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Discovery, SAR, Synthesis, Pharmacokinetic and Biochemical Characterization of A-192411: A Novel Fungicidal Lipopeptide-(I)

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Abstract—The echinocandin class of cyclic lipopepetides has been simplified to discover potent antifungal compounds. Namely A-192411 shows good in vitro activity against common pathogenic yeasts and has an acceptable safety window in vivo. Discovery, limited SAR, synthesis, biochemical and pharmaco-dynamic profiles of A-192411 are presented.

Human fungal infections have come to prominence during the past two decades due to an increase in patients with HIV-infection, chemotherapy-induced neutropenia; organ transplantation, hemodialysis or extensive use of broad-spectrum antibiotics and glucocorticosteroids.[1,2] The echinocandin family of natural hexacyclic-lipopeptides has been of interest in antifungal research over the past three decades due to their fungicidal profiles. Increased interest became apparent during the early-mid eighties after the delineation of their mode of action as β-1,3-glucan synthetase inhibitors. This enzyme is part of a membrane bound protein complex that utilizes UDP-glucose as a substrate to produce oligomeric glucose, which is further processed by a variety of enzymatic steps to mesh the fungal cell wall. Inhibition of such a process induces cell wall breakage, leads to cellular lysis and death.[3] We, and others, have recently reported structure–activity relationship studies, syntheses, in vitro and in vivo antifungal evaluation of a number of cyclic hexapeptides with simplified structures, superior stability and water solubility as compared to the natural product Echinocandin B.[4] As the search of such agents continues, we present in the next two papers, the discovery path to, and synthesis of A-192411 (**1**, Table 1), a structurally simplified cyclic hexapeptide with acceptable in vitro[5] and in vivo profiles (following paper).

Our previous lead compound: A-199930 (Fig. 1) showed in vitro fungicidal activity on several *Candida* strains in the range of 0.2–2 μg/mL and in vivo efficacy in a mouse chronic candidiasis model with ED_{50} <1.25 mg/kg (100% cure and 90% survival rates) and ED_{50} <0.6 mg/kg in a mouse acute infection setting. However, further safety evaluations of A-199930 in rats showed NOEL <0.1 mg/kg. The main side effect was a ~50% drop in mean arterial pressure at low infusion concentrations. While such an event was not observed in the anesthetized dog during CV safety study, A-199930 proved to be unsafe at low doses in cyn. Monkey. The cardiovascular deficiency of A-199930 could be blocked in vivo by the presence of mepyramine, an H1-antagonist. The increased levels of histamine release upon drug treatment were traced to a mast cell degranulation effect, which, was dose-dependently blocked by both benzylalkonium chloride and pertussis toxin. A histamine-radioligand-immunoassay was established and several hundred cyclic hexapeptides were screened for their ability to induce histamine release from mast cells. Limited SAR could be established from such an assay: compounds with high proximal positive charge density (i.e., 4-guanidino, lysine; e.g., A-199930 and several related derivatives) showed high histamine-releasing

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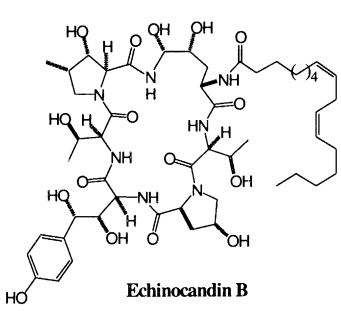
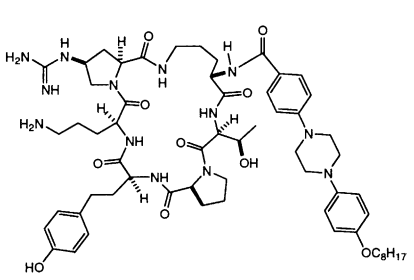
Table 1.

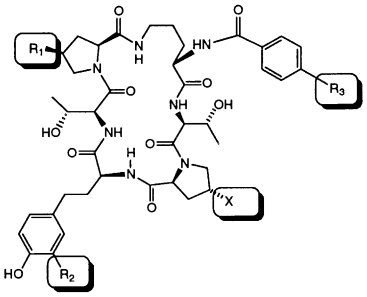
Organism	MIC (μg/mL)										
Compd	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Candida albicans ATCC 10231	0.2	0.39	0.1	0.78	0.78	0.78	0.39	1.56	0.2	0.39	0.1
Candida albicans 579A	0.2	0.2	0.01	0.39	0.39	0.78	0.2	0.78	0.2	0.39	0.1
Candida albicans CCH 442	0.2	0.2	0.01	0.78	0.78	0.78	0.2	0.78	0.1	0.39	0.1
Candida albicans ATCC 38247	0.2	0.2	0.2	0.78	0.39	0.39	0.39	0.78	0.2	0.78	0.2
Candida albicans ATCC 62376	0.2	0.2	0.1	0.39	0.78	0.39	0.2	0.78	0.2	0.39	0.1
Candida zeylanoides NRRL Y-112	0.39	0.39	0.1	1.56	3.12	1.56	0.39	0.78	0.2	0.2	0.1
Candida kefyr ATCC 28838	1.56	1.56	0.78	0.78	0.78	1.56	1.56	0.78	1.56	1.56	1.56
Torulopsis glabrata ATCC 15545	1.56	1.56	0.2	1.56	0.78	1.56	0.39	0.78	1.56	1.56	0.2
Cryptococcus albidus ATCC 34140	12.5	12.5	3.2	6.25	6.25	12.5	25	12.5	12.5	25	6.25
Candida albicans CAF-2	0.2	0.2	0.1	0.39	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Aspergillus niger ATCC 16404	6.25	6.25	0.2	6.25	6.25	12.5	25	25	3.12	25	1.56

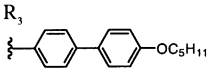
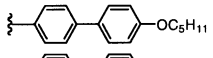
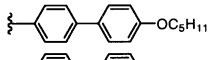
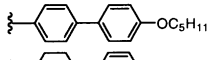
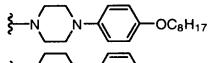
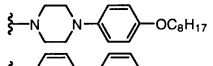
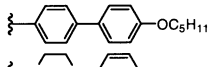
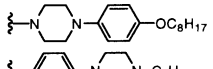
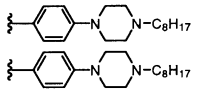
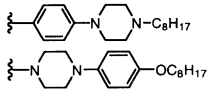


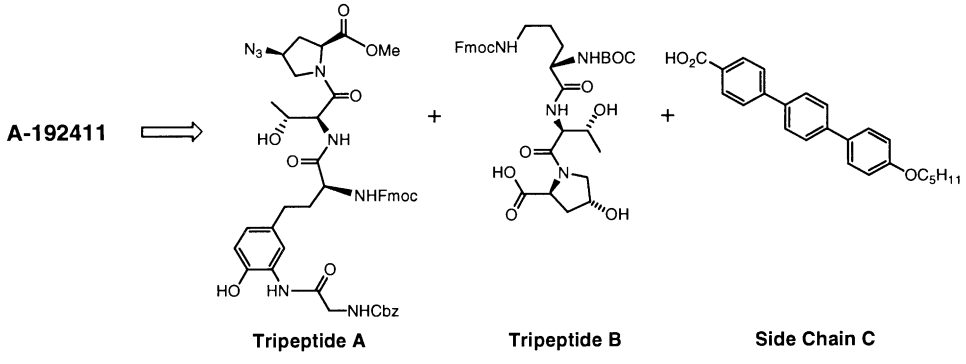
Echinocandin B



A-199930



Compd	X	R_1	R_2	R_3
1	OH	-NH-C(NH$_2$)=NH	-C(O)-CH$_2$NH$_2$	
2	OH	-NH-C(NH$_2$)=NH	-C(O)-(CH$_2$)$_2$NH$_2$	
3	OH	-NH$_2$	-C(O)-CH$_2$NH$_2$	
4	OH	-NH-C(NH$_2$)=NH	-C(O)-CH$_2$NH$_2$	
5	OH	-NH-C(NH$_2$)=NH	-C(O)-CH$_2$NH$_2$	
6	OH	-C(O)-CH$_2$NH$_2$	-C(O)-CH$_2$NH$_2$	
7	H	-NH-C(NH$_2$)=NH	-C(O)-CH$_2$NH$_2$	
8	H	-NH$_2$	-C(O)-CH$_2$NH$_2$	
9	OH	-NH-C(NH$_2$)=NH	-C(O)-CH$_2$NH$_2$	
10	H	-C(O)-CH$_2$NH$_2$	-C(O)-CH$_2$NH$_2$	
11	OH	-NH$_2$	-C(O)-CH$_2$NH$_2$	



A-192411

Tripeptide A

Tripeptide B

Side Chain C

Figure 1.

potency. Overall, compounds showing the best in vitro and in vivo antifungal profiles also turned out to be the most potent histamine-releasing agents. From these studies, we found A-192411 (compound **1**) to possess acceptable in vitro, in vivo and CV safety profiles (Fig. 1). MIC data, generated using NCCLS method, are presented on a small subset of derivatives around compound **1**, Table 1.

While most cyclic peptides maintained relatively interesting potencies as anti-microbial agents (Table 1, **3**, **9**, and **11**), their assessment in mast cell-histamine release directed us towards compound **1** as a preferred entity for further in vivo characterization. A-192411 contains two novel amino acids and a lipophilic side chain attached at the L-ornithine α-amino position. This compound was synthesized through a convergent approach consisting of couplings between two tripeptides **A** and **B** and the 4″-*O*-pentyl terphenyl carboxylate side chain **C**; (Fig. 1). Previous syntheses of Echinocandin B were reported by the Ohfune and Evans groups.[6]

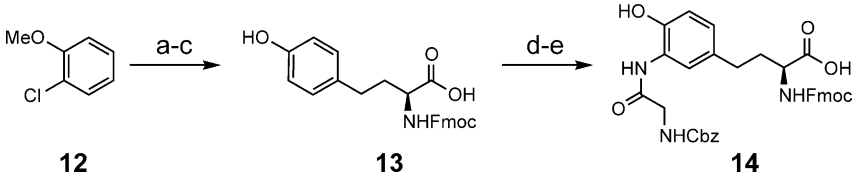
Tripeptide **A** contains two unnatural amino acids, an aryl-*N*-glycine homotyrosine and the 4-(*S*)-azido-proline (Fig. 1). The *aryl*-*N*-glycine homotyrosine moiety **14** was prepared from the reaction of 2-chloroanisole **12** with (L)-*N*-methylcarbamoyl aspartic anhydride, under Friedel–Craft's conditions, to give the corresponding amino acid in 88% yield. Ketone-hydrogenation afforded the desired homotyrosine in 90% yield (not shown). The resulting *N*-methyl carbamate-amino acid (AA) was further treated with HBr and reprotected as the N-Fmoc-AA **13**, obtained in 90% yield. The latter underwent *ortho*-nitration (70% yield) followed by hydrogenation and *N*-acylation with Cbz-protected glycine afforded precursor **14** in 68% yield, (Scheme 1).

Separately, 4-(*R*)-hydroxyproline methyl ester **15**, which, was used in the preparation of both tripeptides A and B, was coupled to (L)-*N*-Boc-threonine to give dipeptide **16** in 98% yield. The latter underwent hydrolysis, *N*-Boc deprotection (84% yield) and coupling to (L)-*N*-α-Boc-ε-Fmoc-Ornithine to give tripeptide **B** (**17**) in 50% yield, (Scheme 2). Similarly, ester **15** was *N*-Boc-protected and treated, in a one-pot reaction, with methanesulfonyl chloride followed by sodium azide, to give the desired derivative **18** in 89% yield. The latter was *N*-deprotected in TFA and coupled to *N*-Boc-threonine to give the corresponding dipeptide **19** in 90% yield. Subsequent TFA deprotection and coupling to *ortho*-*N*-glycyl homotyrosine moiety **14** (Scheme 1), afforded tripeptide **A** (**20**) in an overall yield of 72–82%. The terphenyl side chain was prepared as follows: 4-(4′-bromophenyl)-phenol was *O*-alkylated with iodopentane in the presence of NaOH to give the corresponding pentyl ether in 90% yield. The latter underwent palladium mediated Suzuki-coupling with 4-borono-benzoic diacid ($Pd(PPh_3)_4$, DMF-water, 100 °C) to give the desired side chain C (Fig. 1) in 82% yield.[7]

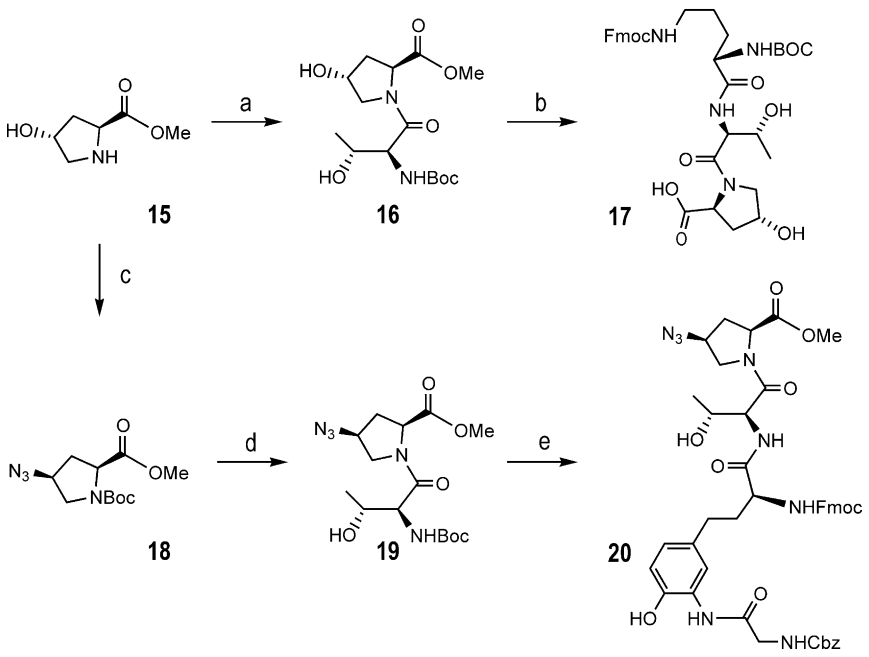
Final couplings

Tripeptides A (**17**) and B (**20**) were first coupled at the hydroxyproline and homotyrosine sites to give the corresponding linear hexapeptide in 57% yield (i) Tripeptide B:diethyl amine (DIEA), CH_3CN; (ii) Tripeptide A, CMC-HOBT; (iii) DIEA, CH_3CN; (iv) LiOH, THF–water; and (v) DPPA-$NaHCO_3$-DMF]. The latter underwent successive *N*-Fmoc deprotection (ornithine), ester hydrolysis (azidoproline) and ring-cyclization to the corresponding peptide 21 in 60% overall yield. Azide reduction and guanidylation proceeded to give the corresponding bis-Cbz-guanidine in 85% overall yield, Scheme 3. *N*-Boc removal, under TFA conditions, followed by *N*-acylation with the terphenyl side chain C and hydrogenation to remove all three *N*-Cbz groups, in a near quantitative yield, gave A-192411 as its acetic acid salt after HPLC purification. This convergent synthesis (27 steps) was used to prepare multi-gram quantities of the antifungal agent A-192411 suitable for in vitro and in vivo preclinical evaluation and characterization.

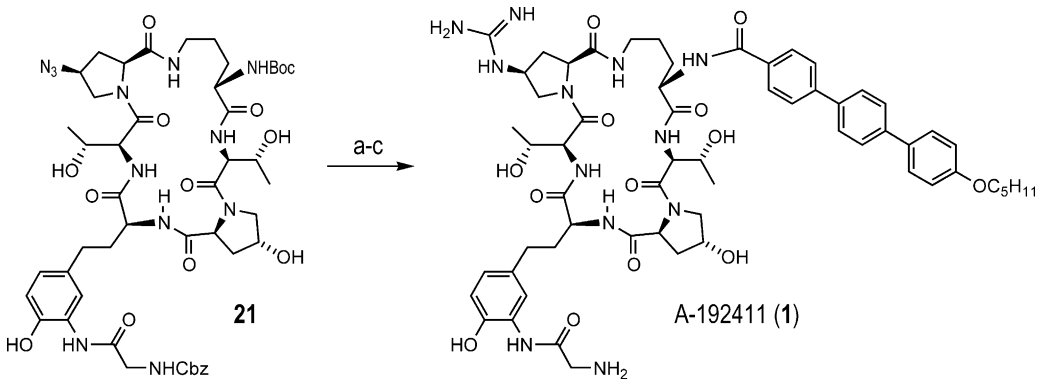
A-192411 showed solubility >3 mg/mL is water, and 0.77 mg/mL phosphate buffered saline. The measured octanol:water partition coefficient (log*D*) is 2.45 and 2.47 at pH 6.5 and 7.4, respectively. The high aqueous solubility allowed ready formulation for iv delivery, as was used in the preclinical animal studies. The in vitro rat, dog and human plasma protein binding at 5, 10, and 50 μg/mL of A-192411 acetate showed no apparent gender-related differences observed for all species. Plasma protein binding did not appear to be concentration-dependent (range 5–50 μg/mL, in rats and in humans) except in dogs, the mean plasma protein binding of A-192411 was lower (91.0%) at 5 μg/mL when compared to the 10 or 50 μg/mL (98.9 and 99.6%, respectively). The pharmacokinetic evaluation of A-192411 was conducted in mouse, rat, dog and monkey at 1 mg/kg and is summarized in Table 2. In monkeys the plasma concentration profile was fit to a two compartmental model, characterized by a terminal elimination half-life of 3.3 h (range 2.2–5.9 h) after a 1 mg/kg intravenous dose. The terminal phase volume of distribution of A-192411 was 0.9 L/kg (range 0.6–1.3 L/kg). A-192411 had a plasma clearance of 0.17 L/h kg (range 0.11–0.26 L/h kg). In dogs, it was characterized by a long terminal elimination half-life of 30.4 h (range 23.1–36.9 h), with a terminal phase volume of the distribution



Scheme 1. (a) $AlCl_3$, (L)-*N*-methylcarbamoyl Asp. Anhydride; (b) H_2, Pd/C; (c) (i) HBr/AcOH; (ii) FmocOSu; (d) $NaNO_2$, AcOH; (e) (i). H_2, Pd/C; (ii) Z-GlyOSu, THF.



Scheme 2. (a) Boc-Thr-OH, EDCI-HOBT; (b) (i) 50% TFA–DCM; (ii) LiOH–THF–water: (iii) Boc-Orn(Fmoc)-Osu; (c) (i) $(Boc)_2O$; (ii) MsCl, Et_3N; (iii) NaN_3; (d) (i) 50% TFA–DCM; (ii) Boc-Thr-OH, EDC-HOBT; (e) (i) 50% TFA-DCM; (ii) **14**, EDC-HOBT.



Scheme 3. (a) (i) PPh_3, THF–water; (ii) ZNHC(=NHZ)-SMe, $HgCl_2$, Et_3N, DMF; (iii) 50% TFA-DCM; (b) Side chain **C** or (**16**); DIPEA, DMF; (c) H_2, $Pd(OH)_2/C$, AcOH, EtOH.

Table 2. Pharmacokinetic evaluation after a 1 mg/kg iv dose in mouse, rat, dog and monkey

Species	$t_{1/2}$ (h)	V_v (L/kg)	Va_β (L/kg)	$AUC_{0-\infty}$ (μg•h/mL)	Clp (L/h•kg)
Mouse	11.5	0.4	1.0	16.84	0.06
Rat*	7.4	0.6	2.2	11.66	0.14
Dog	30.4	0.6	1.3	36.39	0.03
Monkey	3.3	0.2	0.9	6.55	0.17

of 1.3 L/kg (range 0.9–1.7 L/kg) and a plasma clearance of 0.03 L/h•kg. Similarly, it was characterized by a relative long terminal elimination half-life of 11.5 h after intravenous dosing. The terminal phase volume of the distribution was 1.0 L/kg with a plasma clearance of 0.06 L/h•kg. Elimination half-lives, after ip or iv doses, were similar with values of 10.5, 13.6, and 13.2 h for 1.25, 5, and 20 mg/kg ip doses, respectively. Both C_{max} and AUC increased proportionally with the increase in dose over the range of 1.25–20 mg/kg. In conclusion, the pharmacokinetic characteristics of A-192411 were substantially different in different animals species (Table 2). The plasma elimination half lives following a 1 mg/kg intravenous dose were 3.3, 7.4, 11.5, and 30.4 h for monkey, rat, mouse, and dog, respectively. Bioavailability from a single ip-dose of A-192411, in mouse, was constant over the 1.25–20 mg/kg dose range. There was no apparent rapid increase in plasma histamine levels in monkeys following an iv dose of A-192411, with the levels remaining low throughout the sampling interval.

A-192411 inhibits the synthesis of fungal β-1,3-glucan, a major polymer of the fungal cell wall both in vitro and in vivo. The IC_{50} for inhibition of glucan synthesis in vitro is 10–20 μg/mL, depending on the assay preparation conditions (Fig. 2). Glucan synthesis is preferentially inhibited over other macromolecular pathways when whole cells are treated with A-192411. Formal lysis of cells occurred following drug addition, as evidenced by the release of high molecular weight RNA from *C. albicans* (Fig. 2). The 'kill-kinetics' and extent of cellular lysis parallel loss of viability (Fig. 3).

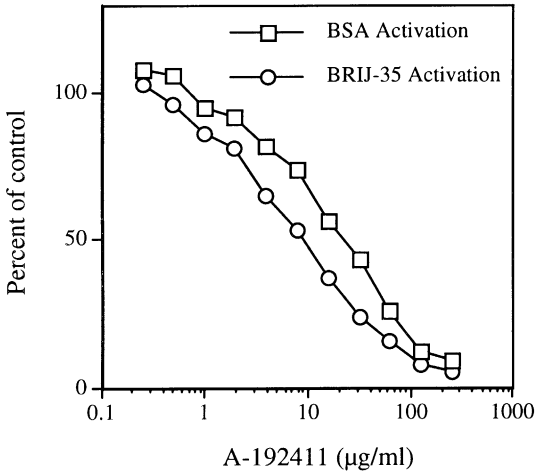


Figure 2. Inhibition of glucan synthesis.

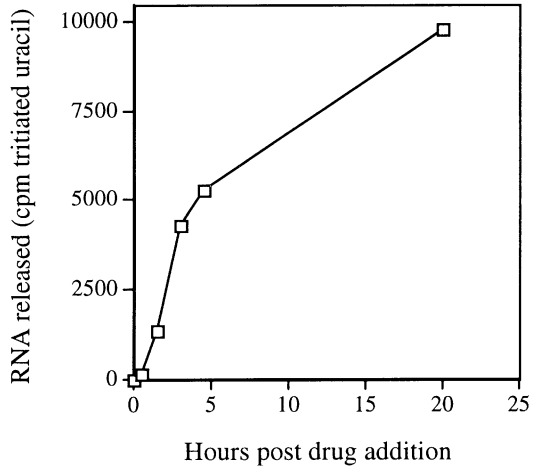


Figure 3. Lysis of *Cand. Alb.* By ^{3}H-RNA release.

In conclusion, we have described the path to select A-192411 from SAR studies on simplified echinocandin-like motifs. This process was guided by unexpected species-specific toxicological findings revolving primarily around cardiovascular deficiencies. We have found that hexacyclic-lipopeptides with clustered basic sites (guanidine and amino groups) potently induced histamine release from mast cells and lead to drop in mean arterial pressure. We have selected **1** for further in-vivo characterization (see following manuscript) based on its in-vitro anti-fungal activity and its safety profile in both rodents and monkeys.

Acknowledgements

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