting it to either the same one-pot procedure as described above for 3-cyanobenzoic acid (Ia) (1) or, alternatively, to a similar non-one-pot procedure: condensation of (Ib) with NH_2OH in EtOH at 100 ºC, coupling of the resulting oxime (IIb) with 2-fluorobenzoyl chloride (III) by means of DIEA in THF, and final cyclization of the isolated oxime ester (IVb) in toluene at 130 ºC (2). Methyl 3-cyanobenzoate (Ib) is prepared from the carboxylic acid (Ia) by methylation with CH_3I in the presence of K_2CO_3 (2). Scheme 1.

Background

Several inherited diseases are caused by “nonsense” mutations – mutations in single nucleotides that result in premature stop codons in the transcribed messenger RNA (UAA, UAG and UGA) and lead to premature translational termination and a truncated, nonfunctional protein product. Therapeutic intervention for genetic disorders linked to nonsense mutations, such as cystic fibrosis (CF) and Duchenne muscular dystrophy (DMD), therefore aims to suppress premature termination of protein synthesis by ignoring or “reading through” premature stop codons (3). Studies have shown that bypassing premature stop codons and boosting specific protein synthesis to as little as 5% of normal levels can greatly reduce the severity of or eliminate the principle manifestations of CF (4). The antibiotic gentamicin has been shown to promote

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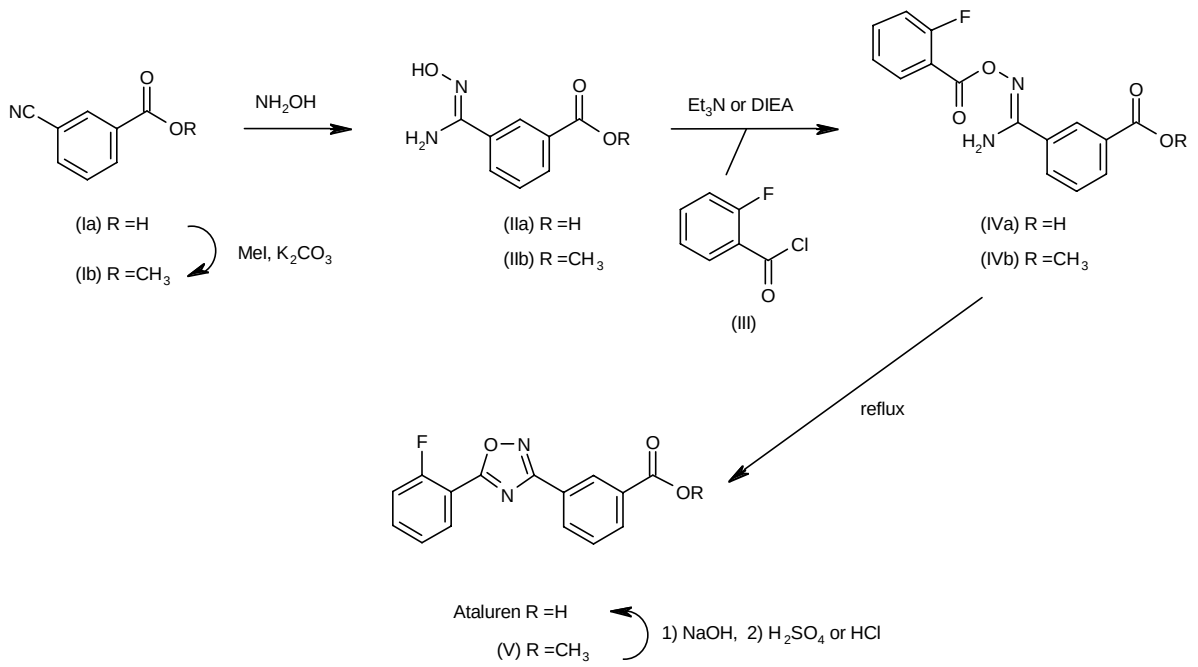
Scheme 1: Synthesis of Ataluren



readthrough of premature stop codons to generate the synthesis of full-length proteins; however, its therapeutic use is limited by poor efficacy and a severe adverse event profile (5).

Nonsense mutations in the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) cause up to 15% of cases of CF, which affects the exocrine (mucus) glands of the lungs, liver, pancreas and intestine, causing progressive disability due to multisystem failure. Seven percent of cases of DMD, characterized by rapidly worsening muscle weakness that starts in the legs and pelvis and later affects the whole body, result from nonsense mutations in the gene for dystrophin (a protein in the muscles) (6).

Ataluren (PTC-124) has emerged as a promising candidate for inducing readthrough of nonsense mutations and has progressed to clinical studies for the treatment of CF and DMD. Two phase II trials are currently evaluating whether the compound can safely increase functional CFTR protein levels in the cells of patients with CF due to nonsense mutations: one is ongoing in the United States (7) and another is active in Israel (8). The effect of ataluren on CFTR levels is also the focus of another study currently recruiting in pediatric CF patients (9). A phase II trial in 38 patients is ongoing to evaluate the safety and efficacy of ataluren in DMD (10; see below) and an extension of this study will evaluate the long-term safety of ataluren, as well as changes in walking, muscle, function, strength and other important clinical and laboratory measures (11). Finally, a larger IIb/III study is recruiting patients in the U.S., Israel and Europe with DMD/Becker muscular dystrophy to determine the clinical benefit of the agent (12).

Preclinical Pharmacology

In vitro studies have confirmed that ataluren suppresses nonsense mutations in HEK 293 cells transfected with a luciferase gene harboring a premature stop codon at Thr190. Suppression was seen at approximately 2 h, with maximal activity at approximately 20 h and loss of activity within 6 h after ataluren removal. Ataluren activity was dependent on the type of stop codon and drug concentration (maximum activity at the UAG codon [approximately 12-fold suppression] and maximal activity at 1.0 μM *versus* > 100 μM for gentamicin). Further investigations also confirmed that ataluren activity is limited to nonsense mutations, without activity at normal stop codons, and additional evidence confirmed a lack of antimicrobial activity against six strains of Gram-positive and Gram-negative bacteria at concentrations used for suppression of nonsense mutations (13, 14). Ataluren has also been shown to elicit its activity via the induction of changes in the chemical footprinting pattern on conserved sites of the large ribosomal RNA (rRNA) that modulate nonsense suppression activity in prokaryotic systems (distinct from the small rRNA sites bound by aminoglycosides) (14).

Studies in a transgenic mouse model of the *CFTR-G542X* mutation demonstrated that ataluren given s.c. once daily over 2-3 weeks was capable of restoring CFTR

expression and function to a level comparable to gentamicin. Suppression of the *CFTR* mutation was also evident following oral administration of ataluren (15).

In *Cftr*$^{-/-}$ mice with intestinal tissues harboring a human *CFTR* transgene under the control of the fatty acid-binding protein promoter and coding for a UGA stop codon (replacing glycine) at position 542 of *CFTR*, ataluren (15-60 mg/kg/day s.c. for 14-21 days) dose-dependently restored hCFTR protein in duodenal submucosal apical epithelium (16).

The efficacy of ataluren was also evaluated in the *mdx* model of DMD (mice with a nonsense mutation in the *dystrophin* gene). Ataluren (1.8 mg/ml mixed with food and given over a period of 8 weeks) partially improved extensor digitorum longus (EDL) specific force, partially prevented EDL eccentric contraction injury and reduced serum creatine kinase. Western blot analyses indicated that ataluren did not induce changes in ribosomal readthrough of normal stop codons (17, 18).

Pharmacokinetics and Metabolism

Phase I studies consisted of single- (N = 18) and multiple-dose (N = 30), randomized, double-blind, placebo-controlled investigations in healthy young adult volunteers. Time to peak plasma levels following single doses of 3-200 mg/kg as an oral suspension was about 1-3 h, with a mean half-life of about 3-6 h. Increases in C_{max} and AUC_{0-24} were somewhat greater than dose-proportional at 3-150 mg/kg, but less than dose-proportional at higher doses. Plasma levels exceeded those associated with activity in preclinical models. Pharmacokinetics were not affected by gender. Absorption was slower (t_{max} about 0.9-2.5 h) following ingestion of a high-fat, high-calorie meal before 50 mg/kg ataluren, while $t_{1/2}$ was not significantly affected. Multiple-dose administration (10-50 mg/kg b.i.d. for 7-14 days) gave mean t_{max} and $t_{1/2}$ values of about 2-4 and 2-6 h, respectively, and C_{max} and AUC_{0-12} increased proportionally to dose. Exposure was similar on days 1, 7 and 14 and no significant gender-related differences were observed (19-21).

Interim analyses of a phase II study to investigate the safety, compliance, pharmacokinetics and pharmacological activity of ataluren in 26 patients with DMD (aged 5-13 years) due to nonsense mutations administered doses of 16-40 mg/kg/day p.o. for 28 days were also presented. Blood samples revealed stable drug exposure over the 28 days of treatment; however, levels did not match those seen in the phase I pharmacokinetic studies conducted in healthy adults (10, 22-24).

Safety

Toxicological analyses demonstrated that the drug was well tolerated in rats and dogs at doses at or above 1500 mg/kg/day p.o. for 1, 7 or 28 days, with no evidence of histopathological, neurological, respiratory or cardiovascular abnormalities, and no mutagenicity, genotoxicity or clastogenic activity (16).

In the phase I studies, it was shown that oral administration of ataluren was well tolerated up to 100 mg/kg, while at 150 and 200 mg/kg the drug was associated with mild headache, dizziness and gastrointestinal adverse events. Multiple-dose studies demonstrated that doses up to 50 mg/kg b.i.d. caused reversible transaminase elevations. No elongation of proteins was seen in peripheral blood mononuclear extracts due to nonspecific ribosomal readthrough of normal stop codons (19-21). It was also well tolerated in the phase II study, with few drug-related adverse events (mild transient abdominal pain/diarrhea in 4 subjects) or laboratory abnormalities, and compliance was high (98%) (10, 22-24).

Clinical Studies

In the phase II study in patients with DMD, ataluren-induced dystrophin expression was confirmed in biopsy-derived cultured myocytes and further characterized qualitatively to show an increase in extensor digitorum brevis muscle biopsies. Serum muscle enzymes also declined significantly during treatment. Twelve additional patients were subsequently evaluated at the higher dose level of 80 mg/kg/day (10, 22-24).

Source

PTC Therapeutics.

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