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(54) Title: ANTIFUNGAL COMBINATION THERAPY

(57) Abrégé/Abstract:
There is described antifungal combination therapy comprising the use of known antifungal agents such as the azoles or polyenes in combination with a pneumocandin derivative antifungal agent. More particularly, the invention relates to antifungal combination therapy comprising the use of azoles such as fluconazole, voriconazole, itraconazole, ketoconazole, miconazole, ER 30346, SCH 56592;polyenes such as amphotericin B nystatin or liposomal and lipid forms thereof such as Abelcet, AmBisome and Amphocil; purine or pyrimidine nucleotide inhibitors such as flucytosine; or polyoxins such as nikkomycins, in particular nikkomycin Z or other chitin inhibitors, elongation factor inhibitors such as sordarin and analogs thereof, mannan inhibitors such as predamycin, bactericidal/permeability-inducing (BPI) protein products such as XMP.97 or XMP.127 or complex carbohydrate antifungal agents such as CAN-296 in combination with a pneumocandin derivative as described herein.

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(54) Title: ANTIFUNGAL COMBINATION THERAPY

(57) Abstract

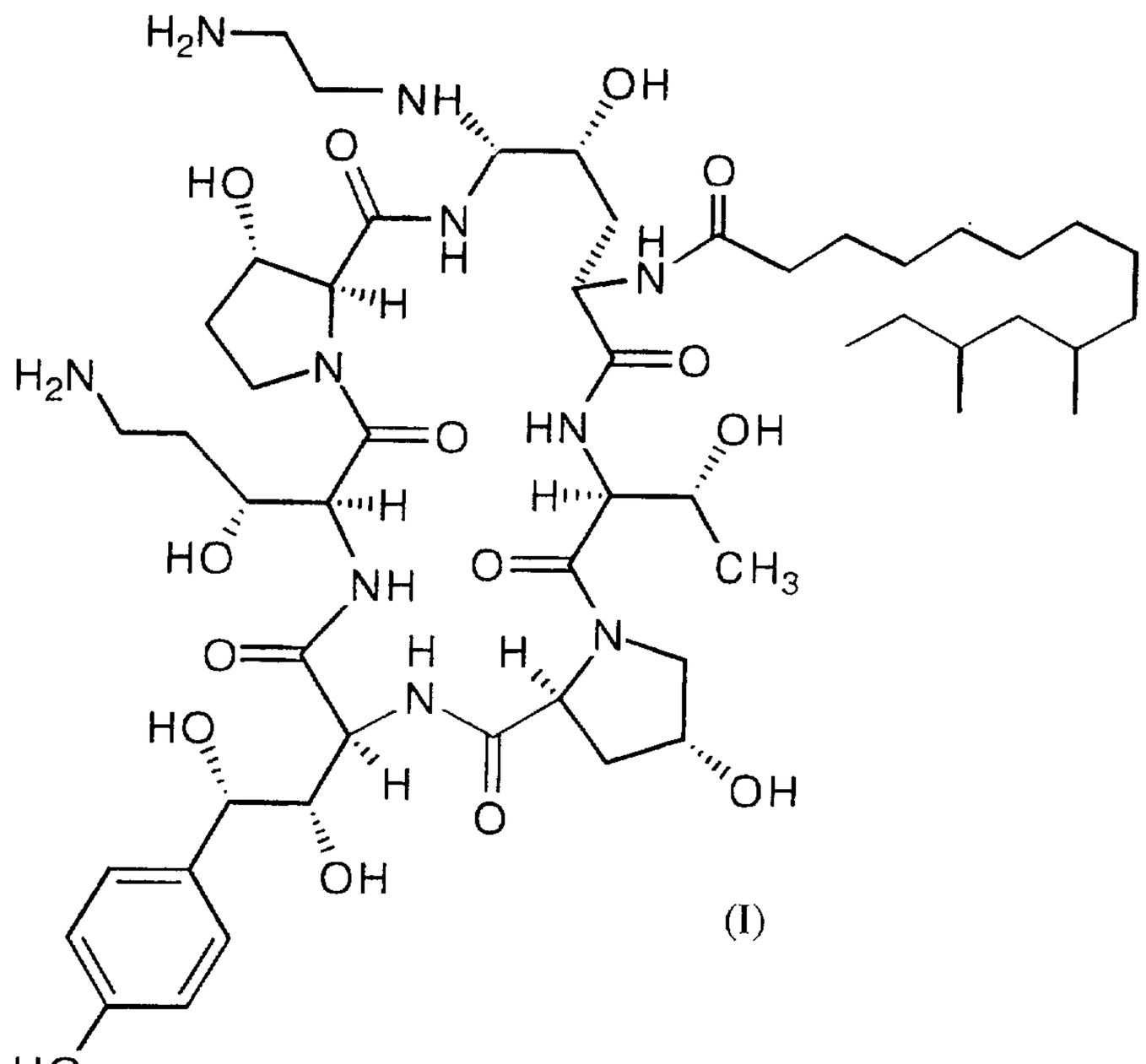
There is described antifungal combination therapy comprising the use of known antifungal agents such as the azoles or polyenes in combination with a pneumocandin derivative antifungal agent. More particularly, the invention relates to antifungal combination therapy comprising the use of azoles such as fluconazole, voriconazole, itraconazole, ketoconazole, miconazole, ER 30346, SCH 56592;polyenes such as amphotericin B, nystatin or liposomal and lipid forms thereof such as Abelcet, AmBisome and Amphocil; purine or pyrimidine nucleotide inhibitors such as flucytosine; or polyoxins such as nikkomycins, in particular nikkomycin Z or other chitin inhibitors, elongation factor inhibitors such as sordarin and analogs thereof, mannan inhibitors such as predamycin, bactericidal/permeability-inducing (BPI) protein products such as XMP.97 or XMP.127 or complex carbohydrate antifungal agents such as CAN-296 in combination with a pneumocandin derivative as described herein.

TITLE OF THE INVENTION

ANTIFUNGAL COMBINATION THERAPY

FIELD OF THE INVENTION

The present invention relates to antifungal combination therapy comprising the use of known antifungal agents such as the azoles or polyenes in combination with a pneumocandin derivative antifungal agent. More particularly, the invention relates to antifungal combination therapy comprising the use of azoles such as fluconazole (hereinafter referred to as FCZ), voriconazole, itraconazole, ketoconazole, miconazole, ER 30346, SCH 56592; polyenes such as amphotericin B (hereinafter referred to as AmB), nystatin or liposomal and lipid forms thereof such as Abelcet (trade-mark), AmBisome (trade-mark) and Amphocil (trade-mark); purine or pyrimidine nucleotide inhibitors such as flucytosine; or polyoxins such as nikkomycins, in particular nikkomycin Z or other chitin inhibitors, elongation factor inhibitors such as sordarin and analogs thereof, mannan inhibitors such as predamycin, bactericidal/permeability-inducing (BPI) protein products such as XMP.97 or XMP.127 or complex carbohydrate antifungal agents such as CAN-296 in combination with a pneumocandin derivative of the structure.



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or a pharmaceutically acceptable salt or other pharmaceutically acceptable formulation thereof.

These combination therapies have been shown to be useful against such opportunistic pathogens as *Cryptococcus* spp., *Candida* spp., *Aspergillus* spp., *Histoplasma* spp., *Coccidioides* spp., *Paracoccidioides* spp. *Blastomyces* spp., *Fusarium* spp., *Sporothrix* spp., *Trichosporon* spp., *Rhizopus* spp., *Pseudallescheria* spp., dermatophytes, *Paeciliomyces* spp., *Alternaria* spp., *Curvularia* spp., *Exophiala* spp., *Wangiella* spp., *Penicillium* spp., *Saccharomyces* spp., *Dematiaceous* fungi and *Pneumocystis carinii*.

BACKGROUND OF THE INVENTION

There is an increasing need for agents which are effective against opportunistic mycotic infections by such agents as *Cryptococcus* spp., *Candida* spp., *Aspergillus* spp., *Histoplasma* spp., *Coccidioides* spp., *Paracoccidioides* spp. *Blastomyces* spp., *Fusarium* spp., *Sporothrix* spp., *Trichosporon* spp., *Rhizopus* spp., *Pseudallescheria* spp.,

dermatophytes, *Paeciliomyces* spp., *Alternaria* spp., *Curvularia* spp., *Exophiala* spp., *Wangiella* spp., *Penicillium* spp., *Saccharomyces* spp., *Dematiaceous* fungi and *Pneumocystis carinii*. The present treatments, i.e., polyenes, such as amphotericin B, cause severe side effects and azoles, such as fluconazole, are only fungistatic. The pneumocandins, which are related to the echinocandins, are cyclic hexapeptides which inhibit cell wall 1,3ß-D-glucan synthesis. The pneumocandins have shown potent *in vivo* activity against *Candida* spp., *Pneumocystis carinii*, *Aspergillus* spp., as well as the other fungal pathogens listed above. However, the pneumocandins, by themselves, have weak activity against *Cryptococcus* spp.

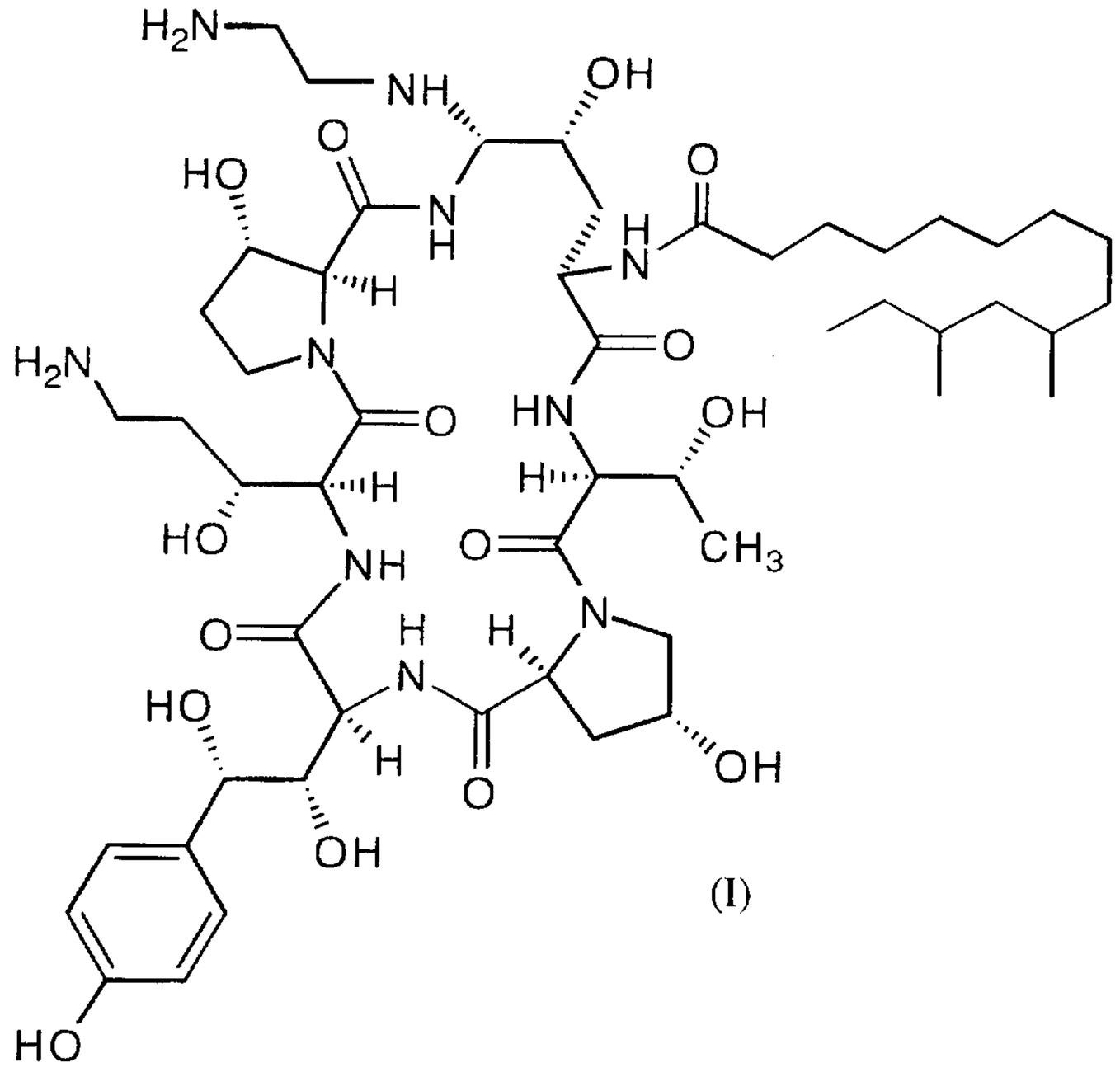
Combination therapy with antifungal drugs may provide additional options for treating *Cryptococcus* and other fungal pathogens.

Previous studies have evaluated the efficacy of other pneumocandin derivatives against *Cryptococcus neoformans* in combination with amphotericin B and fluconazole (Abruzzo et al., Antimicrob. Agents Chemo. 1995, 39:1077-1081 and Bartizal et al., Antimicrob. Agents Chemo. 1995, 39:1070-1076). However, none of these studies have demonstrated the results found using Compound I as the pneumocandin derivative.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to antifungal combination therapy comprising the use of known antifungal agents such as the azoles or polyenes in combination with a pneumocandin derivative antifungal agent. More particularly, the invention relates to antifungal combination therapy comprising the use of azoles such as fluconazole, voriconazole, itraconazole, ketoconazole, miconazole, ER 30346, SCH 56592; polyenes such as amphotericin B, nystatin or liposomal and lipid forms thereof such as Abelcet, AmBisome and Amphocil; purine or pyrimidine nucleotide inhibitors such as flucytosine; or polyoxins such as nikkomycins, in particular nikkomycin Z or other chitin inhibitors, elongation factor inhibitors such as sordarin and analogs thereof, mannan inhibitors such as predamycin , bactericidal/permeability-

inducing (BPI) protein products such as XMP.97 or XMP.127 or complex carbohydrate antifungal agents such as CAN-296 in combination with a compound of the structure



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or a pharmaceutically acceptable salt or other pharmaceutically acceptable formulation thereof.

In particular, this combination therapy has been shown to be useful against such opportunistic pathogens as *Cryptococcus* spp., *Candida* spp., *Aspergillus* spp., *Histoplasma* spp., *Coccidioides* spp., *Paracoccidioides* spp. *Blastomyces* spp., *Fusarium* spp., *Sporothrix* spp., *Trichosporon* spp., *Rhizopus* spp., *Pseudallescheria* spp., dermatophytes, *Paeciliomyces* spp., *Alternaria* spp., *Curvularia* spp., *Exophiala* spp., *Wangiella* spp., *Penicillium* spp., *Saccharomyces* spp., *Dematiaceous* fungi and *Pneumocystis carinii*.

Compound I is disclosed in U.S. Patent No. 5,378,804. Its preparation is described in that patent along with U.S. Patent No. 5,552,521.

It is contemplated that other pneumocandin derivatives such as those disclosed in U.S. Patent No. 5,516,756 and in EP 0 535 967, published 04/07/93; EP 0 535 959, published 04/07/93; WO 94/25050, published 11/10/94; WO 94/25048, published 11/10/94; WO 96/22784, published 08/01/96; and WO 97/17365, published 05/15/97, would be useful in the combination therapy.

The azole, polyene or other antifungal agent may be administered orally or parenterally. Compound I is preferably administered parenterally, but is not limited to that route and may also be administered by other routes such as oral, intramuscular or subcutaneous.

As shown below, the combination therapy results in enhanced effects using sub-inhibitory concentrations of all agents. These effects can be demonstrated *in vitro* and *in vivo* using clinical and environmental strains of *C. neoformans, C. albicans* and *A. fumigatus*.

The invention is further described in connection with the following non-limiting examples.

EXAMPLES

It has been found that combination therapy of Compound I with AmB and FCZ against *C. neoformans* results in enhanced activity against strains of *C. neoformans in vitro*. It has also been found that combination therapy of Compound I with AmB against *C. albicans* and *A. fumigatus* results in enhanced activity *in vitro*. This has been shown using a broth microdilution technique which is the standard method for antifungal susceptibility testing proposed by the NCCLS (protocol M27-T). Sub-inhibitory concentrations of Compound I in combination with sub-inhibitory concentrations of AmB and sub-inhibitory concentrations of FCZ were employed. The minimal inhibitory concentrations (MICs) for AmB and Compound I were defined as the lowest drug concentration at which there was an absence of growth. FCZ MIC was defined as the lowest drug concentration which resulted in a visual turbidity less than or equal to 80% inhibition compared with that produced by the control without antifungal agent.

Results of antifungal susceptibility testing show that colony forming units (CFUs) were markedly reduced when amphotericin B at certain concentrations (0.0075, 0.015 and 0.03 µg/ml) was combined with Compound I at certain concentrations (4, 8 and 16 µg/ml). Additionally, the administration of Compound I significantly enhanced the activity of fluconazole by reducing CFU numbers at certain concentrations (0.25, 0.50 and 1.0 µg/ml).

Additional drug combination testing *in vitro* was performed to evaluate combinations of Compound I with AmB and FCZ against clinical isolates. There was no antagonism evident between Compound I and AmB against *C. albicans*, *A. fumigatus* and *C. neoformans*. Fractional inhibitory indices (FIC) were approximately 0.50 or lower, indicative of additive or synergistic activity. Results suggest that Compound I can enhance the activity of FCZ and AmB and indicate a potential role for Compound I in combination regimes against those fungi less sensitive or insensitive to Compound I when used alone.

Drug interaction and efficacy studies with Compound I combined with either AmB or FCZ against disseminated candidiasis, aspergillosis and cryptococcosis were performed. Results showed no adverse effects with combinations at high, use or lower concentrations and no antagonism of efficacy with either AmB or FCZ combined with Compound I. Against *C. albicans*, Compound I doses of 0.03 mg/kg and lower plus 0.03 mg/kg and lower of AmB appeared more efficacious than either agent administered alone. With FCZ similar results were found at doses of 0.31 mg/kg and lower of FCZ when combined with 0.03 mg/kg of Compound I. Against *A. fumigatus*, significant improvements in efficacy (10 to >800-fold in ED values) with combinations were noted with Compound I titrated between 0.03 and 2 mg/kg and with AmB between 0.03 and 0.5 mg/kg. Significant improvement in survival was seen with Compound I at 0.008 mg/kg combined with AmB at 0.12 mg/kg over the compounds administered alone.

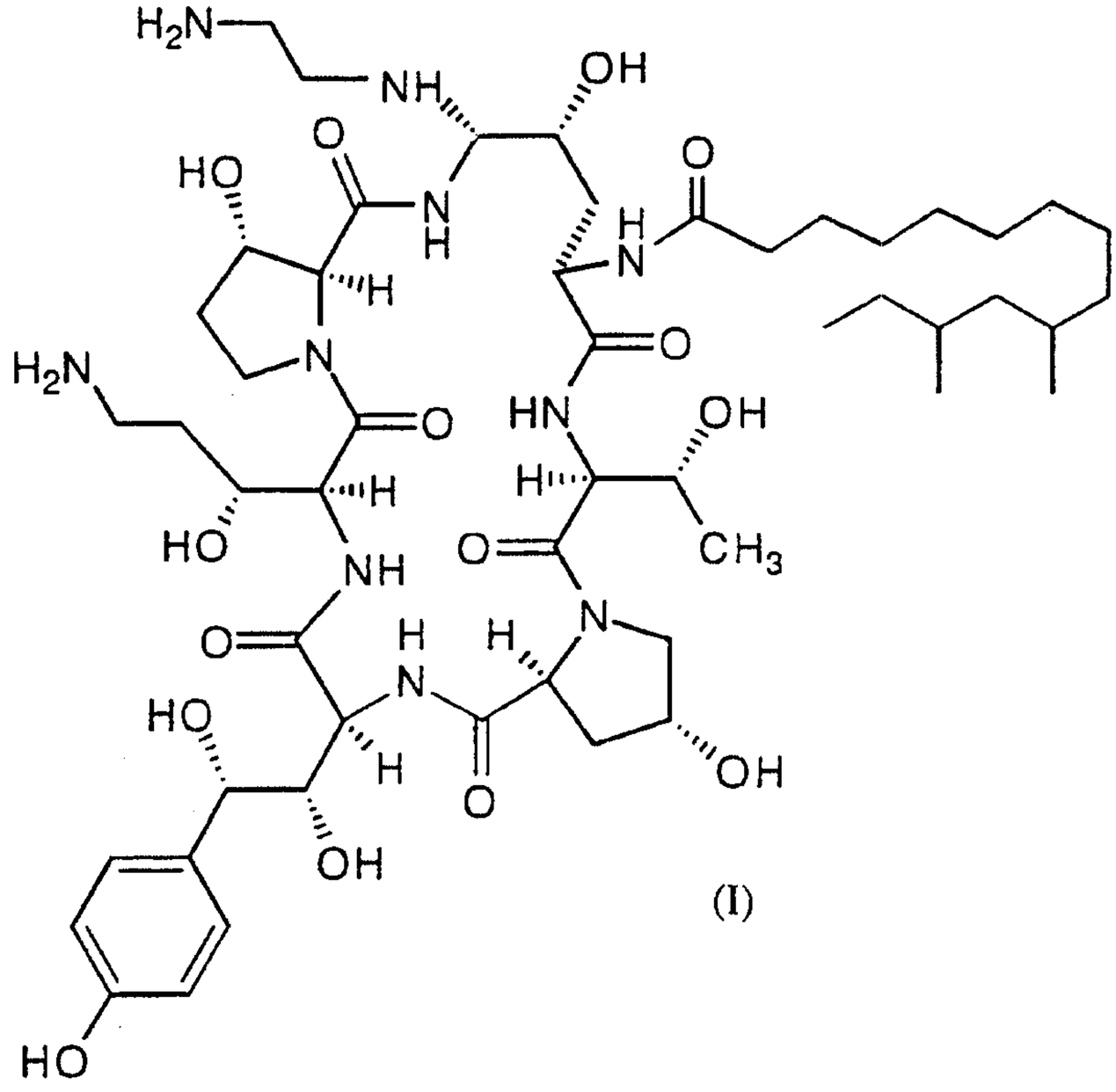
As found in drug combination studies *in vitro* with Compound I and AmB, FIC values were approximately 0.50, suggesting

additive or synergistic activity *in vivo*. Additionally, no antagonism of efficacy with FCZ in combination with Compound I was seen in studies against cryptococcosis in mice. Beneficial effects on efficacy were observed against *C. neoformans* with combinations of Compound I and FCZ at certain concentrations.

Given the above disclosure, it is thought that variations will occur to those skilled in the art. For example, it is thought that combination therapy using azoles other than fluconazole and pneumocandin derivatives other than Compound I may also be effective against fungal infections caused by the fungal pathogens noted. Accordingly, it is intended that the above examples should be construed as illustrative and that the invention disclosed herein should be limited only by the following claims.

CLAIMS:

1. An antifungal combination for treating fungal infection comprising a therapeutically effective amount of a pneumocandin derivative of formula (I):



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or a pharmaceutically acceptable salt thereof and a therapeutically effective amount of an azole, polyene, purine nucleotide inhibitor, pyrimidine nucleotide inhibitor, mannan inhibitor, protein elongation factor inhibitor, bactericidal/permeability inducing protein product or polyoxin.

2. A combination of claim 1 which comprises a therapeutically effective amount of said pneumocandin derivative and a therapeutically effective amount of a polyene.

3. A combination of claim 1 which comprises a therapeutically effective amount of said pneumocandin derivative and a therapeutically effective amount of said azole.

4. A combination of claim 1 wherein the azole is selected from the group consisting of fluconazole, voriconazole, itraconazole, ketoconazole, miconazole, ER 30346 and SCH 56592; the polyene is selected from the group consisting of amphotericin B, nystatin and liposomal and lipid forms thereof; the purine or pyrimidine nucleotide inhibitor is flucytosine; the polyoxin is nikkomycin Z, the elongation factor inhibitor is sordarin and analogs thereof and the mannan inhibitor is predamycin.

5. A combination of claim 1, 3 or 4 wherein the azole is fluconazole.

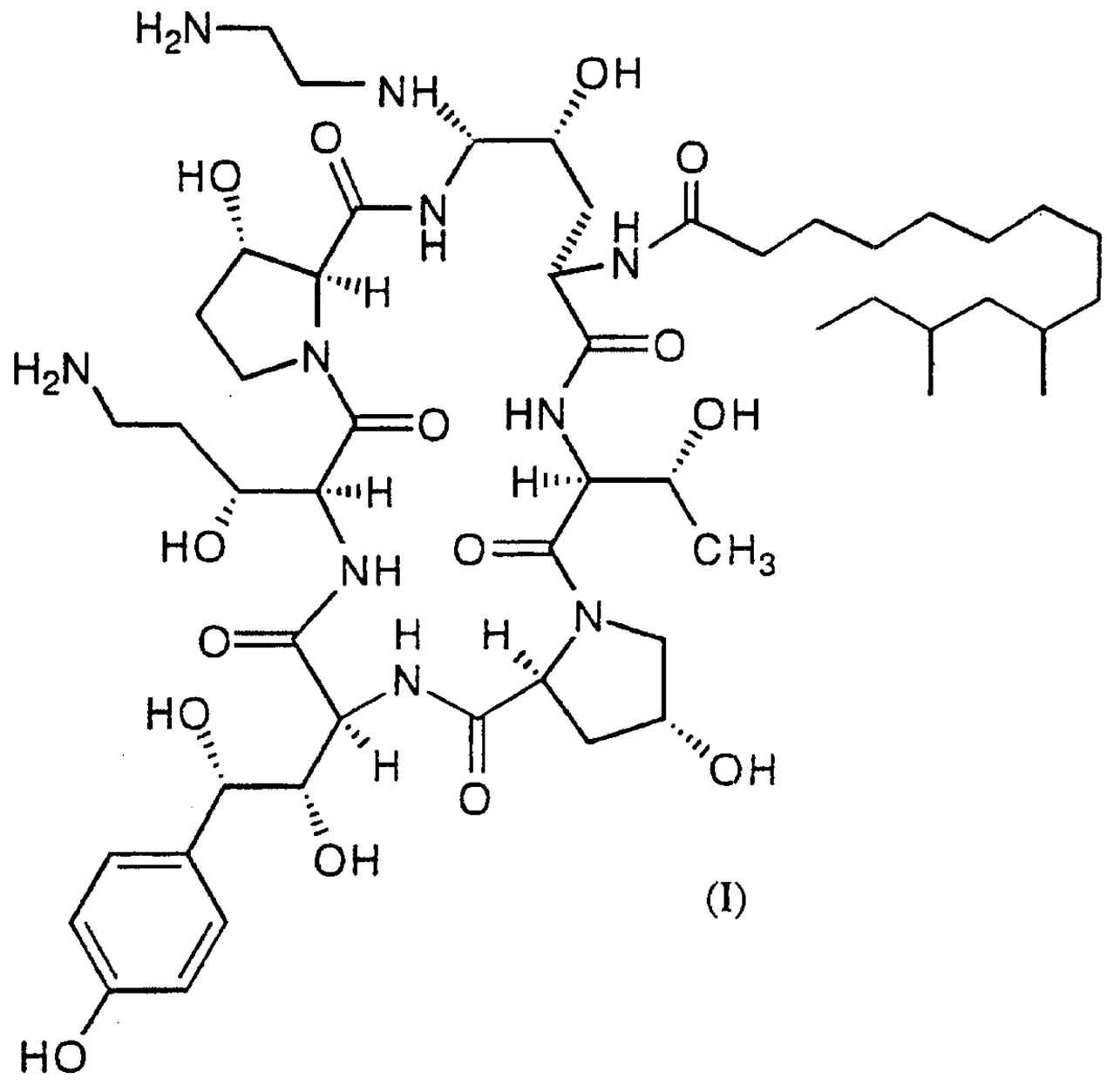
6. A combination of claim 1, 2 or 4 wherein the polyene is amphotericin B.

7. A combination of claim 1, 2, 3, 4, 5, or 6, wherein the infection is caused by a fungal pathogen selected from *Cryptococcus* spp., *Candida* spp., *Aspergillus* spp., *Histoplasma* spp., *Coccidioides* spp., *Paracoccidioides* spp., *Blastomyces* spp., *Fusarium* spp., *Sporothrix* spp., *Trichosporon* spp., *Rhizopus* spp., *Pseudallescheria* spp., dermatophytes, *Paeciliomyces* spp., *Alternaria* spp., *Curvularia* spp., *Exophiala* spp., *Wangiella* spp., *Penicillium* spp., *Saccharomyces* spp., *Dematiaceous* fungi and *Pneumocystis carinii*.

8. A combination of claim 7 wherein the fungal pathogen is selected from *Cryptococcus* spp., *Candida* spp. and *Aspergillus* spp.

9. A combination according to any one of claims 1 to 8 wherein each effective amount is associated with a pharmaceutically acceptable carrier.

10. Use of a pneumocandin derivative of formula (I)



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or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the combination therapy treatment with an antifungal agent selected from an azole, polyene, purine nucleotide inhibitor, pyrimidine nucleotide inhibitor, mannan inhibitor, protein elongation factor inhibitor, bactericidal/permeability inducing protein product and polyoxin, of a fungal infection.

11. Use of claim 10 wherein said antifungal agent is a polyene.

12. Use of claim 10 wherein said antifungal agent is an azole.

13. Use of claim 10, wherein the azole is selected from the group consisting of fluconazole, voriconazole, itraconazole, ketoconazole, miconazole, ER 30346 and SCH 56592; the polyene is selected from the group consisting of amphotericin B, nystatin and liposomal and lipid forms thereof; the purine or pyrimidine nucleotide inhibitor is flucytosine; the polyoxin is

nikkomycin Z, the elongation factor inhibitor is sordarin and analogs thereof and the mannan inhibitor is predamycin.

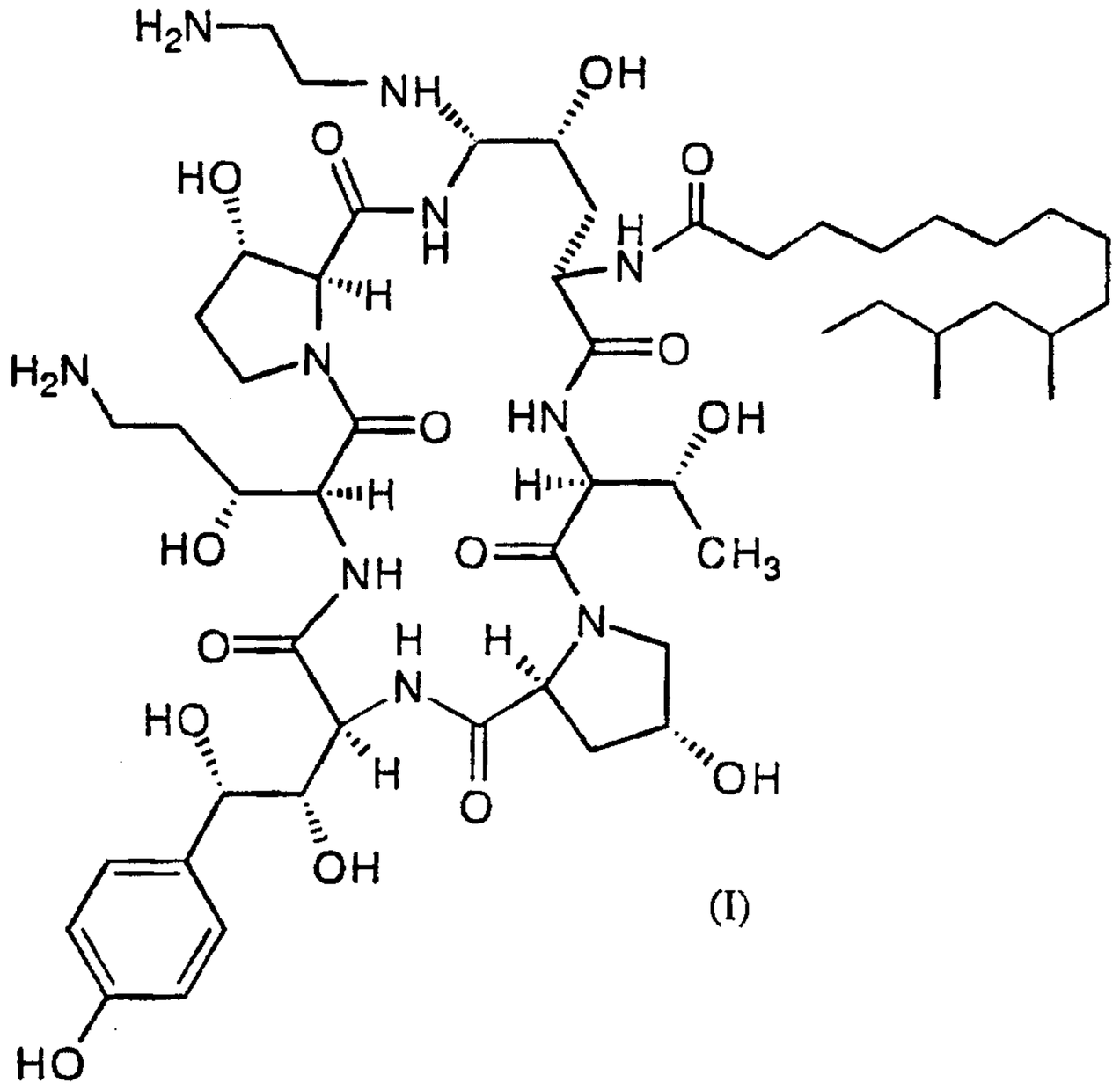
14. Use of claim 10, wherein the antifungal agent is fluconazole.

15. Use of claim 10, wherein the antifungal agent is amphotericin B.

16. Use of any one of claims 10 to 15 wherein the infection is caused by a fungal pathogen selected from *Cryptococcus* spp., *Candida* spp., *Aspergillus* spp., *Histoplasma* spp., *Coccidioides* spp., *Paracoccidioides* spp., *Blastomyces* spp., *Fusarium* spp., *Sporothrix* spp., *Trichosporon* spp., *Rhizopus* spp., *Pseudallescheria* spp., dermatophytes, *Paeciliomyces* spp., *Alternaria* spp., *Curvularia* spp., *Exophiala* spp., *Wangiella* spp., *Penicillium* spp., *Saccharomyces* spp., *Dematiaceous* fungi and *Pneumocystis carinii*.

17. Use of claim 16 wherein the fungal pathogen is selected from *Cryptococcus* spp., *Candida* spp. and *Aspergillus* spp.

18. A pneumocandin derivative of formula (I):



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or a pharmaceutically acceptable salt thereof for use in combination therapy, with an antifungal agent, for treating a fungal infection, said antifungal agent being an azole, polyene, purine nucleotide inhibitor, pyrimidine nucleotide inhibitor, mannan inhibitor, protein elongation factor inhibitor, bactericidal/permeability inducing protein product or polyoxin.

19. A pneumocandin derivative of claim 18, wherein said antifungal agent is a polyene.

20. A pneumocandin derivative of claim 18, wherein said antifungal agent is an azole.