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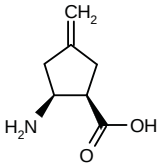
CCC: 0377-8282/2002

PLD-118

Antifungal
Isoleucyl-tRNA Synthetase Inhibitor

BAY-10-8888

(–)-(1*R*,2*S*)-2-Amino-4-methylenecyclopentane-1-carboxylic acid



$C_7H1_1NO_2$

Mol wt: 141.1689

CAS: 198022-65-0

CAS: 156292-47-6 (as hydrochloride)

EN: 268135

Abstract

The incidence of nosocomial fungal infections, a life-threatening problem for immunocompromised individuals, has increased over the past 15 years. *Candida* spp. are particularly common in intensive care units, with *Candida albicans* the most frequently isolated strain. Although numerous antifungal agents are currently available, they are frequently associated with adverse events, resistance development and/or poor bioavailability. Thus, the search for novel antifungals displaying increased efficacy and tolerability is ongoing. Cyclic β-amino acids are one class of potent antifungals that have a dual mode of action. These compounds inhibit protein synthesis after concentrative uptake and interfere with self-regulatory mechanisms of amino acid metabolism. One novel agent of this class is PLD-118 (Bay-10-888) which competitively inhibits isoleucyl-tRNA synthetase after concentrative uptake. PLD-118 disrupts fungal protein biosynthesis and cell growth. Because of its potent specific activity observed against *Candida* spp. including azole-resistant strains *in vitro* and *in vivo*, in addition to its excellent pharmacokinetics, solubility and safety, PLD-118 was chosen for further development for systemic treatment of *Candida* infections.

Synthesis

PLD-18 can be obtained by three related ways:

1) Basic hydrolysis of *cis*-4-methylenecyclopentane-1,2-dicarboxylic acid diethyl ester (I) with LiOH gives the free acid (II), which by refluxing with propionic anhydride yields the cyclic anhydride (III). Rearrangement using trimethylsilyl azide in dioxane at 80 °C produces the oxazinedione (IV), which is hydrolyzed to (±)-*cis*-2-amino-4-methylenecyclopentane-1-carboxylic acid ethyl ester (V) by means of acetyl chloride in EtOH. Further hydrolysis of ester (V) with aqueous HCl provides the racemic amino acid (VI), which is coupled with *N*-(9-fluorenylmethoxycarbonyl)succinimide (VII) to afford the Fmoc-amino acid (VIII). Racemic (VIII) is resolved by treatment with (+)-(*R*)-1-phenylethylamine (IX) and crystallization of the resulting salt (X) in EtOH/methyl *tert*-butyl ether. Finally, acidification of salt (X) and extraction of the (1*R*,2*S*)-free acid, followed by removal of the Fmoc group with liquid ammonia, provides PLD-118 isolated as its hydrochloride salt (1). Scheme 1.

2) Hydrolysis of diester (I) with KOH in water/EtOH at 55 °C gives the dicarboxylic acid (II), which is subjected to cyclization by refluxing with propionic anhydride to provide anhydride (III). Cyclic anhydride (III) is opened with allyl alcohol (XI) in diethyl ether in the presence of (–)-quinine as asymmetric inducer to yield (–)-1,2-*cis*-4-methylenecyclopentane-1,2-dicarboxylic acid monoallyl ester (–)-(XII), which is then converted into the amide derivative (–)-(XIII) by activation of the carboxylic acid group with isobutyl chloroformate in ethyl acetate in the presence of *N*-ethylmorpholine at –6 °C, followed by reaction with aqueous ammonia. Removal of the allyl group of (–)-(XIII) with triphenylphosphine, tetrakis(triphenylphosphine)palladium and 2-ethylhexanoic acid sodium salt in ethyl acetate affords (–)-1,2-*cis*-2-(aminocarbonyl)-4-methylenecyclopentane-1-carboxylic acid sodium salt (–)-(XIV). Compound (–)-(XIV) is subjected to a Hofmann rearrangement with KOH and KOCl in water to provide a crude aqueous solution containing PLD-118, which is finally purified by protection of the free amine group with

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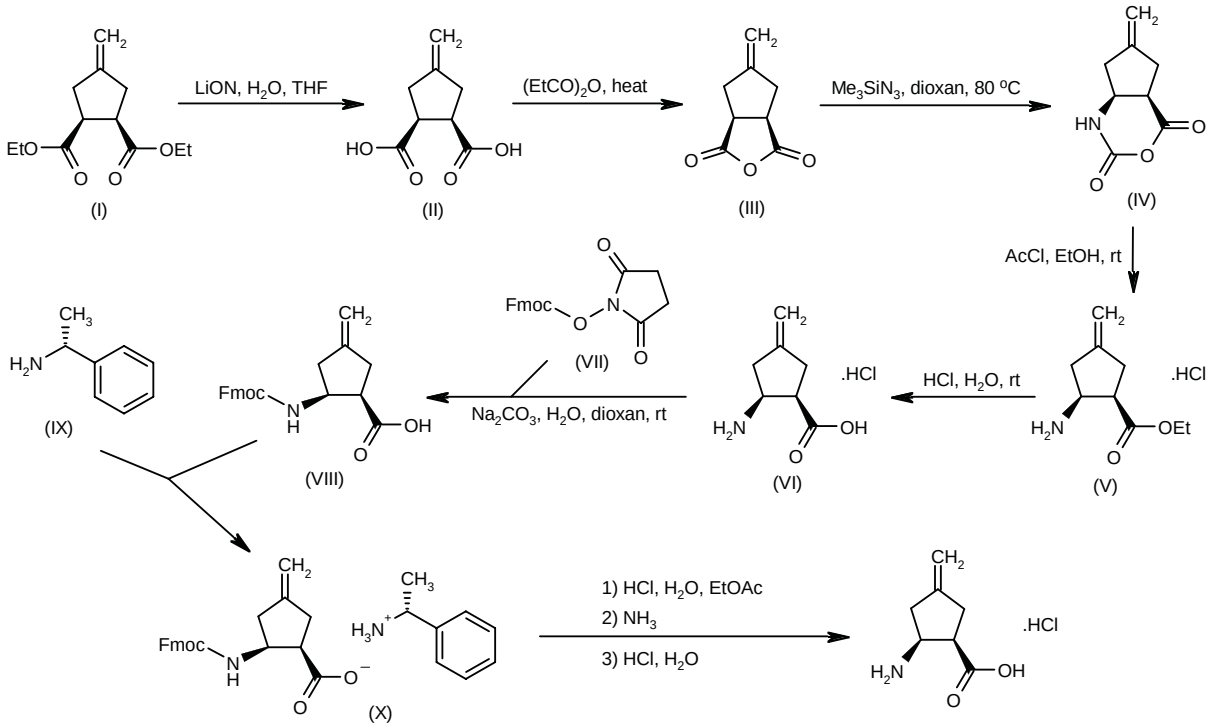
Scheme 1: Synthesis of PLD-118



N-(9-fluorenylmethoxycarbonyloxy)succinimide by means of Na_2CO_3 in dioxane followed by Fmoc removal with piperidine in diethyl ether (2). Scheme 2.

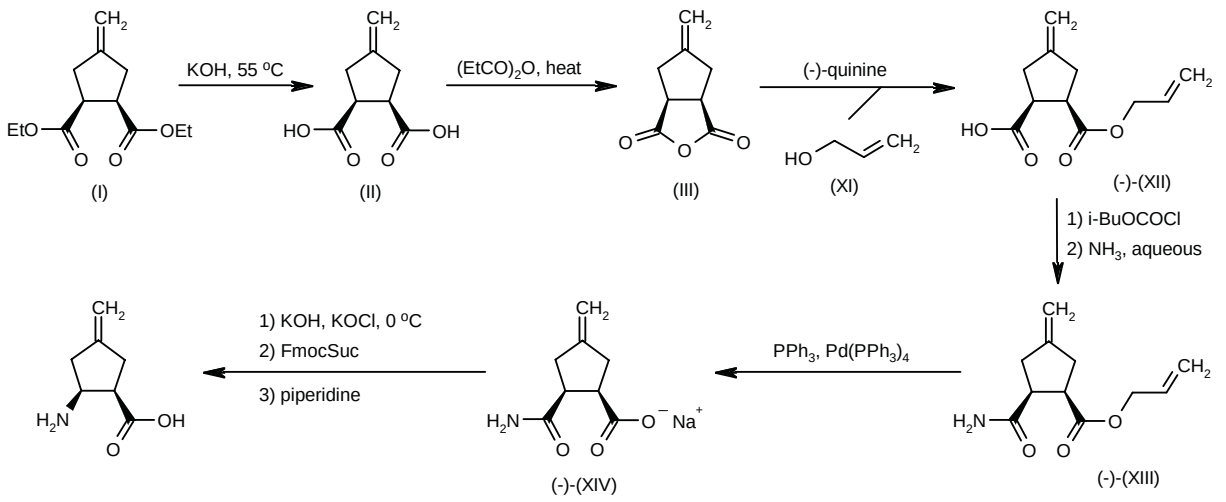
3) Cyclization of the tetracarboxylic acid (XV) gives 4-oxocyclopentane-1,2-dicarboxylic acid (XVI), which is esterified with ethanol and sulfuric acid to yield the diester (XVII). Reaction of diester (XVII) with methyl-(triphenyl)phosphonium bromide and *t*-BuOK in THF affords 4-methylenecyclopentane-1,2-dicarboxylic acid diethyl ester (XVIII), which is hydrolyzed with KOH in THF/water to provide the corresponding free acid (XIX). The anhydrization of (XIX) by means of propionic anhydride at 135 °C gives anhydride (III), which is submitted to an enantioselective quinine-mediated alcoholysis with 3-phenyl-2-propen-1-ol (XX) to yield (1*R*,2*S*)-2-amino-4-methylenecyclopentane-1-carboxylic acid 3-phenyl-2-propenyl monoester (XXI). Degradation of the free carboxylic acid group of (XXI) by means of DPPA, TEA and alcohol (XX) in hot toluene affords carbamate (XXII), which is finally fully deprotected with $Pd(OAc)_2$ and PPh_3 in ethanol (3, 4). Scheme 3.

Introduction

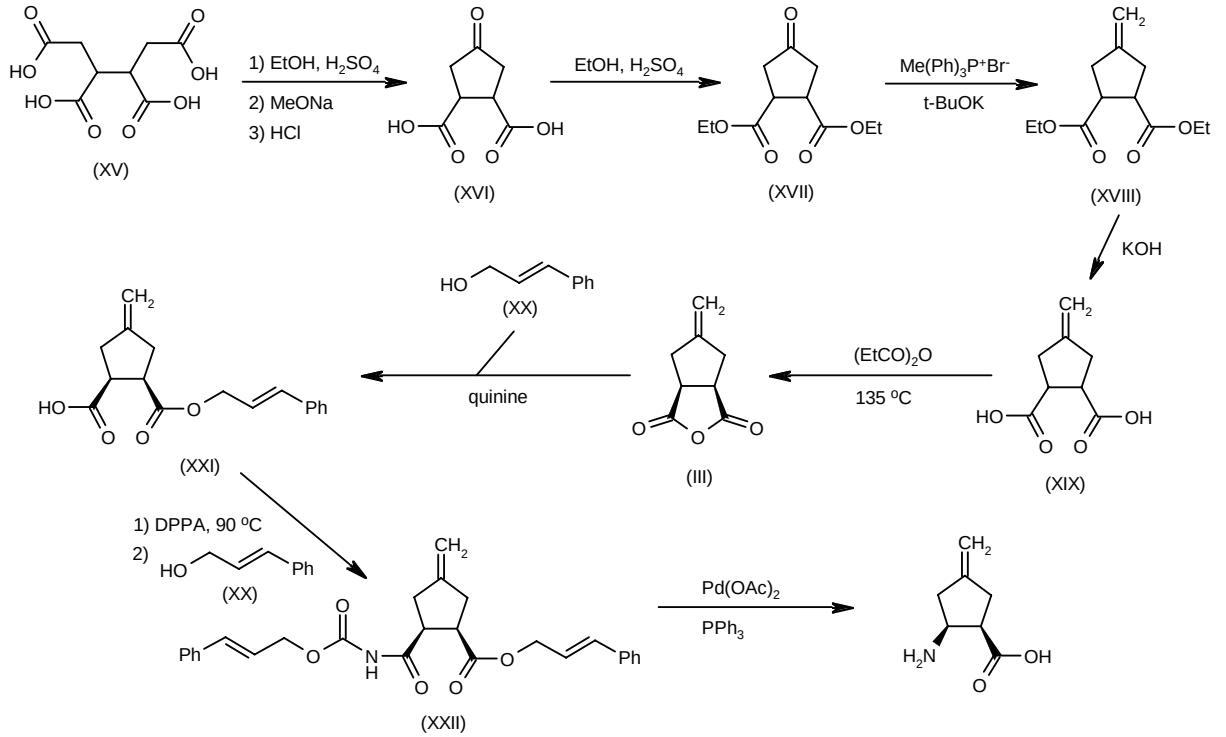
Systemic mycosis poses an extreme, life-threatening danger for immunocompromised AIDS, cancer and chemotherapy patients, patients in intensive care units and recipients of organ transplants, and the incidence of nosocomial fungal infections has increased over the past 15 years. *Candida* spp. in particular are commonly found in intensive care units, with *Candida albicans* being the most frequently isolated strain. An increase has also been observed in the incidence of non-albicans infections (5-9). Although numerous antifungal agents are currently available, they are frequently associated with adverse events, resistance development and/or poor bioavailability (10, 11). Thus, the search for novel antifungals displaying increased efficacy and tolerability is ongoing.

Inhibition of translation which involves initiation, elongation and termination phases, is an attractive antifungal target and several antifungal agents with protein biosynthesis inhibitory effects are actively being developed (Fig. 1). Cyclic β-amino acids are one class of potent antifungals that appear to a have a dual mode of action: inhibition of protein synthesis after concentrative uptake and interference with self-regulatory mechanisms of amino acid metabolism (12, 13). Cispentacin is a naturally occurring cyclic β-amino acid with potent *in vitro* and *in vivo* anti-*Candida* activity including inhibitory activity against *C. albicans*, *Candida krusei* and *Candida tropicalis* (MIC range for *Candida* spp. = 8-50 μg/ml); the agent is inactive against *Aspergillus* spp. Following concentrative uptake, cispentacin was found to inhibit prolyl-tRNA

Scheme 2: Synthesis of PLD-118



Scheme 3: Synthesis of PLD-118



synthetase (14-18). Discovery of this compound led to derivatization programs with the goal of identifying novel cyclic β-amino acid derivatives possessing improved efficacy and tolerability. One novel derivative identified was PLD-118 (Bay-10-8888). PLD-118 competitively inhibits isoleucyl-tRNA synthetase after concentrative uptake resulting in suppression of fungal translation. The agent serves as an artificial substrate with a low affinity for

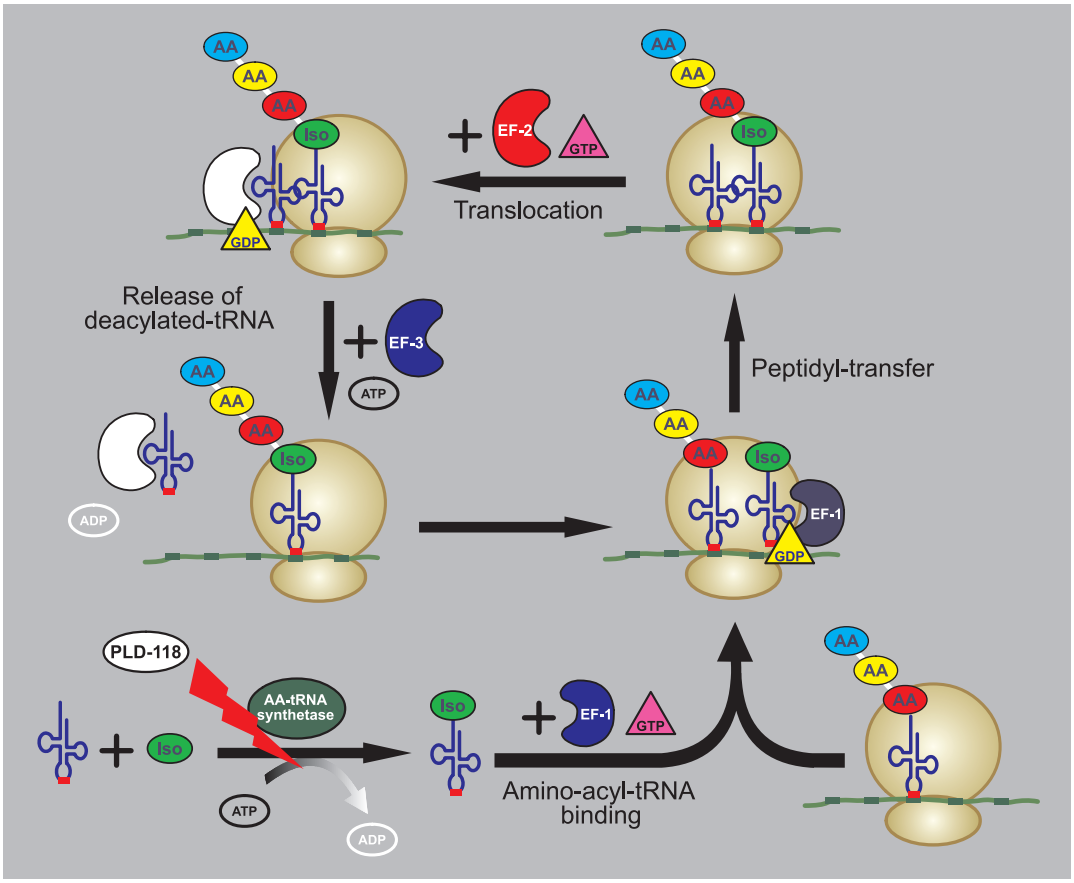


Fig. 1. Inhibition of translation as a target for developing antifungal compounds.

isoleucyl-tRNA its carrier. It is actively accumulated by a H^+-coupled symporter specific for branch-chain amino acids (isoleucine, leucine and valine). PLD-118 disrupts fungal protein biosynthesis and cell growth. Because of its potent specific activity (the agent is inactive against *Aspergillus* spp.) observed against *Candida* spp. including azole-resistant strains *in vitro* (MIC range for *Candida* spp. ≤ 0.25-> 32 μg/ml) and *in vivo*, in addition to its excellent pharmacokinetics, solubility and safety, PLD-118 was chosen for further development for systemic treatment of *Candida* infections (4, 13, 19).

Pharmacological Actions

PLD-118 (500 μM) was shown to have no hydrophobic interactions with boiled *C. albicans*. The agent was actively transported into *C. albicans* cells by an H^+-coupled amino acid transporter with specificity for branched-chain amino acids (isoleucine, leucine and valine); transport was unidirectional and efflux was not carrier-mediated but via diffusion. The Michaelis constant of the transport reaction (KT) and the V_{max} obtained were 0.96 mM and 18.9 mmol/min x 10^7 cells, respectively. PLD-118 was accumulated 100- to 200-fold. Once inside the cell, PLD-118 (50 μM resulting in intracellular concentrations of 10 and 6.5 mM at 1 and 2 h, respectively) competitively inhibited isoleucyl-tRNA synthetase which subsequently resulted in inhibition of protein synthesis and growth. The growth inhibitory effects of the agent were reversed by intracellular L-isoleucine. PLD-118 inhibiteed isoleucyl in a concentration-dependent manner, with a K_i of 1 mM obtained, suggesting that the interaction of the agent with its substrate is not very specific (13, 20).

Further analysis of the mechanism of action of PLD-118 revealed that accumulation of the agent into selected *C. albicans* mutants was reduced while isoleucyl-tRNA synthetase activity was increased. Similarly, although naturally resistant *C. tropicalis* strains displayed concentrations of PLD-118 in the millimolar range after treatment, isoleucyl-tRNA synthetase activity was 4-fold higher as compared to *C. albicans* (21).

An *in vitro* study using more than 90 clinical isolates of *C. albicans* reported MIC values for PLD-118 ranging from 4-32 mg/l. A defined medium (YNG: yeast nitrogen base, glucose, pH 7.0) and an inoculum of 50-100 cfu/microtiter well (100 μl total volume) incubated for 24 h at 30 °C, was required for optimum activity of the agent due to its mode of action (*i.e.*, active transport into yeast and inhibition of protein synthesis) (22).

The *in vitro* activity of PLD-118 was compared with amphotericin B and fluconazole against *Candida* non-*albicans* spp. (*C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. guilliermondii*) in a study using the microtiter plate dilution assay described in the above study. PLD-118 was the most active compound against *C. glabrata* and *C. krusei* isolates (MIC = 0.25-8 μg/ml after 24-48 h) (23).

The antifungal efficacy of PLD-118 (5 and 10 mg/kg b.i.d. p.o. or 2 and 4 mg/kg b.i.d. p.o. for 3 days in mice and rats, respectively, starting 30 min postinfection) against *C. albicans* infection was demonstrated *in vivo* in mice and rats. Animals were experimentally infected with fluconazole-susceptible (MIC < 4 mg/l) or fluconazole-resistant (MIC > 64 mg/l) clinical *C. albicans* isolates. Survival rates (up to 7 days) in mice and rats were 50-100 and 80-100%, respectively, as compared to the 100% mortality rate seen in untreated controls. PLD-118 was effective at a dose of 10 mg/kg b.i.d. in mice infected with fluconazole-resistant strains. Fluconazole only had antifungal effects in animals infected with fluconazole-susceptible strains of *C. albicans* (24).

An *in vivo* study in mice demonstrated the efficacy of oral PLD-118 (5 and 10 mg/kg b.i.d. for 4 days starting 30 min postinfection) against systemic infection with clinical isolates of several *Candida* spp. Survival rates (up to 10 days) for animals with lethal systemic *C. albicans* infections and treated with PLD-118 were 80 and 100% for the respective doses; unlike fluconazole, the agent was effective against both fluconazole-susceptible and -resistant strains. Infections with non-*albicans* species including *C. glabrata* and *C. krusei* were not lethal and resulting kidney infections spontaneously resolved within 2 days. However, a single dose of PLD-118 given 30 min postinfection significantly reduced kidney cfu as compared to untreated controls (25).

PLD-118 (4, 10 and 25 mg/kg/day b.i.d i.v. for 7 days) was also effective *in vivo* in immunocompromised rabbits with experimental fluconazole-resistant esophageal candidiasis (*C. albicans* clinical isolates from patients with refractory esophageal candidiasis). A dose-dependent clearance in yeast from the tongue, oropharynx and esophagus was observed with PLD-118 treatment as compared to untreated controls. No significant effects were observed in animals treated with fluconazole (1 mg/kg/day b.i.d. i.v. for 7 days) (26).

Pharmacokinetics

An *in vitro* study using pooled liver microsomes and the cytochrome P450 (CYP) substrates 7-ethoxyresorufin, coumarin, 7-ethoxy-4-(trifluoromethyl)coumarin, diclofenac (*S*)-mephenytoin, bufuralol, chloroxazone and testosterone directed against human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A, respectively, showed that PLD-118 did not inhibit any of the enzymes tested. Enzyme activities relative to controls after incubation with PLD-118 were 99, 96, 94, 104, 99, 97, 101 and 103%, respectively. It was concluded that PLD-118 has a very low propensity to interact with drugs metabolized by these enzymes (27).

The pharmacokinetics of PLD-118 (1.26-126 mg/kg) were examined in mice (p.o. and s.c.) and rats, rabbits and dogs (p.o. and i.v.). The distribution of [^{14}C]-PLD-118 was also examined in rats (10 mg/kg p.o. or i.v.). Oral bioavailability was high in all species with a rate of 60% obtained for mice and 100% for rats, rabbits and dogs. Dose-proportional increases in plasma concentrations ranging from 1-100 mg/kg were observed in rats and dog. Plasma half-life values were 3 and 10 h in rats and dogs, respectively. Plasma protein binding was negligible (< 1%) and gender did not affect the pharmacokinetics of the agent. PLD-118 was distributed homogeneously to all tissues. Elimination was slow from the outer renal medulla, pancreas, seminal vesicles and eye lens; no affinity was observed for retinal melanin-bearing tissues. In rats and dogs, elimination of the unchanged compound was almost completely renal, with no major metabolites being detected (28).

The safety, tolerability and pharmacokinetics of oral PLD-118 were examined in 2 double-blind, randomized, placebo-controlled, phase I studies involving healthy male volunteers. The first study was a 3-way crossover study conducted in 18 subjects who were given 2 doses of PLD-118 (17.5, 35, 70, 140 and 280 mg after fasting or 70 mg after a meal) and 1 dose of placebo. The agent was well tolerated. In fasted subjects, peak plasma PLD-118 concentrations were achieved after about 1 h of dosing and were 0.5 and 8.5 mg/l with the 17.5 and 280 mg doses, respectively. Dose-proportional increases in C_{max} and AUC_{∞} values were observed. Elimination half-lives were 6-7 h with 70-90% of the unchanged compound found in urine within 72 h of dosing. Delayed absorption and reductions in peak plasma concentrations were observed in fed subjects A total of 16 mild to moderate adverse events were reported with only 8 concluded to be related to PLD-118. Headache was the most common side effect seen in both treatment and placebo groups. The agent had no significant effects on vital signs or EEG/laboratory parameters (29).

The second study was a sequentially ascending dose study involving a total of 48 healthy male subjects who received multiple oral doses of the agent (50, 100, 150 or 200 mg t.i.d or 150 or 300 mg b.i.d.) or placebo for 7 days; each dose was separated by a 72-h washout period. Two subjects receiving placebo and 1 subject receiving PLD-118 withdrew due to adverse events. The agent was well tolerated with dry mouth the most frequently reported adverse effects reported by 6 PLD-118-treated subjects. Abnormalities in EEG parameters were seen in 2 subjects on placebo and 2 PLD-118-treated subjects. No significant changes in vital signs or EEG/laboratory parameters were associated with the agent. Steady-state plasma levels were dose-proportional and were reached after 2-3 days of dosing. Mean C_{max} (μg/ml) values on the last day of dosing with 50 and 200 mg t.i.d. ranged from 2.4-8.6 μg/ml; the C_{max} after dosing with 300 mg b.i.d.

was 10.9 μg/ml. $T_{1/2}$ values for days 1 and 11 for all doses were approximately 7-8 h for all dosing schedules (30, 31).

Clinical Studies

PLD-118 is currently undergoing phase II trials in patients with candidiasis (32).

Source

Bayer AG (DE); licensed to Pliva d.d. (HR).

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