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BAL-4815/BAL-8557

Antifungal Agent

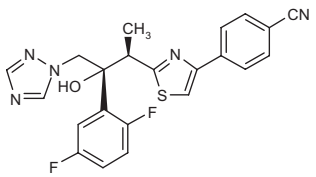
Abstract

As the number of immunocompromised individuals increases, fungi such as *Candida* spp. and *Aspergillus* spp. frequently cause severe infections. Some of these organisms are becoming resistant to commonly used drugs such as fluconazole. Research efforts are therefore focusing on the development of additional therapeutic agents to combat these fungal infections. One such agent is BAL-4815, a member of the triazole class of antifungal drugs. BAL-4815 and its water-soluble prodrug BAL-8557 are currently being tested for their antifungal activity against a number of microorganisms. The active drug BAL-4815 has shown good *in vitro* activity when compared to fluconazole, itraconazole and voriconazole, as well as several other antifungal agents. Based on the results of pharmacokinetic studies, initial intravenous infusion followed by oral administration of BAL-8557 is the dosing regimen suggested in order to rapidly achieve active concentrations of BAL-4815.

BAL-4815

RO-0094815

4-[2-[2(*R*)-(2,5-Difluorophenyl)-2-hydroxy-1(*R*)-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]thiazol-4-yl]benzonitrile



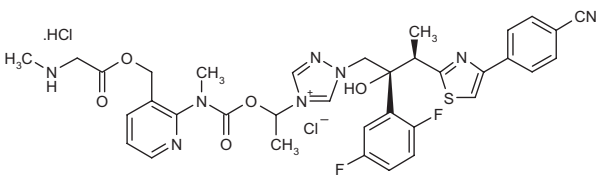
$C_{22}H_{17}F_2N_5OS$
Mol wt: 437.4663
CAS: 241479-67-4

EN: 281150

BAL-8557

RO-0098557

1-[3(*R*)-[4-(4-Cyanophenyl)thiazol-2-yl]-2(*R*)-(2,5-difluorophenyl)-2-hydroxybutyl]-4-[1-[*N*-methyl-*N*-[3-[2-(methylamino)acetoxymethyl]pyridin-2-yl]carbamoyloxy]ethyl]-1*H*-1,2,4-triazol-4-ium chloride, hydrochloride



$C_{35}H_{36}Cl_2F_2N_8O_5S$
Mol wt: 789.6795
CAS: 497235-79-7
CAS: 338990-84-4 (as dihydrochloride)

EN: 311257

Synthesis of BAL-4815

Addition of 2,5-difluorophenylmagnesium bromide (I) to the lactic acid amide derivative (II) in THF affords the propiophenone derivative (III), which by reaction with trimethylsulfoxonium iodide and either NaH in DMSO or BuLi in THF/DMPU provides oxirane (IV). Epoxide ring opening of compound (IV) with 1,2,4-triazole (V) by means of NaH yields the triazolyl alcohol (VI) as a mixture of diastereoisomers. Deprotection of intermediate (VI) in acidic media and purification of the corresponding diol mixture by column chromatography yields the desired (*R,R*)-diastereoisomer (VII), which by reaction with methanesulfonyl chloride in the presence of TEA in CH_2Cl_2 gives the mesylate (VIII). Epoxide formation of derivative (VIII) by means of NaOMe in MeOH affords intermediate (IX), which by reaction with either trimethylsilyl cyanide in xylene followed by desilylation with Bu_4NF in THF, or with acetone cyanohydrin and LiH in THF, provides the cyano derivative (X). Reaction of compound (X) with diethyl dithiophosphate in H_2O yields

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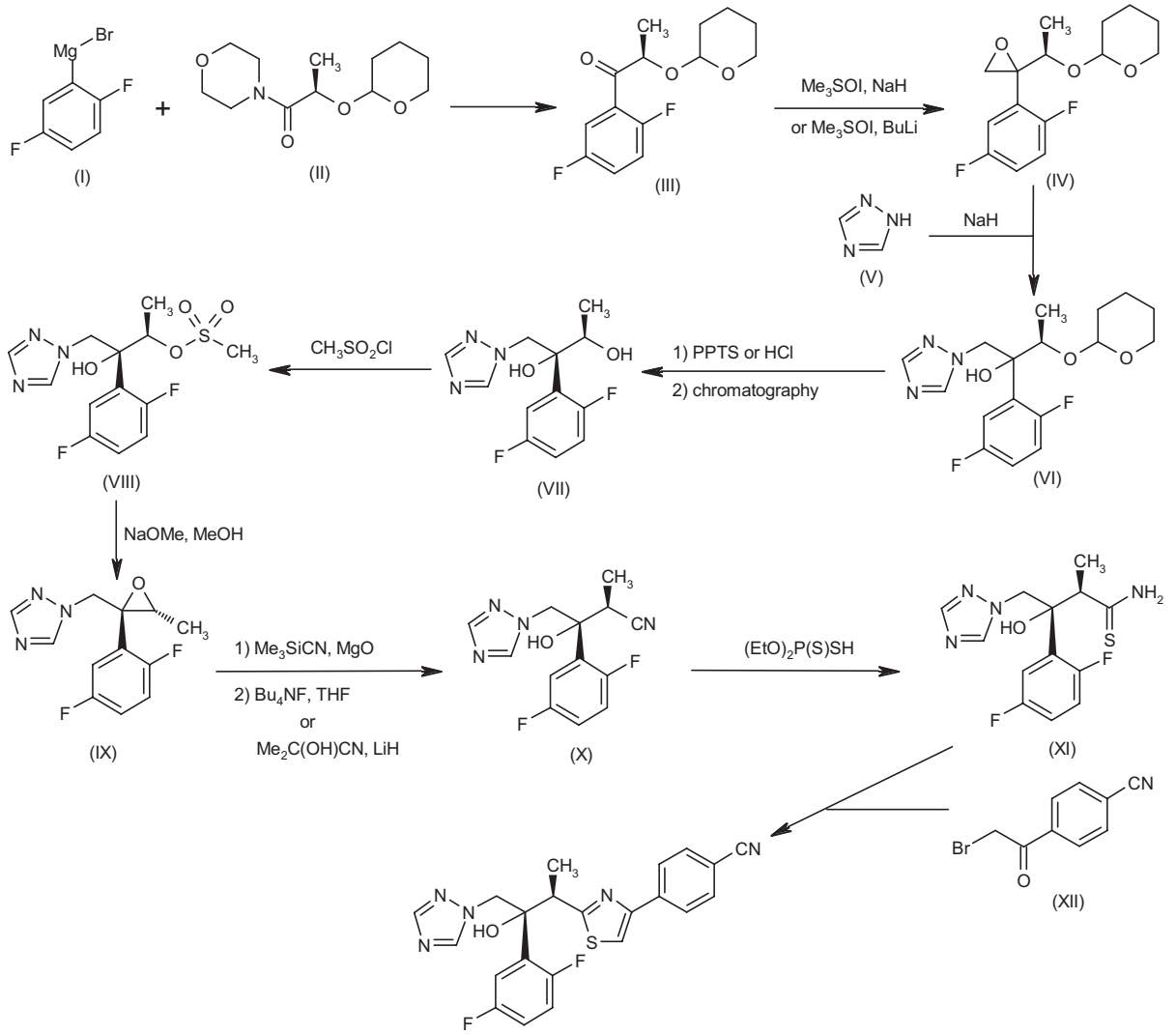
Scheme 1: Synthesis of BAL-4815



the thioamide (XI), which is finally cyclized with 4-cyanophenacyl bromide (XII) in refluxing EtOH (1). Scheme 1.

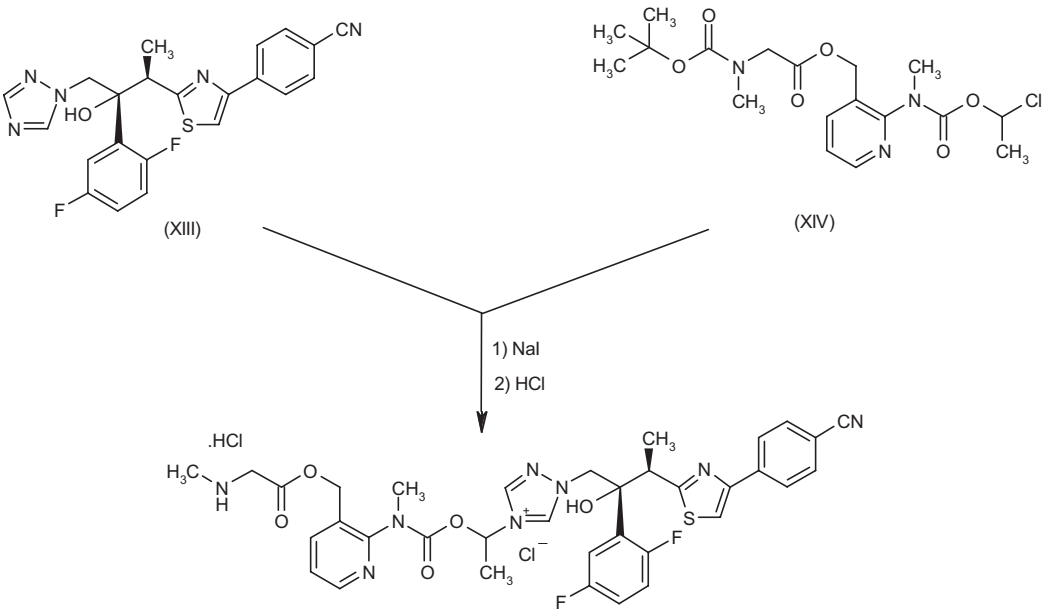
Synthesis of BAL-8557

BAL-8557 is prepared through a quaternization reaction of BAL-4815 (XIII) with the alkylating compound (XIV) in the presence of NaI in CH_3CN, followed by BOC deprotection in AcOEt and acidic media (2-6). Scheme 2.

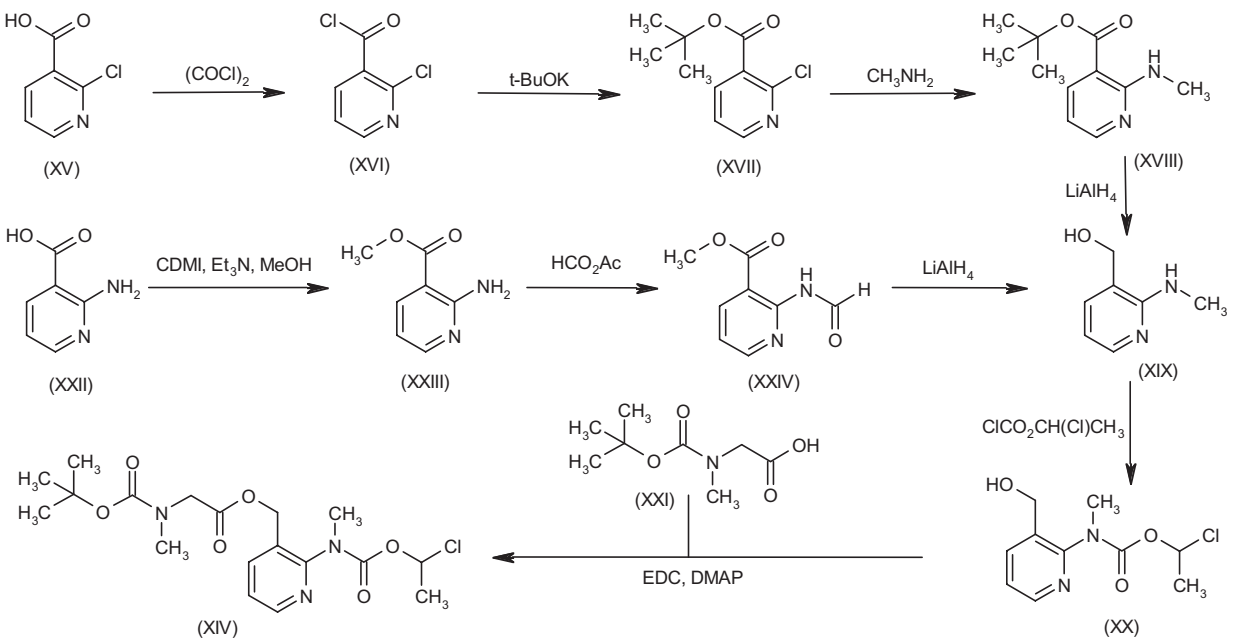
The chloroethyl carbamate (XIV) is prepared as follows:

Reaction of 2-chloronicotinic acid (XV) with oxalyl chloride in DMF/CH_2Cl_2 affords the corresponding acid chloride (XVI), which by treatment with potassium *tert*-butoxide in THF yields the *tert*-butyl ester (XVII). Displacement of the chloride group of intermediate (XVII) with $MeNH_2$ in MeOH renders the 2-(methylamino)nicotinic ester (XVIII), which after reduction by means of $LiAlH_4$ in THF provides the amino alcohol (XIX). Acylation of compound (XIX) with 1-chloroethyl chloroformate in the presence of DIEA in CH_2Cl_2 yields the carbamate derivative (XX), which is subsequently esterified with *N*-Boc-sarcosine (XXI) by means of EDC and DMAP in CH_2Cl_2 (2, 4-6). Alternatively, the amino alcohol (XIX) can be prepared by esterification of 2-aminonicotinic acid (XXII) by means of 2-chloro-1,3-dimethylimidazolinium chloride (CDMI) and TEA in MeOH to give intermediate (XXIII), which is formylated with formic-acetic mixed anhydride in THF to afford formamide (XXIV) and finally reduced with $LiAlH_4$ in THF (2, 6). Scheme 3.

Scheme 2: Synthesis of BAL-8557



Scheme 3: Synthesis of Intermediate (XIV)



Background

Candida species are ubiquitous in nature and are often associated commensally with humans. The infections caused by *Candida* spp., *i.e.*, candidiasis, can range from minor, self-limiting infection to serious, life-threatening disease. Due to the growing number of immunocompromised individuals in recent years, the frequency and severity of infections caused by *Candida* spp. have increased substantially. Candidiasis may affect many areas of the body, including the skin, nails, vagina, mouth, esophagus, blood or organs. *Candida albicans* is the most common pathogen of this genus, but other species such as *Candida glabrata, Candida tropicalis, Candida kefyr, Candida parapsilosis, Candida krusei* and *Candida guilliermondii* can also cause serious infections. Organisms resistant to fluconazole, an azole drug often used to treat candidiasis, are becoming increasingly frequent, especially in AIDS patients and in individuals who have received organ transplants. In addition to fluconazole, other compounds used to treat candidiasis include triazole antifungals such as itraconazole and voriconazole, echinocandins such as caspofungin, and amphotericin B (7).

Infections caused by the airborne saprophytic fungi belonging to *Aspergillus* spp., *i.e.*, aspergillosis, are also on the rise in immunocompromised individuals. Invasive aspergillosis is associated with high mortality rates. Amphotericin B is currently used to treat these infections, but toxicity remains high even with the newer lipid formulations (8).

BAL-8557 is a water-soluble triazolium salt-type prodrug with high bioavailability (intravenous and oral) that exhibits fast and quantitative bioconversion to the active metabolite BAL-4815 (Fig. 1). A member of the triazole class of antifungal agents, BAL-4815 has shown broad-spectrum *in vitro* activity against *C. albicans* and other *Candida* spp., including fluconazole-resistant strains, as well as against *Aspergillus* spp. and other fungal pathogens. BAL-8557 is being investigated for the oral and i.v. treatment of serious and life-threatening systemic fungal infections, with phase III trials set to begin this year (3-5, 9).

Preclinical Pharmacology

The *in vitro* activity of BAL-4815 was tested against 118 isolates of *Aspergillus* spp., including 16 isolates with resistance to either amphotericin B or itraconazole. MIC values were reported for BAL-4815, amphotericin B, caspofungin, itraconazole and voriconazole against *Aspergillus fumigatus* (n=62), *Aspergillus terreus* (n=18), *Aspergillus flavus* (n=20) and *Aspergillus niger* (n=18). The respective MIC_{50} and MIC_{90} values against *A. fumigatus* were 0.5 and 2.0 mg/l for BAL-4815, 0.25 and 0.5 mg/l for amphotericin B, 0.5 and 0.5 mg/l for caspofungin, 0.25 and > 8.0 mg/l for itraconazole, and 0.25 and 0.5 mg/l for voriconazole. Testing against *A. terreus* yielded respective MIC_{50} and MIC_{90} values of 0.5 and 0.5 mg/l for BAL-4815, 1.0 and 1.0 mg/l for amphotericin B, 0.5 and 0.5 mg/l for caspofungin, 0.125 and 0.25 mg/l for itraconazole, and 0.25 and 0.5 mg/l for voriconazole. The

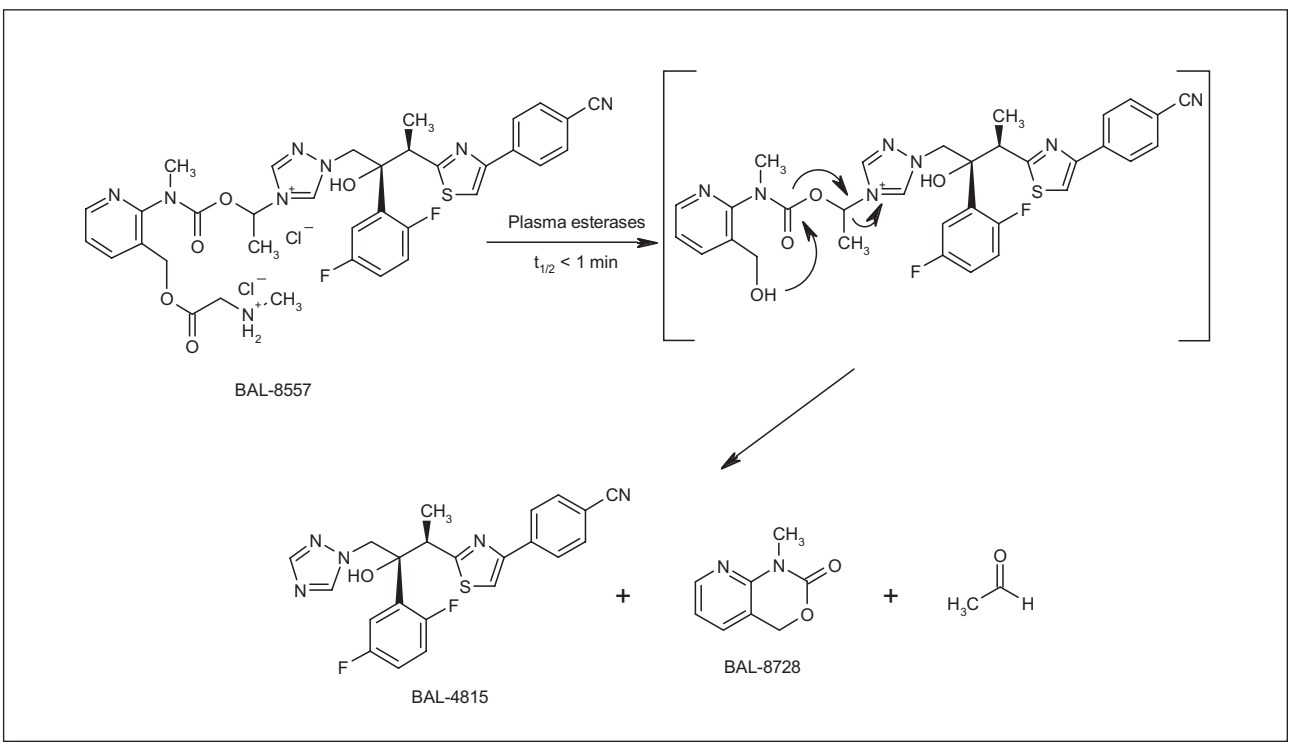


Fig. 1. Suggested pathway for the conversion of BAL-4815 in plasma.

respective MIC_{50} and MIC_{90} values against *A. flavus* were 0.5 and 1.0 mg/l for BAL-4815, 1.0 and 1.0 mg/l for amphotericin B, 0.25 and 0.25 mg/l for caspofungin, 0.125 and 0.5 mg/l for itraconazole and 0.5 and 0.5 mg/l for voriconazole. Against *A. niger*, the respective MIC_{50} and MIC_{90} values were 0.5 and 2.0 mg/l for BAL-4815, 0.5 and 0.5 mg/l for amphotericin B, 0.25 and 0.25 mg/l for caspofungin, 0.5 and 2.0 mg/l for itraconazole, and 0.25 and 1.0 mg/l for voriconazole. Overall against all 118 isolates tested, MIC_{50} values were 0.5 mg/l for BAL-4815 and amphotericin B and 0.25 mg/l for caspofungin, itraconazole and voriconazole. The overall MIC_{90} values against all isolates were 2.0 mg/l for BAL-4815, 1.0 mg/l for amphotericin B, 0.5 mg/l for caspofungin, > 8.0 mg/l for itraconazole and 1.0 mg/l for voriconazole. When the geometric mean minimum fungicidal concentrations (MFCs) against all isolates were calculated, the values obtained were 1.68 mg/l for BAL-4815, 1.78 mg/l for itraconazole, 1.09 mg/l for voriconazole and 0.98 mg/l for amphotericin B (10, 11).

The *in vitro* antifungal activity of BAL-4815 was tested against *Candida* isolates preselected for decreased susceptibility to fluconazole. Overall, the MIC_{50} values of BAL-4815 were generally lower than those of voriconazole. Against fluconazole-susceptible isolates of *C. albicans*, the MIC_{50} values were 0.004-0.008 and 0.008 μg/ml for BAL-4815 and voriconazole, respectively, as compared to 0.25-0.5 μg/ml for fluconazole. When fluconazole-resistant isolates of *C. albicans* were tested, the MIC_{50} values were 0.25 μg/ml for BAL-4815 and 0.125-0.25 μg/ml for voriconazole compared to 64 μg/ml for fluconazole. Against other *Candida* spp., BAL-4815, voriconazole and fluconazole showed the following respective MIC_{50} values: < 0.03-0.25, 0.125-1 and 2-16 μg/ml against *C. glabrata*; 0.25, 0.5 and 32-64 μg/ml against *C. krusei*; 0.015-0.03, 0.03-0.06 and 2 μg/ml against *C. parapsilosis*; 0.015-0.25, 0.03-0.06 and 1-2 μg/ml against *C. tropicalis*; 0.125, 0.06-0.125 and 4 μg/ml against *C. guilliermondii*; and < 0.001-0.03, 0.008-0.06 and 0.5 μg/ml against *C. lusitaniae* (12, 13).

Another study determined the MIC_{50} values for BAL-4815 and for several other antifungal drugs against isolates of the dermatophytes *Trichophyton rubrum* (n=9), *Trichophyton mentagrophytes* (n=19) and *Microsporum canis* (n=12). The overall MIC_{50} values for all strains tested were 1, 0.125, 16, 0.5, 0.5 and 0.5 mg/l for amphotericin B, terbinafine, fluconazole, itraconazole, voriconazole and BAL-4815, respectively. Against *T. rubrum*, the MIC_{50} values were 0.5, 4, 0.5, 0.25, 0.063 and 0.25 mg/l for amphotericin B, fluconazole, itraconazole, voriconazole, terbinafine and BAL-4815, respectively. The MIC_{50} values against *T. mentagrophytes* were 2, 32, 1, 0.5, 0.063 and 1 mg/l for the respective agents. Against *M. canis* isolates, MIC_{50} values were 1, 8, 0.5, 0.5, 0.125 and 0.25 mg/l, respectively (14).

The antifungal activity of BAL-4815 was also determined against dermatophytes and filamentous fungi that cause subcutaneous disease, pheohyphomycosis and opportunistic infections. Results showed that BAL-4815 had an MIC of 0.06-1.0 μg/ml against dermatophytes, 0.06-2.0 μg/ml against subcutaneous isolates, 2.0-8.0 μg/ml against *Sporothrix schenckii* isolates, 0.5-16 μg/ml against agents that cause pheohyphomycosis and 0.125-1 μg/ml against zygomycetes. The *in vitro* activity of BAL-4815 was compared with that of amphotericin B against several invasive and emerging pathogens. The MIC_{50} values for BAL-4815 and amphotericin B, respectively, were 0.125 and 0.25 μg/ml against *Bipolaris* isolates (n=5), 0.06 and 0.5 μg/ml against *Cladosporium* isolates (n=5), 0.5 and 1 μg/ml against *Curvularia* isolates (n=5), 0.06 and 2 μg/ml against *Exophiala* isolates (n=5), 0.06 and 0.5 μg/ml against *Fonsecaea* isolates (n=5), 0.06 and 1 μg/ml against *Phialophora* isolates (n=5), 4 and 2 μg/ml against *Sporothrix* isolates (n=5), 2 and > 16 μg/ml against *Scedosporium* isolates (n=5), 16 and 4 μg/ml against *Fusarium* isolates (n=5), and 0.5 and 0.5 μg/ml against zygomycetes (n=5). The antifungal activity of BAL-4815 against dermatophytes was compared to that of terbinafine. MIC_{50} values for BAL-4815 and terbinafine, respectively, were 0.06 and 0.004 μg/ml against terbinafine-susceptible strains of *T. rubrum* (n=4), 0.06 and 4 μg/ml against terbinafine-resistant strains of *T. rubrum* (n=6), 0.06 and 0.008 μg/ml against *T. mentagrophytes* (n=10), 0.125 and 0.015 μg/ml against *Trichophyton tonsurans* (n=10), 0.06 and 0.008 μg/ml against *Epidermophyton floccosum* (n=10), and 0.06 and 0.015 μg/ml against *M. canis* (n=10) (15, 16).

BAL-4815 exhibited good activity against clinical *Candida* isolates, including azole-resistant strains, according to results from a study comparing the *in vitro* antifungal activity of the compound against the activities of several comparators. Results were reported for amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and BAL-4815 against 44 *Candida* isolates and 19 fluconazole-resistant strains from a culture collection. The overall MIC_{50} values against the clinical and fluconazole-resistant strains, respectively, were 0.25 and 1 μg/ml for amphotericin B, 0.03 and 0.125 μg/ml for flucytosine, 0.25 and 64 μg/ml for fluconazole, 0.047 and 2 μg/ml for itraconazole, 0.008 and 2 μg/ml for voriconazole, and 0.008 and 2 μg/ml for BAL-4815. When grouped by species, the MIC_{50} values for the respective antifungal agents were 0.25, 0.125, 0.25, 0.031, 0.008 and 0.008 μg/ml against all strains of *C. albicans* (n=26); 0.25, 0.125, 0.25, 0.012, 0.008 and 0.008 μg/ml against susceptible strains of *C. albicans* (n=18); 1, 0.03, 16, 1, 1 and 0.5 μg/ml against resistant strains of *C. albicans* (n=8); 1, 8, 64, 0.5, 0.5 and 0.25 μg/ml against *C. krusei* (n=5); 1, 0.03, 16, 1, 1 and 0.5 μg/ml against *C. glabrata* (n=19); and 0.25, 0.063, 0.25, 0.031, 0.016 and 0.008 μg/ml against *C. dublinensis* (17).

BAL-4815 and BAL-8557 showed good antifungal activity when tested *in vitro* and *in vivo*, respectively. The *in vitro* efficacy of BAL-4815 was tested against 122 reference strains and 83 clinical isolates. Results showed geometric mean MIC values of 0.008 μg/ml for *C. albicans*, 0.09 μg/ml for *C. glabrata*, 0.10 μg/ml for *C. krusei* and 0.1 μg/ml for *A. fumigatus*. *In vivo* testing against sys-

temic candidiasis in normal rats demonstrated that BAL-8557 had efficacy superior to that of voriconazole and equivalent to that of itraconazole. The ED_{50} values in this model ranged between 1.4 and 4.2 μmol/kg for BAL-8557 (i.v. and oral), 0.7 and 1.9 μmol/kg for fluconazole (oral), 1.0 and 4.7 μmol/kg for itraconazole (oral), and 1.7 and > 22 μmol/kg for voriconazole (i.v. and oral). When tested against aspergillosis in rats, the ED_{50} values for BAL-8557 ranged between 6 and 14 μmol/kg for systemic infections and between 6.5 and 12 μmol/kg for pulmonary infections, similar to those found for voriconazole and itraconazole in this model. In plasma, the minimal effective concentrations (MECs) were 0.05 μg/ml for both the candidiasis and aspergillosis models (18).

The i.v. and oral *in vivo* efficacies of BAL-8557 were tested in various rat models of candidiasis and aspergillosis. In rats infected systemically with the *C. albicans* strain CY1002, the ED_{50} values measured on day 14 were 4.0 μmol/kg for both oral and i.v. BAL-8557 compared to 4.7 μmol/kg for oral itraconazole. In a similar model involving the *C. albicans* strain CY3003, ED_{50} values of 1.5 μmol/kg for i.v. BAL-8557, 2.6 μmol/kg for oral BAL-8557 and 1.7 μmol/kg for oral itraconazole were found. The ED_{50} values against systemic experimental candidiasis caused by the *C. tropicalis* strain CY5042 were 0.9 μmol/kg for i.v. BAL-8557, 0.8 μmol/kg for oral BAL-8557 and 1.7 μmol/kg for oral itraconazole. The efficacy of the compound was also tested in rat models of aspergillosis in both normal and immunosuppressed hosts. Testing in normal rats with systemic infection caused by *A. fumigatus* strain CF1003 yielded ED_{50} values of 12 μmol/kg for i.v. BAL-8557, 14 μmol/kg for oral BAL-8557 and 8.9 μmol/kg for oral itraconazole. In immunosuppressed rats infected systemically with *A. fumigatus* strain CF924390, the ED_{50} values were 6.0 μmol/kg for i.v. BAL-8557, 9.9 μmol/kg for oral BAL-8557 and 7.2 μmol/kg for oral itraconazole. Finally, in rats with pulmonary infection caused by *A. fumigatus* strain CF924390, the ED_{50} values were 6.8 μmol/kg for i.v. BAL-8557, 8.9 μmol/kg for oral BAL-8557 and 2.5 μmol/kg for oral itraconazole (3, 5).

In a model of disseminated candidiasis in neutropenic mice, researchers determined the dose-response curves for BAL-8557 (0.125-50 mg/kg/day s.c.) and investigated the effects of dose fractionation. In this study, male CD1 mice were infected with the *C. albicans* isolate FA6862. The tissue burden in the kidneys of control animals was 5.8 $\log_{10}$ CFU/g, which was maximally reduced to 2.82 $\log_{10}$ CFU/g in animals treated with BAL-8557. Results from the dose-fractionation studies indicated that divided doses were as effective at reducing tissue burden as single daily doses when the total daily dose was above 6 mg/kg (19).

In another neutropenic murine model involving infection with the *A. flavus* strain AFL8, researchers examined the *in vivo* efficacy of BAL-8557 (5, 10, 25 and 50 mg/kg p.o.) in comparison to voriconazole (10 and 25 mg/kg i.v.), itraconazole (10 and 25 mg/kg p.o.) and caspofungin (1 mg/kg i.v.), administered 2, 4 or 24 h after infection. Tissue burden was measured by both CFU determination and by a newly developed quantitative polymerase chain reaction (PCR) method. Results showed a mean fungal burden in untreated mice of 1.3×10^4 CFU/g tissue and 3.4×10^4 genome equivalents. Treatment with BAL-8557 reduced the mean fungal burden to 424 CFU/g tissue and genome equivalents to 201. For comparison, treatment with voriconazole, itraconazole and caspofungin reduced the mean culture burdens to 260, 1340 and 301 CFU/g, respectively, and the genome equivalents to 194, 151 and 108, respectively. The ability of BAL-8557 to reduce tissue burden was observed even when treatment was delayed until 24 h after *A. flavus* infection (20). Also in this study, a survival rate of > 80% was obtained when BAL-8557 was administered before infection at a dose of 10 mg/kg or more or when the compound was given after infection at a dose of 25 mg/kg or more, as compared to 80-100% mortality observed in control mice. In mice receiving treatment before infection with *A. flavus*, the protection offered by BAL-8557 was similar to that with itraconazole and caspofungin, and superior to that with voriconazole. Mice treated at 4 or 24 h postinfection showed excellent (> 80%) survival with BAL-8557 (25 or 50 mg/kg), with similar survival rates observed in mice treated with the same doses of voriconazole or itraconazole. Caspofungin treatment generally yielded excellent survival rates, except for the group treated 4 h postinfection, which had survival rates of only 50% (21).

Pharmacokinetics and Metabolism

Administration of BAL-8557 to monkeys yielded $t_{1/2}$ values for BAL-4815 of 9.8 and 12.8 h for i.v. and oral administration, respectively, and an oral bioavailability of 87% (18).

A non-neutropenic murine model of disseminated *C. albicans* (UC820) infection was used to study the pharmacodynamic properties of BAL-4815 (1.25-160 mg/kg/day i.p.). Study results showed an MIC_{50} of 0.004 μg/ml and a half-life of 3.8 h. The volume of distribution was 3.2 l/kg and protein binding was 95.4%. The kidneys from untreated mice showed a tissue burden of 7.42 $\log_{10}$ CFU at 72 h, in comparison with the values for treated mice, which were 2.16-5.60, 2.15-5.77 and 3.58-5.23 $\log_{10}$ CFU, respectively, when the drug was administered every 6, 12 and 24 h. The activity of BAL-4815 against *C. albicans* in this model was independent of the dosing interval. The AUC/MIC of the unbound fraction of the drug showed the best correlation with outcome (22).

An *in vitro* and *in vivo* study was conducted to predict the pharmacokinetics of BAL-4815 in humans. When incubated with heparinized rat, cynomolgus monkey and human plasma, BAL-8557 (10 μg/ml) was rapidly converted to BAL-4815, and incubation with liver microsomes from the same species showed that < 10% of BAL-4815 was metabolized during a 120-min period. BAL-4815 exhibited strong binding to plasma proteins, with a free fraction of 3.5% in rats, 3.6% in cynomolgus monkeys and 2.2% in humans. *In vivo* pharmacokinetic results for

BAL-4815 in rats after i.v. administration of BAL-8557 (5 mg/kg) revealed a C_{max} of 0.624 μg/ml, a t_{max} of 0.083 h, a $t_{1/2}$ of 5.07 h and an $AUC_{0-\infty}$ of 1.39 μg.h/ml. Oral administration of BAL-8557 (10 mg/kg) to rats gave a C_{max} of 0.307 μg/ml, a t_{max} of 2.00 h, a $t_{1/2}$ of 3.47 h and an $AUC_{0-\infty}$ of 1.72 μg.h/ml for BAL-4815. In cynomolgus monkeys, i.v. administration of the prodrug (3 mg/kg) yielded the following pharmacokinetic data for BAL-4815: C_{max} = 1.03 μg/ml; t_{max} = 0.083 h; $t_{1/2}$ = 9.84 h; and $AUC_{0-\infty}$ = 5.08 μg.h/ml. Oral administration of BAL-8557 (3 mg/kg) to cynomolgus monkeys gave a C_{max} of 0.36 μg/ml, a t_{max} of 3.33 h, a $t_{1/2}$ of 12.8 h and an $AUC_{0-\infty}$ of 4.39 μg.h/ml for BAL-4815. The volume of distribution for BAL-4815 was 15 l/kg in rats and 5 l/kg in cynomolgus monkeys following i.v. and oral BAL-8557 and oral bioavailability was 62% and 87%, respectively. The half-life for BAL-4815 in humans was predicted to be over 30 h due to the expected low intrinsic clearance of the drug combined with its large volume of distribution. Testing in humans confirmed the half-life of the drug to be 76.9 ± 34.4 h (23).

The metabolic profiles of BAL-8557 and BAL-4815 were determined in liver microsomes from mice, rats, rabbits, dogs, cynomolgus monkeys and humans, as well as in rat hepatocytes. The same metabolic pattern was observed in all species. Specifically, researchers reported the formation of mono-oxidized BAL-4815. However, almost no metabolic biotransformation was observed in dogs, cynomolgus monkeys or humans, in contrast to the results found in mice, rats and rabbits. An additional di-oxidized metabolite of BAL-4815 was observed in rabbits. Higher drug concentrations were associated with decreased metabolism in liver microsomes from all species. Only the lowest concentration of BAL-4815 (1 μg/ml) was metabolized in rat hepatocytes, and glutathione, cysteine and *N*-acetylcysteine conjugates of mono-oxidized BAL-4815 were identified. No specific metabolite was detected for BAL-8557 (24).

The pharmacokinetic properties of BAL-4815 after i.v. and oral administration of BAL-8557 were also determined in human volunteers. A randomized, double-blind, placebo-controlled study assessed the pharmacokinetics of BAL-4815 in 24 healthy male volunteers after 1-h i.v. infusions of BAL-8557 at dose equivalents corresponding to 50, 100 and 200 mg of the active drug. Results from this single-ascending-dose study showed C_{max} values for the active drug of 0.446, 1.03 and 2.47 μg/ml, respectively, after infusions of 50-, 100- or 200-mg dose equivalents of the prodrug. The corresponding $AUC_{0-\infty}$ values for the respective doses were 11.3, 26.6 and 73.2 μg.h/ml, and the mean elimination half-lives were 76, 104 and 80 h. Thus, both C_{max} and AUC values appeared to increase in a slightly greater than dose-proportional manner, unlike mean elimination half-life, which appeared to be independent of the dose administered. The volume of distribution was large, systemic clearance was low and little active drug was recovered in urine. BAL-8557 was detectable in the plasma only during the first 2 h following the start of infusion. In general, prodrug concentrations were 10-fold lower than active drug concentrations, and systemic exposure to the prodrug was < 0.4% of that observed for the active drug. All doses tested were well tolerated, with only mild adverse events observed, and ECG and laboratory parameters showed no abnormalities (25, 26).

Data from the study discussed above were used to perform Monte Carlo simulations, a technique that can be employed to determine the probability target attainment (PTA) for pharmacodynamic indices. Monte Carlo simulations performed using the results for the dose of 200 mg predicted that steady state would be attained after 10-14 days and that steady-state trough levels of > 4 mg/l would be maintained. At fAUC/MIC values of 25, 50 and 100, MICs showing probabilities of at least 100% target attainment were 0.06, 0.03 and 0.015 mg/l, respectively. Given the long time required to reach steady-state concentrations, loading doses and/or more frequent administration at the start of therapy appear advisable. One such regimen could involve the administration of 400-mg doses every 12 h for the first 2 days. A 400-mg dose of BAL-4815 shows a 100% probability of attaining a target fAUC/MIC ratio of 25 in patients infected with *Candida* strains having an MIC of up to 0.06 mg/l. Thus, loading doses followed by maintenance doses permit the rapid achievement of active concentrations of BAL-4815, according to results from Monte Carlo simulations of a once-daily dosing regimen (27).

A randomized, double-blind, placebo-controlled, single-ascending-dose study evaluated the pharmacokinetics of BAL-4815 after the oral administration of BAL-8557. In this study, 15 healthy male subjects received oral doses of 100-, 200- or 400-mg equivalents of BAL-4815 administered as the prodrug. Following oral administration, the C_{max} values were 1.45, 2.59 and 5.57 μg/ml, respectively, for the three doses and the $AUC_{0-\infty}$ values were 37.0, 78.4 and 215 μg.h/ml, respectively. The mean elimination half-lives for the three doses were 63, 77 and 56 h, respectively. The C_{max} and AUC values for BAL-4815 increased in a dose-proportional manner, whereas the elimination half-life appeared to be independent of dose. The volume of distribution was large and systemic clearance was low. All doses were well tolerated, and no serious adverse events were reported. Since the minimum effective concentrations of BAL-4815 needed to treat systemic candidiasis and aspergillosis are predicted to be between 125 and 350 ng/ml, the researchers suggested that a single loading dose of 100- or 200-mg equivalents of BAL-4815, followed by either a once-weekly dose of 400 mg or a once-daily dose of 50 mg, should be sufficient for the treatment of these systemic fungal infections in humans (25, 28).

A multiple-ascending-dose study was conducted in 32 healthy male volunteers to examine the pharmacokinetics of BAL-4815 after i.v. and oral administration of BAL-8557. This randomized, double-blind, placebo-controlled trial grouped participants into 4 cohorts with the following dosing regimens: 1) C1: 100-mg oral loading dose followed by once-daily maintenance doses of 50 mg up to day 21; 2) C2: 200-mg oral loading dose followed by maintenance doses of 100 mg; 3) C3: 1-h i.v. infusion of

100 mg as loading dose followed by a maintenance dose of 50 mg; and 4) C4: 1-h i.v. infusion of 200 mg as loading dose followed by a maintenance dose of 100 mg. All doses represent BAL-4815 equivalents administered as BAL-8557. After oral administration, the AUC_{0-24h} values for BAL-4815 were 8.75 and 21.6 μg.h/ml for C1 on days 1 and 21, respectively, and for C2, the values were 18.5 and 40.3 μg.h/ml, respectively. After i.v. administration, the AUC_{0-24h} values on days 1 and 14 were 7.32 and 14.3 μg.h/ml, respectively, for C3 and 12.9 and 33.6 μg.h/ml, respectively, for C4. A 4-5-fold accumulation of BAL-4815 was observed after both i.v. and oral administration, which was somewhat lower than that predicted by the elimination half-life of 85-117 h. The systemic clearance at steady state was 2.4-4.1 l/h and the volume of distribution was 308-542 l. Plasma levels of BAL-4815 showed dose-proportional increases after repeated oral and i.v. administration, and no indications of inhibition or induction of cytochrome P-450 CYP3A4-metabolizing enzymes were observed. Adverse events were mild or moderate, with the exception of 1 case of severe rhinitis which was not related to the trial drug. Furthermore, absolute oral bioavailability appeared to be excellent (29, 30).

Safety

The toxicities of oral and i.v. BAL-8557 were assessed in cynomolgus monkeys. BAL-8557 was administered orally at doses of 0, 10, 30 and 90 mg/kg over a 4-week period, and by continuous i.v. infusion at doses of 0, 10, 30 and 60 mg/kg/day over a 2-week period. An additional study involving oral administration of BAL-4815 at doses of 0, 10, 30 and 90 mg/kg for 2 weeks was also performed. The major toxicities were observed in the liver and adrenals. The lethal dose of BAL-8557 in the 4-week oral toxicity study was 90 mg/kg, but no mortality was observed in the 2-week continuous i.v. infusion study. At doses of 10 and 30 mg/kg, BAL-8557 was rapidly converted to BAL-4815 in a dose-dependent manner. In addition, plasma C_{max} and AUC values closely correlated with toxicity at these doses. However, at doses of 60 and 90 mg/kg, BAL-8557 showed exposure that was greater than dose-proportional. The results from this study suggest that initial i.v. infusion followed by oral administration would provide an effective concentration of the compound without producing excessive toxicity (31).

Clinical Studies

A multicenter, randomized, double-blind phase II trial investigated the efficacy, safety and tolerability of BAL-8557 in the treatment of 160 immunocompromised adults with esophageal candidiasis. In this study, BAL-8557 was administered at BAL-4815 equivalents of 50 or 100 mg/day or 400 mg weekly, and fluconazole was administered at 100 mg/day as comparator drug. Following a loading dose on day 1, treatment continued for 14 or 21 days. Endoscopically confirmed cure rates were 90%, 90% and 95% at 14-day follow-up, 28-day follow-up and the end of treatment, respectively, in patients (n=38) receiving 50 mg/day BAL-4815 equivalents. In patients (n=38) receiving 100 mg/day, the respective cure rates were 92%, 90% and 95%. Weekly dosing with 400 mg yielded respective cure rates of 95%, 90% and 98%. In comparison, fluconazole produced cure rates of 95%, 84% and 95% at 14-day follow-up, 28-day follow-up and the end of treatment, respectively. BAL-4815 had MIC values ranging from 0.000125 mg/l to 0.008 mg/l (MIC_{50} = 0.00025 mg/l *vs.* 0.001 and 0.25 mg/l, respectively, for voriconazole and fluconazole). BAL-8557 was generally well tolerated, with the most frequent adverse events being intercurrent infectious diseases (32).

Source

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