Published in final edited form as:

Medchemcomm. 2014 May 1; 5(5): 671–676. doi:10.1039/C4MD00041B.

Synthesis and Antimicrobial Activities of His(2-aryl)-Arg and Trp-His(2-aryl) Classes of Dipeptidomimetics

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Abstract

In this communication, we report the design, synthesis and *in vitro* antimicrobial activity of ultra short peptidomimetics. Besides producing promising antibacterial activities against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), the dipeptidomimetics exhibited high antifungal activity against *C. neoformans* with IC₅₀ values in the range of 0.16-19 µg/mL. The most potent analogs exhibited 4-fold higher activity than the currently used drug amphotericin B, with no apparent cytotoxicity in a panel of mammalian cell lines.

Invasive antimicrobial infections are devastating and often classified as opportunistic or primary.¹ Opportunistic infections develop mainly in immunocompromised hosts, whereas primary infections can develop in immunocompetent hosts.² The causes of immunocompromisation include AIDS, azotemia, diabetes mellitus, bronchiectasis, emphysema, TB, lymphoma, leukemia, other hematologic cancers, burns, and therapy with corticosteroids or immunosuppressants.

Many fungi are opportunistic and are usually not pathogenic except in an immunocompromised host.³ Despite state-of-the-art antifungal therapy, the mortality rates for invasive infections with the three most common species of human fungal pathogens are *Candida albicans* (20-40%),⁴ *Aspergillus fumigatus* (50-90%),⁵ and *Cryptococcus neoformans* (20-70%).⁶ *C. neoformans* is one of the leading causes of opportunistic fungal infections in immunocompromised individuals worldwide.⁷ *Cryptococcus* is responsible for over 625,000 deaths annually within a growing cohort of susceptible individuals, particularly the AIDS population;⁸ therefore, the need for new agents to target this growing threat is vital.

The few available antimycotic agents target a limited repertoire of fungal-specific cell wall or membrane components and have high toxicity and poor efficacy.⁹ Drugs for systemic antifungal treatment include amphotericin B (and its lipid formulations), various azole derivatives, echinocandins, and flucytosine.¹⁰ Amphotericin B, an effective but relatively toxic drug, has long been the mainstay of antifungal therapy for invasive and serious mycoses.¹¹ However, newer potent and less toxic triazoles and echinocandins are now often recommended as first-line drugs for many invasive fungal infections.¹² These drugs have markedly changed the approach to antifungal therapy, sometimes even allowing oral treatment of chronic mycoses.

Unfortunately, the present repertoire of antifungal agents is limited, particularly in comparison to the number of agents available for bacterial infections.¹³ In fact, it took 30 years for the newest class of antifungal drugs, the echinocandins, to progress from bench-to-bedside.¹⁴ Furthermore, it is sobering to consider that the gold standard therapy for cryptococcal meningitis is based on medications (amphotericin B and flucytosine) that were discovered nearly 50 years ago. Although no “off-the-shelf” antifungal drugs have emerged from “repurposing” studies, the antifungal scaffolds with known pharmacological properties could serve as useful leads for further development.¹⁵

Over the past several years, peptides have formed the basis for the vast majority of anti-infective therapies in current clinical use.¹⁶ For example, the broad-spectrum antimicrobial peptide pexiganan (a 22-amino acid membrane disruptor analog of the *Xenopus* peptide magainin), used for the topical treatment of diabetic foot ulcers, has reached phase III in clinical trials.¹⁷ A synthetic mimic of indolicidin named omiganan has reached clinical trial phase II.¹⁸ Novexatin, the lead product of NovaBiotics, UK, a cyclic and highly cationic (arginine-rich) peptide based on human α - and β -defensins (among others), targets stubborn fungal infections in toenails.¹⁹ Other well-known peptides in various stages of clinical trials include OP-145, NVB302, and arenicin.²⁰ Some of the challenges facing these peptide-based drugs are poor metabolic stability, oral bioavailability, and membrane permeability as well as high production costs.

One possible answer for these problems is to design peptides of shorter length, while keeping the essential pharmacophore intact. In this direction, Svendsen and co-workers have synthesized a range of peptides of variable chain length.²¹ More recently, they disclosed a short synthetic peptidomimetic, LTX-109, exhibiting potent antimicrobial activity.²²⁻²³ In the recent past, we have reported the dipeptides having the motifs His-Arg and Trp-His as potent antimicrobial agents and tripeptides Arg-His(2-aryl)-Arg as potent antifungal agents.²⁴⁻²⁵ Keeping these facts in mind, we developed a series of dipeptides without increasing the length of the lead peptide. In this regard, we kept the arginine and tryptophan residues intact in the respective dipeptide motif and modified the histidine residue by placing a substitution at the C-2 position of the imidazole ring. It was reasoned that aryl substitution at the C-2 would provide the required bulk and hydrophobicity for membrane insertion without increasing the overall sequence length. The general scaffolds of the designed peptides are shown in Figure 1. A wide variety of lipophilic aryl substituents at the C-2 position of L-histidine were explored. To observe the effect of C-terminus capping, four series of peptides were synthesized having an NHBzl group and an OMe group at the C-terminus.

Starting materials *N*- α -Boc-2-aryl-L-histidines **3a-g** required for the synthesis of series 1-4 peptides were obtained regioselectively from *N*- α -trifluoroacetyl-L-histidine methyl ester by a recently developed homolytic free radical reaction using arylboronic acids.²⁶ The synthesis of designed peptides was accomplished using a recently developed environmentally benign microwave (MW)-assisted peptide synthesis protocol under solvent-free conditions.²⁷⁻²⁹ This method provides a new paradigm in solvent-free peptide synthesis assisted by microwave irradiation, using DIC-HONB as the coupling reagent combination. Key features of this original protocol are solvent-free synthesis, very short reaction time, and racemization-free synthesis in high purity. To confirm the racemization predicament, Boc-L-His-Arg-OMe, Boc-D-His-Arg-OMe, and Boc-D,L-His-Arg-OMe were synthesized under solvent-free MW irradiation. As evident from the HPLC chromatograms, purified peptides were free of racemization (see Supporting Information).

The synthesis of L-arginine benzylamide (**2**) was achieved by coupling of L-arginine (**1**) with benzylamine using 1,1'-carbonyldiimidazole (CDI) in water as described earlier.³⁰ L-Arginine benzylamide (**2**) upon solvent-free reaction with Boc-L-His(2-Ar)-OH (**3a-g**) using a coupling reagent combination of DIC, DIEA and HONB at 60°C for 15 min under MW irradiation gave protected dipeptides **4a-g**. The removal of the Boc group using aqueous 3N HCl at ambient temperature for 15 min cleanly afforded the designed dipeptides **5a-g** (Scheme 1).

L-arginine methyl ester dihydrochloride (**6**) required for the synthesis of **8a-g** was obtained by the reaction of L-arginine (**1**) with anhydrous HCl gas at 4°C in methanol. Compound **6** was first neutralized *in situ* using DIEA as a base and then subjected to coupling with Boc-His(2-Ar)-OH using a coupling reagents combination of DIC and HONB at 60°C for 15 min under MW irradiation to afford dipeptides **7a-g**. The removal of the Boc group as described above afforded the designed dipeptides **8a-g** (Scheme 2).

N- α -Boc-2-aryl-L-histidines **3a-g** upon condensation reaction with benzylamine in the presence of DIC and HONB afforded *N*- α -Boc-2-aryl-L-histidine benzylamides (**9a-g**). Compounds **9a-g**, upon acidolysis afforded the salts of 2-aryl-L-histidine benzylamide **10a-g**, which were neutralized *in situ* with DIEA and then coupled with Boc-Trp-OH using DIC and HONB at 60°C for 15 min under MW irradiation to give protected dipeptides **11a-g**. The latter compounds **11a-g** upon acidolysis afforded the desired peptides **12a-g** (Scheme 3).

In a similar fashion, 2-aryl-L-histidine methyl ester·2HCl (**14a-g**) were obtained by passing HCl gas into a mixture of 2-aryl-L-histidine (**13a-g**) in methanol at 4°C for 2 h followed by *in situ* neutralization using DIEA. The coupling of the latter compounds with Boc-Trp-OH using DIC and HONB at 60°C for 15 min under MW irradiation gave protected dipeptides **15a-g**, which upon acidolysis produced **16a-g** (Scheme 4).

Both free and Boc-protected peptides (**4**, **5**, **7**, **8**, **11**, **12**, **15**, and **16**) were evaluated for *in vitro* growth inhibition activity against fungal (*Candida albicans*, *C. glabrata*, *C. krusei*, *Aspergillus fumigatus* and *C. neoformans*) and bacterial (*Escherichia coli*, *Staphylococcus aureus* and methicillin-resistant *S. aureus*) strains (Tables 1-3). All the peptides were found

to be inactive against *Candida* species, *A. fumigatus*, and *E. coli* (results not included). The minimum inhibitory concentration (MIC) was measured using a protocol suggested by the Clinical and Laboratory Standard Institute (previously known as the National Committee for Clinical Laboratory Standards, NCCLS).³¹ Amphotericin B and ciprofloxacin served as positive controls in these studies.³²

The results of antifungal evaluation of His(2-aryl)-Arg peptides (Series 1 and 2) against *C. neoformans* are shown in Table 1. In general, we observed that the peptides with an NHBzl group at the C-terminus are more potent compared to their counterparts having a methyl ester linkage. For example, peptide **5e** (Ar = 4-*tert*-butylphenyl, R₁= NHBzl) displayed a much lower IC₅₀ value of 0.16 µg/mL as compared to peptide **8e** (Ar = 4-*tert*-butylphenyl, R₁= OMe), which exhibited an IC₅₀ value of 10.16 µg/mL. This difference in potency appears to be a consequence of enhanced hydrophobicity imparted by the NHBzl group. We also examined the effect of substitution at the C-2 position of L-histidine residue. It is noteworthy that peptides containing bulky substituents like 4-*tert*-butylphenyl, biphenyl and naphthyl groups (**5e**, **5f**, **5g**) exhibited high activity against *C. neoformans* with IC₅₀ values in the range of 0.16-0.62 µg/mL. The most potent peptide **5e**, which contained 4-*tert*-butylphenyl at the C-2 position of imidazole of histidine and an NHBzl group at the C-terminus, exhibited an IC₅₀ value of 0.16 µg/mL and MIC and MFC values of 0.31 µg/mL. The activity of **5e** is >4-fold higher than amphotericin B (IC₅₀ = 0.69 µg/mL, MIC = 1.25 µg/mL, and MFC = 1.25 µg/mL).

The relatively less bulky peptide **5f** (Ar = biphenyl, R₁= NHBzl) showed the second highest antifungal potency against *Cryptococcus* with an IC₅₀ value of 0.20 µg/mL and MIC and MFC values of 0.31 µg/mL. Moreover, peptide **5g** (Ar = naphthyl, R₁= NHBzl) showed activity (IC₅₀ = 0.62 µg/mL, MIC = 1.25 µg/mL, MFC = 1.25 µg/mL) comparable to that of amphotericin B. Other peptides of the series, **5b** (Ar = phenyl, R₁= NHBzl) and **5c** (Ar = tolyl, R₁= NHBzl), showed good activity with IC₅₀ values of 2.20 and 1.22 µg/mL, respectively. Surprisingly, peptide **5d** (Ar = anisoyl, R₁= NHBzl) was inactive against *C. neoformans*. The remaining peptides **4b-4g**, **7b**, **7c**, **7f**, **7g**, **8b**, and **8e-8g** also showed promising activity with IC₅₀ values in the range of 1-17 µg/mL.

The results for the Trp-His(2-aryl) peptides (Series 3 and 4) are shown in Table 2. These peptides in general are less active against *C. neoformans* as compared to the His(2-aryl)-Arg class of peptides. A possible explanation for this observation is the significant reduction in cationicity of peptides due to the incorporation of Trp residue. In a nut shell, the most potent peptides from these series exhibited activity against *C. neoformans* with IC₅₀ values in the range of 0.54-19 µg/mL. Apart from promising activities against *C. neoformans*, the peptides also showed encouraging activity against *S. aureus* and methicillin-resistant *S. aureus* (MRSA) as shown in Table 3. Analogs **4e**, **4f** and **12f** produced promising activity against *S. aureus* with IC₅₀ values of 5.95, 7.30 and 2.60 µg/mL, respectively. At the same time, analogs **12f** and **4e** were also effective against MRSA with IC₅₀ values of 4.31 and 9.32 µg/mL, respectively.

All the synthesized peptides were also evaluated for cytotoxicity in a panel of mammalian cell lines to determine their safety profile. The *in vitro* cytotoxicity was determined against

four human cancer cell lines (SK-MEL, KB, BT-549, and SK-OV-3) and two noncancerous mammalian cells (VERO and LLC-PK₁) by neutral red uptake assay.³³ The results demonstrated that the synthesized peptides were non-toxic up to a concentration of 10 µg/mL, which is indicative of a higher selectivity index for some compounds and their safety against mammalian cells.

To measure the hydrophobicity of the synthesized peptides (series 1 and 2), ClogP values were measured using ACD labs 12 software and compiled in Table 4. As expected, dipeptides having an ester linkage at C-terminus were found to be less hydrophobic than their amidated counterparts. From Table 4, we can clearly understand the hydrophobic nature of various aryl substituents and their effect on the activity against *C. neoformans*. From the values, it is indicated that the high hydrophobic characteristic was imparted by the 4-*tert*butylphenyl among all the incorporated aryl groups at the C-2 position of L-histidine. From the results, it can be concluded that among the biaryl groups (biphenyl and naphthyl), biphenyl imparts more hydrophobicity as compared to the naphthyl group. In conclusion, peptide **5e** being most hydrophobic in nature displays the highest activity with an IC₅₀ value of 0.16 µg/mL against *C. neoformans*, and the results are in good correlation.

Conclusions

In summary, we have prepared four series of dipeptides that were based on the pharmacophore model of short antimicrobial peptides. The peptides exhibited potent antifungal activity against *C. neoformans*. The results also demonstrated that the peptides of the His(2-aryl)-Arg class are more potent compared to Trp-His(2-aryl) class, owing to a delicate balance required between hydrophobicity and hydrophilicity in the peptidic structure. A combination of dual hydrophobic-hydrophilic amino acid (His), highly hydrophilic Arg residue, and placement of NHBzl group at the C-terminus appeared to be ideal for strong antimicrobial activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Amit Mahindra thanks the Council of Scientific and Industrial Research (CSIR), New Delhi, for the award of Senior Research Fellowship. The authors wish to thank Ms. Marsha Wright and Mr. John Trott for biological testing. This work was supported by the NIH, NIAID, Division of AIDS, Grant No. AI 27094 (antifungal), and the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-1-603 (antibacterial).

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Series 1. $R_1 = \text{NHBzl}$, $R_2 = \text{H}$
Series 2. $R_1 = \text{OMe}$, $R_2 = \text{H}$

Series 3. $R_1 = \text{NHBzl}$, $R_2 = \text{H}$
Series 4. $R_1 = \text{OMe}$, $R_2 = \text{H}$

Fig. 1.
Generalized structures of the synthesized dipeptides

Reagents and conditions: (i) $\text{C}_6\text{H}_5\text{CH}_2\text{NH}_2$, CDI, H_2O , 25 °C; (ii) DIEA, Boc-His(2-aryl)-OH (3), DIC, HONB, 15 min, 60 °C, MW; (iii) 3N HCl, rt, 15 min.

Scheme 1.
Synthesis of His(2-aryl)-Arg-NHBzl (5a-g, series 1)

Reagents and conditions: (i) MeOH, HCl gas, 4 °C, 2h, DIEA, 15 min; (ii) Boc-His(2-aryl)-OH (**3**), DIC, HONB, 15 min, 60 °C, MW; (iii) 3N HCl, rt, 15 min.

Scheme 2.

Synthesis of His(2-aryl)-Arg-OMe (8a-g, series 2)

Scheme 3.
Synthesis of Trp-His(2-aryl)-NHBzl (12a-g, series 3)

Reagents and conditions: (i) HCl gas, MeOH, 4 °C, 2h, DIEA, 15 min; (ii) Boc-Trp-OH, DIC, HONB, 60 °C, 15 min, MW; (iii) 3N HCl, rt, 15 min.

Scheme 4.

Synthesis of Trp-His(2-aryl)-OMe (16a-g, series 4)

Table 1

Anti-cryptococcal activity of dipeptides (Series 1 and 2)

Peptide	Ar	R ₁	R ₂	<i>C. neoformans</i> ^d			Cytotoxicity ^e	Selectivity Index ^f
				IC ₅₀ ^a	MIC ^b	MFC ^c	CTX (µg/mL)	<i>C. neoformans</i>
4a	H	NHBzl	Boc	NA	NA	NA	>10	–
4b	C ₆ H ₅	NHBzl	Boc	2.56	5	5	>10	>3.9
4c	4-CH ₃ -C ₆ H ₄	NHBzl	Boc	6.04	10	10	>10	>1.7
4d	4-OCH ₃ -C ₆ H ₄	NHBzl	Boc	5.77	10	10	>10	>1.7
4e	4-C(CH ₃) ₃ -C ₆ H ₄	NHBzl	Boc	2.42	2.50	2.50	>10	>4.1
4f	4-C ₆ H ₅ -C ₆ H ₄	NHBzl	Boc	1.09	2.5	2.5	>10	>9.2
4g	1-Naphthyl	NHBzl	Boc	2.79	5	5	>10	>3.6
5a	H	NHBzl	H	NA	NA	NA	>10	–
5b	C ₆ H ₅	NHBzl	H	1.22	2.5	2.5	>10	>8.2
5c	4-CH ₃ -C ₆ H ₄	NHBzl	H	2.11	2.50	2.50	>10	>4.7
5d	4-OCH ₃ -C ₆ H ₄	NHBzl	H	NA	NA	NA	>10	–
5e	4-C(CH ₃) ₃ -C ₆ H ₄	NHBzl	H	0.16	0.31	0.31	>10	>62.5
5f	4-C ₆ H ₅ -C ₆ H ₄	NHBzl	H	0.2	0.31	0.31	>10	>50
5g	1-Naphthyl	NHBzl	H	0.62	1.25	1.25	>10	>16.1
7a	H	OMe	Boc	NA	NA	NA	>10	–
7b	C ₆ H ₅	OMe	Boc	16.58	NA	NA	>10	–
7c	4-CH ₃ -C ₆ H ₄	OMe	Boc	16.94	NA	NA	>10	–
7d	4-OCH ₃ -C ₆ H ₄	OMe	Boc	NA	NA	NA	>10	–
7e	4-C(CH ₃) ₃ -C ₆ H ₄	OMe	Boc	NA	NA	NA	>10	–
7f	4-C ₆ H ₅ -C ₆ H ₄	OMe	Boc	4.91	10	10	>10	>2.0
7g	1-Naphthyl	OMe	Boc	1.25	2.5	2.5	>10	>8
8a	H	OMe	H	NA	NA	NA	>10	–
8b	C ₆ H ₅	OMe	H	10.56	NA	NA	>10	–
8c	4-CH ₃ -C ₆ H ₄	OMe	H	NA	NA	NA	>10	–
8d	4-OCH ₃ -C ₆ H ₄	OMe	H	NA	NA	NA	>10	–
8e	4-C(CH ₃) ₃ -C ₆ H ₄	OMe	H	10.16	20	20	>10	–
8f	4-C ₆ H ₅ -C ₆ H ₄	OMe	H	5.49	10	10	>10	>1.8
8g	1-Naphthyl	OMe	H	1.38	2.5	2.5	>10	>7.3
Amphotericin B				0.69	1.25	1.25		

For cytotoxicity experiments, the CTX value of the peptides was set to >10 µg/mL, in order to calculate a selectivity index. NA, not active.

^aIC₅₀ is the concentration (µg/mL) that affords 50% inhibition of growth

^bMIC (Minimum Inhibitory Concentration) is the lowest test concentration (µg/mL) that allows no detectable growth

^cMFC (Minimum Fungicidal Concentration) is the lowest test concentration (µg/mL) that kills the organism

^dHighest tested concentration was 20 µg/mL

^e highest tested concentration was 10 µg/mL

^f selectivity index was calculated as CTX divided by IC₅₀ values for *C. neoformans*.

Table 2

Anti-cryptococcal activity of dipeptides (Series 3 and 4)

Peptide	Ar	R ₁	R ₂	<i>C. neoformans</i> ^d			Cytotoxicity ^e	Selectivity Index ^f
				IC ₅₀ ^a	MIC ^b	MFC ^c		
							CTX (µg/mL)	<i>C. neoformans</i>
11a	H	NHBzl	Boc	NA	NA	NA	>10	–
11b	C ₆ H ₅	NHBzl	Boc	NA	NA	NA	>10	–
11c	4-CH ₃ -C ₆ H ₄	NHBzl	Boc	NA	NA	NA	>10	–
11d	4-OCH ₃ -C ₆ H ₄	NHBzl	Boc	NA	NA	NA	>10	–
11e	4-C(CH ₃) ₃ -C ₆ H ₄	NHBzl	Boc	NA	NA	NA	>10	–
11f	4-C ₆ H ₅ -C ₆ H ₄	NHBzl	Boc	NA	NA	NA	>10	–
11g	1-Naphthyl	NHBzl	Boc	NA	NA	NA	>10	–
12a	H	NHBzl	H	NA	NA	NA	>10	–
12b	C ₆ H ₅	NHBzl	H	2.04	5	5	>10	>4.9
12c	4-CH ₃ -C ₆ H ₄	NHBzl	H	7.84	20	NT	>10	>1.3
12d	4-OCH ₃ -C ₆ H ₄	NHBzl	H	10.82	10	NA	>10	–
12e	4-C(CH ₃) ₃ -C ₆ H ₄	NHBzl	H	4.53	10	10	>10	>2.21
12f	4-C ₆ H ₅ -C ₆ H ₄	NHBzl	H	0.54	1.25	1.25	>10	>18.5
12g	1-Naphthyl	NHBzl	H	NA	NA	NA	>10	–
15a	H	OMe	Boc	NA	NA	NA	>10	–
15b	C ₆ H ₅	OMe	Boc	NA	NA	NA	>10	–
15c	4-CH ₃ -C ₆ H ₄	OMe	Boc	NA	NA	NA	>10	–
15d	4-OCH ₃ -C ₆ H ₄	OMe	Boc	NA	NA	NA	>10	–
15e	4-C(CH ₃) ₃ -C ₆ H ₄	OMe	Boc	18.68	NA	NA	>10	–
15f	4-C ₆ H ₅ -C ₆ H ₄	OMe	Boc	NA	NA	NA	>10	–
15g	1-Naphthyl	OMe	Boc	NA	NA	NA	>10	–
16a	H	OMe	H	NA	NA	NA	>10	–
16b	C ₆ H ₅	OMe	H	7.97	20	20	>10	>1.3
16c	4-CH ₃ -C ₆ H ₄	OMe	H	11.88	NA	NA	>10	–
16d	4-OCH ₃ -C ₆ H ₄	OMe	H	NA	NA	NA	>10	–
16e	4-C(CH ₃) ₃ -C ₆ H ₄	OMe	H	18.84	NA	NA	>10	–
16f	4-C ₆ H ₅ -C ₆ H ₄	OMe	H	3.82	10	20	>10	>2.6
16g	1-Naphthyl	OMe	H	17.3	NA	NA	>10	–
Amphotericin B				0.69	1.25	1.25		

For cytotoxicity experiments, the CTX value of peptides was set to >10 µg/mL, in order to calculate a selectivity index. NA, not active. NT, not tested.

^aIC₅₀ is the concentration (µg/mL) that affords 50% inhibition of growth

^bMIC (Minimum Inhibitory Concentration) is the lowest test concentration (µg/mL) that allows no detectable growth

^cMFC (Minimum Fungicidal Concentration) is the lowest test concentration (µg/mL) that kills 100% of the organism

^d Highest tested concentration was 20 µg/mL

^e Highest tested concentration was 10 µg/mL

^f selectivity index was calculated as CTX divided by IC₅₀ values for *C. neoformans*.

Table 3Antibacterial activity of peptides against *S. aureus* and *MRSA*

Peptide	<i>S. aureus</i>			Methicillin-resistant <i>S. aureus</i> (MRSA)		
	IC ₅₀	MIC	MBC ^a	IC ₅₀	MIC	MBC ^a
4e	5.95	10	NA	9.32	20	NA
5e	15.86	NA	NA	NA	NA	NA
4f	7.30	10	20	8.97	20	NA
5f	12.84	NA	NA	NA	NA	NA
4g	16.37	20	20	13.20	20	NA
15f	18.98	NA	NA	NA	NA	NA
15e	15.06	NA	NA	NA	NA	NA
12f	2.60	5	20	4.31	10	20
12e	11.65	20	NA	NA	NA	NA
11e	10.15	NA	NA	NA	NA	NA
Cipro	0.08	0.25	0.50	0.09	0.25	0.50

^a MBC (Minimum Bactericidal Concentration) is the lowest test concentration (µg/mL) that kills 100% of the organisms. NA, not active.

Table 4

Correlation of hydrophobicity (ClogP) with anti-cryptococcal activity

Peptide	ClogP	<i>C. neoformans</i> IC ₅₀ (µg/mL)
5a	−1.36	NA
5b	0.68	1.22
5c	1.14	2.11
5d	0.85	NA
5e	2.37	0.16
5f	2.33	0.2
5g	1.92	0.62
8a	−2.51	NA
8b	−0.47	10.56
8c	−0.01	NA
8d	−0.30	NA
8e	1.22	10.16
8f	1.18	5.49
8g	0.76	1.38