Discovery of short peptides exhibiting high potency against *Cryptococcus neoformans*

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SUPPORTING INFORMATION

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1. General information

All starting materials were purchased from Sigma-Aldrich/Chem-Impex and used further without any additional purification. Analytical thin-layer chromatography (TLC) was performed using aluminum plates precoated with silica gel (0.25 mm, 60 Å pore-size) impregnated with a fluorescent indicator (254 nm). Visualization on TLC was achieved by the use of UV light (254 nm), treatment with 10% ninhydrin in ethanol or stained with iodine vapors. Flash column chromatography was undertaken on silica gel (230-400 mesh). Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on AVANCE III 400 Bruker (400 MHz). Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent (CD_3OD, δ 3.31). The following abbreviations were used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. Coupling constants, *J*, were reported in Hertz unit (Hz). Carbon 13 nuclear magnetic resonance spectroscopy (^{13}C NMR) was recorded on AVANCE III 400 Bruker (100 MHz) and was fully decoupled by broad band decoupling. Chemical shifts were reported in ppm referenced to the centre line at a 49.0 ppm of CD_3OD. High resolution mass spectra were taken with MAXIS-Bruker using ESI-TOF method. HPLC analysis was performed on the SHIMADZU-prominence using Supelcosil^{TM} LC-18 column (25 cm × 4.6 mm, 5µm) run for 40 min with a flow of 1 mL/min, using a gradient of 85-5% (A:B) where buffer A was 0.1% TFA in H_2O and buffer B was 0.1% TFA in CH_3CN and detection at 220 nm. All MW-irradiation reactions were carried out on a CEM Discover® microwave reactor.

2. Experimental procedure

2.1. General experimental procedure for *N*-α-Boc-2-aryl-L-histidines (5a-e)

To a solution of *N*-α-trifluoroacetyl-L-histidine methyl ester (**1**, 1.89 mmol, 1.0 equiv) in dichloromethane (CH_2Cl_2, 5 mL), trifluoroacetic acid (TFA, 2.83 mmol, 1.5 equiv) was added. After 5 min, a solution of silver nitrate (0.37 mmol, 0.2 equiv) in water (5 mL) was added, followed by arylboronic acid (3.77 mmol, 2 equiv). Ammonium persulfate (3.77 mmol, 2.0 equiv) was then added in one portion and the solution was stirred vigorously at ambient temperature for 10-18 h, and progress of the reaction was monitored by thin-layer chromatography analysis. Upon the completion of reaction, the pH of the reaction mixture was adjusted to 11-12 by the addition of aqueous ammonia solution. The residual CH_2Cl_2 was evaporated followed by dilution with water, and extraction of the reaction mixture with ethyl acetate (3 × 30 mL). The organic layer was washed with brine. The resulting organic solution

was dried over sodium sulfate, and filtered. The filtrate was concentrated, and the residue was purified by flash column chromatography to provide **3a-e**. 2-Aryl-L-histidines (**4a-e**) were synthesized by refluxing a solution of **3a-e** in 6N HCl for 16-24 h. 2-Aryl-L-histidine (**4a-e**, 1 mmol) was suspended in a mixture of water-dioxane (2:3). A solution of 4N NaOH was added and pH of the reaction mixture was adjusted to 12. After that *di-tert*-butyl dicarbonate (2 mmol) was added to the reaction mixture in one portion. After 10 min, pH was again adjusted to 12 and *di-tert*-butyl dicarbonate (2 mmol) was added in second portion. Reaction was allowed to stir at ambient temperature for 12 h. Solvent was removed under reduced pressure and methanol (10 mL) was added to the viscous residue. The resulting solution was stirred at ambient temperature for additional 12 h. The complete removal of solvent followed by treatment of resulting *N*-α-Boc-His(2-aryl)-O^-Na^+ with saturated aqueous solution of $KHSO_4$ to pH 3.75 generated the free *N*-α-Boc-2-aryl-L-histidine. The solvent was removed under reduced pressure and the resulting residue was extraction with *t*-butanol (4 × 50 mL). The evaporation of the solvent under reduced pressure afforded **5a-e**.[1]

2.2. General experimental procedure for dipeptides (7a-f and 8a-f)

In a 10 mL MW vial equipped with a magnetic stir bar, amino acid Arg-NHBzl/OMe (1 mmol) was dissolved in DMF (2.5 mL) followed by DIEA (3 mmol). Boc-2-aryl-His-OH (1.2 mmol) was added followed by DIC (1.2 mmol) and HONB (1.2 mmol). Mixture was subjected to MW-irradiation (CEM Discover® microwave reactor) with gas cooling (pressure of 40 psi was maintained during irradiation) for 28 min at 40W with magnetic stirring, and a temperature limit of 50 ˚C (reaction time refers to the hold time at the desired set temperature). DMF was evaporated and the reaction mixture was purified using CH_2Cl_2:MeOH (9:1), on a automated flash column chromatography system (Biotage®) to give Boc-His(2-aryl)-Arg-NHBzl/OMe.[2]

2.3. General experimental procedure for *N*-α-Boc-protected tripeptides (9a-f and 10a-f)

In a 10 mL MW vial equipped with a magnetic stir bar, Boc-His(2-aryl)-Arg-NHBzl/OMe(1 mmol) was subjected to 3N HCl in MeOH (5 mL) at 25 ˚C for 15 min. Dihydrochloride salt of dipeptide was neutralized with DIEA (3 mmol). The resulting His(2-aryl)-Arg-NHBzl/OMe (1 mmol) was dissolved in DMF (2.5 mL). Boc-Arg-OH (1.2 mmol) was added followed by HATU (1.2 mmol) and HOAt (1.2 mmol). Mixture was subjected to MW-irradiation (CEM Discover® microwave reactor) with gas cooling (pressure of 40 psi was maintained during irradiation) for 28 min at 40W with magnetic stirring, and a temperature limit of 50 ˚C (reaction time refers to the hold time at the desired set temperature). DMF was

evaporated and the reaction mixture was purified on automated flash column chromatography system (Biotage®) to afford tripeptides using CH_2Cl_2:MeOH (8:2). [2]

2.4. General experimental procedure for the synthesis of tripeptides (11a-f and 12a-f)

Boc-Arg-His(2-aryl)-Arg-NHBzl/OMe (1 mmol) upon reaction with 3N HCl in MeOH (5 mL) at 25 ˚C for 15 min resulted in the removal of Boc group to give desired tripeptides.

3. Product Characterization Data

Boc-His-Arg-NHBzl (7a): Yield: 50%; ^{1}H NMR (CD_3OD): δ 7.59 (s, 1H), 7.25-7.35 (m, 5H), 6.93 (s, 1H), 4.41 (s, 3H), 4.31-4.34 (m, 2H), 3.19-3.36 (m, 2H), 3.07 (dd, *J* = 5.90, 14.68 Hz, 1H), 2.95 (dd, *J* = 7.78, 14.81 Hz, 1H), 1.93 (d, *J* = 6.27 Hz, 1H), 1.75-1.78 (m, 1H), 1.60-1.65 (m, 2H), 1.35 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.4, 173.7, 157.8, 139.7, 136.3, 129.4, 128.5, 128.2, 81.0, 56.2, 54.3, 44.2, 42.1, 30.6, 30.1, 28.7, 26.2; HRMS (ESI-TOF): calculated for *m/z* 501.2938 [M+H$^+$], found 501.2938; HPLC: t_R= 25.16 min, 99.7%.

Boc-His(phenyl)-Arg-NHBzl (7b): Yield: 47%; ^{1}H NMR (CD_3OD): δ 7.82 (d, *J* = 7.28 Hz, 2H), 7.36-7.44 (m, 3H), 7.20-7.28 (m, 5H), 6.96 (s, 1H), 4.35-4.38 (m, 2H), 4.30-4.34 (m, 2H), 3.12-3.19 (m, 2H), 3.06-3.11 (m, 1H), 2.95 (dd, *J* = 8.16, 14.93 Hz, 1H), 1.83-1.90 (m, 1H), 1.72 (dt, *J* = 4.39, 9.10 Hz, 1H), 1.56-1.66 (m, 2H), 1.37 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.5, 173.6, 158.6, 147.8, 139.6, 131.0, 130.0, 129.6, 128.5, 128.3, 126.5, 80.9, 56.3, 54.3, 44.1, 42.0, 30.2, 28.7, 26.3; HRMS (ESI-TOF): calculated for *m/z* 577.3251 [M+H$^+$], found 577.3251; HPLC: t_R= 28.18 min, 97.3%.

Boc-His(4-methylphenyl)-Arg-NHBzl (7c): Yield: 45%; ^{1}H NMR (CD_3OD): δ 7.75 (d, *J* = 8.03 Hz, 2H), 7.27-7.32 (m, 4H), 7.23-7.26 (m, 3H), 6.99 (s, 1H), 4.41 (d, *J* = 3.76 Hz, 2H), 4.36 (d, *J* = 2.76 Hz, 2H), 3.17-3.23 (m, 2H), 3.13 (dd, *J* = 5.52, 15.06 Hz, 1H), 2.94-3.04 (m, 1H), 2.38 (s, 3H), 1.92 (d, *J* = 6.78 Hz, 1H), 1.77 (td, *J* = 4.52, 13.80 Hz, 1H), 1.61-1.68 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.4, 173.7, 158.6, 148.0, 140.4, 139.6, 130.6, 129.6, 128.5, 128.3, 128.0, 126.5, 81.0, 56.2, 54.4, 44.1, 42.0, 30.4, 28.7, 26.3, 21.4; HRMS (ESI-TOF): calculated for *m/z* 591.3407 [M+H$^+$], found 591.3407; HPLC: t_R= 30.34 min, 96.9%.

Boc-His(4-methoxyphenyl)-Arg-NHBzl (7d): Yield: 45%; ^{1}H NMR (CD_3OD): δ 7.79 (d, *J* = 8.78 Hz, 2H), 7.23-7.30 (m, 5H), 7.00 (m, 3H), 4.39-4.41 (m, 2H), 4.36 (s, 2H), 3.85 (s, 3H), 3.12-3.31 (m, 3H), 2.99-3.03 (m, 1H), 1.91(br.s., 1H) 1.76-1.79 (m, 1H), 1.63-1.65 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.4, 173.6, 162.2, 158.6, 139.7, 129.6, 128.5,

128.1, 115.3, 81.1, 56.0, 54.8, 54.3, 44.1, 41.9, 30.2, 28.6, 26.4; HRMS (ESI-TOF): calculated for *m/z* 606.3278 [M+H$^+$], found 606.3278; HPLC: t_R= 29.61 min, 98.1%.

Boc-His(4-*t*-butylphenyl)-Arg-NHBzl (7e): Yield: 48%; ^{1}H NMR (CD_3OD): δ 7.87-7.90 (m, 2H), 7.53 (d, *J* = 8.53 Hz, 2H), 7.31-7.35 (m, 2H), 7.23-7.26 (m, 3H), 6.93 (s, 1H), 4.93-4.98 (m, 1H), 4.65-4.68 (m, 1H), 4.46 (s, 2H), 3.03-3.10 (m, 2H), 2.64-2.67 (m, 2H), 1.84-1.87 (m, 2H), 1.63-1.69 (m, 2H), 1.41 (s, 9H), 1.33 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.4, 173.7, 158.6, 153.6, 139.6, 129.6, 128.5, 128.3, 126.9, 126.4, 81.0, 56.2, 54.4, 44.1, 42.0, 35.6, 31.7, 30.2, 28.7, 27.7, 26.3; HRMS (ESI-TOF): calculated for *m/z* 633.6877 [M+H$^+$], found 633.6871; HPLC: t_R= 36.86 min, 95.6%.

Boc-His(biphenyl)-Arg-NHBzl (7f): Yield: 47%; ^{1}H NMR (CD_3OD): δ 7.92 (d, *J* = 8.53 Hz, 1H), 7.85 (d, *J* = 7.28 Hz, 1H), 7.66 (d, *J* = 8.53 Hz, 1H), 7.61-7.64 (m, 2H), 7.39-7.48 (m, 3H), 7.31-7.39 (m, 1H), 7.20-7.28 (m, 5H), 6.99 (d, *J* = 11.04 Hz, 1H), 4.40-4.43 (m, 2H), 4.33-4.38 (m, 2H), 3.16-3.23 (m, 2H), 3.10-3.14 (m, 1H), 2.97-3.05 (m, 1H), 1.88-1.93 (m, 1H), 1.76 (td, *J* = 4.74, 9.35 Hz, 1H), 1.60-1.70 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (CD_3OD):δ 174.5, 173.7, 158.6, 142.8, 141.5, 139.6, 130.0, 129.6, 128.8, 128.5, 128.3, 127.9, 127.0, 126.5, 81.0, 54.9, 54.5, 44.1, 42.0, 30.3, 28.7, 26.3; HRMS (ESI-TOF): calculated for *m/z* 653.3564 [M+H$^+$], found 653.3564; HPLC: t_R= 34.91 min, 97.9%.

Boc-His-Arg-OMe (8a): Yield: 56%; ^{1}H NMR (CD_3OD): δ 7.71 (s, 1H), 6.93 (s, 1H), 4.48 (s, 1H), 4.32 (s, 1H), 3.73 (s, 3H), 3.22-3.28 (m, 2H), 3.04-3.08 (m, 1H), 2.87-2.92 (m, 1H), 1.94 (d, *J* = 7.0 Hz, 1H), 1.77 (br.s., 1H), 1.66 (d, *J* = 7.0 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.8, 173.5, 158.6, 136.2, 129.7, 80.9, 56.1, 53.3, 52.9, 41.8, 30.6, 29.8, 28.7, 26.0; HRMS (ESI-TOF): calculated for *m/z* 426.2465 [M+H$^+$], found 426.2465; HPLC: t_R= 22.14 min, 95.3%.

Boc-His(phenyl)-Arg-OMe (8b): Yield: 58%; ^{1}H NMR (CD_3OD): δ 7.87 (d, *J* = 7.53 Hz, 1H), 7.67 (s, 1H), 7.37-7.53 (m, 3H), 6.97 (s, 1H), 4.48 (dd, *J* = 4.52, 8.53 Hz, 1H), 4.38-4.44 (m, 1H), 3.66 (s, 3H), 3.21 (d, *J* = 6.53 Hz, 2H), 3.13 (dd, *J* = 5.52, 15.06 Hz, 1H), 2.97 (d, *J* = 8.53 Hz, 1H), 1.89-2.01 (m, 1H), 1.77 (d, *J* = 8.03 Hz, 1H), 1.62-1.73 (m, 2H), 1.30 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.6, 173.5, 158.6, 157.8, 131.1, 127.9, 126.9, 126.4, 80.8, 56.2, 53.1, 41.9, 30.7, 29.7, 28.7, 26.1; HRMS (ESI-TOF): calculated for *m/z* 502.2778 [M+H$^+$], found 502.2778; HPLC: t_R= 24.16 min, 96.7%.

Boc-His(4-methylphenyl)-Arg-OMe (8c): Yield: 57%; ^{1}H NMR (CD_3OD): δ 7.75 (d, *J* = 7.53 Hz, 2H), 7.28 (d, *J* = 7.78 Hz, 2H), 6.98 (s, 1H), 4.48 (dd, *J* = 4.64, 8.66 Hz, 1H), 4.40 (t, *J* = 6.65 Hz, 1H), 3.70 (s, 3H), 3.21 (d, *J* = 6.53 Hz, 2H), 3.12 (dd, *J* = 5.02, 14.81 Hz,

1H), 2.94 (dd, *J* = 8.66, 14.43 Hz, 1H), 2.38 (s, 3H), 1.89-1.98 (m, 1H), 1.77 (d, *J* = 7.78 Hz, 1H), 1.63-1.71 (m, 2H), 1.34 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.6, 173.5, 158.6, 140.4, 130.6, 126.5, 80.8, 56.1, 54.9, 53.1, 41.9, 30.6, 29.7, 28.7, 26.2, 21.4; HRMS (ESI-TOF): calculated for *m/z* 516.2934 [M+H$^+$], found 516.2939; HPLC: t_R= 26.24 min, 98.0%.

Boc-His(4-methoxyphenyl)-Arg-OMe (8d): Yield: 55%; ^{1}H NMR (CD_3OD): δ 7.76 (d, *J* = 8.03 Hz, 2H), 7.01 (d, *J* = 8.03 Hz, 2H), 6.97 (s, 1H), 4.49 (dd, *J* = 4.77, 8.53 Hz, 1H), 4.39 (t, *J* = 6.65 Hz, 1H), 3.86 (s, 3H), 3.70 (s, 3H), 3.17-3.26 (m, 2H), 3.11 (dd, *J* = 5.27, 14.81 Hz, 1H), 2.93 (dd, *J* = 8.91, 14.18 Hz, 1H), 1.89-2.02 (m, 1H), 1.74-1.81 (m, 1H), 1.63-1.71 (m, 2H), 1.37 (s, 9H); ^{13}C NMR (CD_3OD): δ 173.5, 162.0, 158.6, 130.9, 128.1, 115.4, 80.8, 55.9, 54.3, 53.1, 41.9, 29.7, 28.7, 26.1; HRMS (ESI-TOF): calculated for *m/z* 532.2883 [M+H$^+$], found 532.2887; HPLC: t_R= 23.76 min, 97.6%.

Boc-His(4-*t*-butylphenyl)-Arg-OMe (8e): Yield: 55%; ^{1}H NMR (CD_3OD): δ 7.82-7.87 (m, 2H), 7.53 (d, *J* = 8.03 Hz, 2H), 6.90 (s, 1H), 4.90-4.93 (m, 1H), 4.63-4.68 (m, 1H), 3.61 (s, 3H), 3.03-3.08 (m, 2H), 2.61-2.67 (m, 2H), 1.84-1.89 (m, 2H), 1.58-1.63 (m, 2H), 1.37 (s, 9H), 1.35 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.6, 173.5, 158.6, 140.4, 130.6, 126.5, 80.8, 56.1, 54.9, 53.1, 41.9, 30.6, 29.7, 28.7, 26.2, 31.4; HRMS (ESI-TOF): calculated for *m/z* 558.3404 [M+H$^+$], found 558.3408; HPLC: t_R= 29.78 min, 96.4%.

Boc-His(biphenyl)-Arg-OMe (8f): Yield: 60%; ^{1}H NMR (CD_3OD): δ 7.93 (d, *J* = 8.03 Hz, 2H), 7.71 (d, *J* = 7.78 Hz, 2H), 7.67 (d, *J* = 7.53 Hz, 2H), 7.46 (t, *J* = 7.40 Hz, 2H), 7.34-7.40 (m, 1H), 7.03 (s, 1H), 4.50 (dd, *J* = 4.77, 8.78 Hz, 1H), 4.42 (t, *J* = 6.53 Hz, 1H), 3.70 (s, 3H), 3.19-3.27 (m, 2H), 3.11-3.17 (m, 1H), 2.97 (dd, *J* = 8.53, 14.56 Hz, 1H), 1.95 (dd, *J* = 6.02, 13.55 Hz, 1H), 1.75-1.83 (m, 1H), 1.64-1.71 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.6, 173.5, 158.6, 142.8, 141.5, 130.0, 129.9, 128.8, 128.5, 127.9, 126.9, 80.8, 56.1, 53.1, 41.9, 29.8, 28.7, 26.2; HRMS (ESI-TOF): calculated for *m/z* 578.3091 [M+H$^+$], found 578.3091; HPLC: t_R= 28.86 min, 95.9%.

Boc-Arg-His-Arg-NHBzl (9a): Yield: 45%; ^{1}H NMR (CD_3OD): δ 8.70 (s, 1H), 7.38 (s, 1H), 7.27-7.36 (m, 5H), 4.74 (t, *J* = 6.02 Hz, 1H), 4.44 (s, 2H), 4.35-4.38 (m, 1H), 4.01-4.05 (m, 1H), 3.18-3.23 (m, 6H), 1.86-1.92 (m, 1H), 1.78-1.82 (m, 2H), 1.64-1.70 (m, 5H), 1.46 (s, 9H); ^{13}C NMR (CD_3OD): δ 175.0, 173.6, 173.1, 172.1, 158.7, 145.9, 133.5, 131.3, 131.2, 130.9, 130.8, 127.9, 124.4, 119.6, 81.0, 61.6, 55.7, 53.6, 53.4, 53.0, 42.0, 41.8, 30.0, 29.4, 28.7, 28.4, 26.3, 20.9; HRMS (ESI-TOF): calculated for *m/z* 657.3949 [M+H$^+$], found 657.3956; HPLC: t_R= 16.41 min, 97.5%.

Boc-Arg-His(phenyl)-Arg-NHBzl (9b): Yield: 43%; ^{1}H NMR (CD_3OD): δ 7.89-7.91 (m, 2H), 7.34-7.42 (m, 3H), 7.12-7.27 (m, 5H), 6.96 (s, 1H), 4.61 (s, 2H), 4.35 (d, *J* = 13.55 Hz, 2H), 3.88-3.91 (m, 1H), 3.12 (br.s., 4H), 2.82-3.00 (m, 2H), 1.93-2.02 (m, 1H), 1.90 (br.s., 1H), 1.38-1.45 (m, 6H), 1.27 (s, 9H); ^{13}C NMR (CD_3OD): δ 175.4, 173.7, 158.6, 148.1, 139.6, 131.4, 130.0, 129.8, 129.5, 128.9, 128.5, 128.2, 126.5, 81.1, 56.4, 55.4, 54.4, 44.1, 42.0, 41.9, 29.9, 28.6, 26.3, 26.2, 22.9; HRMS (ESI-TOF): calculated for *m/z* 733.4262 [M+H$^+$], found 733.4115; HPLC: t_R= 17.63 min, 98.7%.

Boc-Arg-His(4-methylphenyl)-Arg-NHBzl (9c): Yield: 43%; ^{1}H NMR (CD_3OD): δ 7.83 (d, *J* = 6.02 Hz, 2H), 7.34-7.42 (m, 3H), 7.26.7.30 (m, 4H), 7.12 (s, 1H), 4.78-4.81 (m, 1H), 4.60 (s, 2H) 4.44 (dd, *J* = 5.02, 8.53 Hz, 1H), 4.04 (d, *J* = 7.03 Hz, 1H), 3.12-3.26 (m, 6H), 2.45 (s, 3H), 1.91-2.00 (m, 1H), 1.74-1.81 (m, 2H), 1.60-1.72 (m, 5H), 1.32 (s, 9H); ^{13}C NMR (CD_3OD): δ 175.1, 174.5, 172.0, 163.1, 158.7, 139.8, 133.8, 131.4, 130.7, 129.6, 128.6, 127.9, 121.4, 119.7, 81.0, 55.7, 54.8, 54.6, 51.9, 44.2, 44.1, 41.9, 31.2, 30.2, 28.7, 26.4, 26.3, 21.6; HRMS (ESI-TOF): calculated for *m/z* 747.4418 [M+H$^+$], found 747.4415; HPLC: t_R= 19.41 min, 98.1%.

Boc-Arg-His(4-methoxyphenyl)-Arg-NHBzl (9d): Yield: 40%; ^{1}H NMR (CD_3OD): δ 7.88 (d, *J* = 8.03 Hz, 2H), 7.39 (s, 1H), 7.15-7.29 (m, 5H), 7.05 (d, *J* = 8.53 Hz, 2H), 5.01 (br.s., 1H), 4.80 (s, 1H), 4.35-4.47 (m, 2H), 4.06 (s, 1H), 3.87 (s, 3H), 3.19-3.28 (m, 5H), 1.89 (br.s., 1H), 1.76-1.86 (m, 2H), 1.65-1.67 (m, 6H), 1.37 (s, 9H); ^{13}C NMR (CD_3OD): δ 175.1, 174.1, 172.0, 164.3, 158.6, 139.6, 130.3, 129.8, 129.5, 128.3, 119.4, 116.1, 81.0, 56.1, 54.7, 53.2, 44.2, 41.9, 30.2, 30.0, 28.6, 26.4; HRMS (ESI-TOF): calculated for *m/z* 763.4367 [M+H$^+$], found 763.4369; HPLC: t_R= 18.93 min, 96.4%.

Boc-Arg-His(4-*t*-butylphenyl)-Arg-NHBzl (9e): Yield: 45%; ^{1}H NMR (CD_3OD): δ 7.87-7.90 (m, 2H), 7.53 (d, *J* = 7.53 Hz, 2H), 7.29-7.31 (m, 5H), 6.93 (s, 1H), 4.91-4.93 (m, 1H), 4.59-4.68 (m, 2H), 4.46 (s, 2H), 3.01-3.03 (m, 5H), 2.61-2.67 (m, 2H), 1.84-1.89 (m, 2H), 1.63-1.67 (m, 5H), 1.35 (s, 9H), 1.29 (s, 9H); ^{13}C NMR (CD_3OD): δ 175.7, 173.7, 158.6, 153.1, 148.3, 129.5, 128.6, 128.5, 128.2, 126.9, 126.4, 81.1, 56.5, 55.5, 54.4, 42.0, 37.9, 35.6, 31.7, 29.9, 29.6, 28.6, 26.3; HRMS (ESI-TOF): calculated for *m/z* 789.4888 [M+H$^+$], found 789.4867; HPLC: t_R= 24.41 min, 97.3%.

Boc-Arg-His(biphenyl)-Arg-NHBzl (9f): Yield: 41%; ^{1}H NMR (CD_3OD): δ 8.02 (d, *J* = 7.03 Hz, 1H), 7.93 (d, *J* = 6.53 Hz, 1H), 7.71 (d, *J* = 7.53 Hz, 1H), 7.66 (d, *J* = 7.03 Hz, 1H), 7.44-7.51 (m, 3H), 7.39 (d, *J* = 6.53 Hz, 1H), 7.24-7.35 (m, 6H), 7.02 (d, *J* = 10.54 Hz, 1H), 4.67-4.71 (m, 1H), 4.43-4.38 (m, 2H), 4.25 (s, 2H), 3.18-3.27 (m, 5H), 3.00 (d, *J* = 15.56 Hz,

1H), 1.96 (br.s., 1H), 1.73-1.81 (m, 2H), 1.61-1.72 (m, 5H), 1.33 (s, 9H); ^{13}C NMR (CD_3OD): δ 176.7, 175.4, 173.6, 163.3, 158.6, 142.7, 139.6, 130.0, 129.5, 128.7, 128.5, 128.2, 127.9, 127.0, 126.5, 122.9, 117.7, 103.1, 81.1, 73.9, 60.2, 54.4, 42.0, 29.9, 29.6, 28.6, 26.2, 8.5; HRMS (ESI-TOF): calculated for *m/z* 809.4575 [M+H$^+$], found 809.4878; HPLC: t_R= 24.04 min, 99.1%.

Boc-Arg-His-Arg-OMe (10a): Yield: 42%; ^{1}H NMR (CD_3OD): δ 7.69 (s, 1H), 6.93 (s, 1H), 4.51 (q, *J* = 6.6 Hz, 1H), 4.28 (dd, *J* = 4.9, 9.2 Hz, 1H), 3.81-3.92 (m, 1H), 3.63 (s, 3H), 3.02-3.12 (m, 4H), 2.92-3.02 (m, 2H), 1.66-1.82 (m, 1H), 1.51-1.62 (m, 2H), 1.40-1.49 (m, 5H), 1.31 (s, 9H); ^{13}C NMR (CD_3OD): δ 178.1, 174.5, 173.3, 171.6, 156.6, 155.5, 135.7, 135.4, 132.2, 117.5, 82.0, 54.8, 53.2, 52.9, 52.2, 40.6, 28.7, 28.2, 27.5, 24.3, 22.5; HRMS (ESI-TOF): calculated for *m/z* 582.3476 [M+H$^+$], found 582.3476; HPLC: t_R= 10.75 min, 98%.

Boc-Arg-His(phenyl)-Arg-OMe (10b): Yield: 40%; ^{1}H NMR (CD_3OD): δ 7.97 (d, *J* = 6.5 Hz, 2H), 7.59-7.76 (m, 3H), 7.48 (s, 1H), 4.82 (t, *J* = 6.5 Hz, 1H), 4.47 (dd, *J* = 4.6, 8.9 Hz, 1H), 4.02-4.08 (m, 1H), 3.67 (s, 3H), 3.16-3.29 (m, 5H), 1.96 (d, *J* = 3.5 Hz, 2H), 1.77 (d, *J* = 9.3 Hz, 2H), 1.59-1.74 (m, 5H), 1.42 (s, 9H); ^{13}C NMR (CD_3OD): δ 175.0, 173.6, 173.1, 172.1, 158.7, 145.9, 133.5, 131.3, 131.2, 130.9, 130.8, 127.9, 124.4, 119.6, 81.0, 61.6, 55.7, 53.6, 53.4, 53.0, 42.0, 41.8, 30.0, 29.4, 28.7, 28.4, 26.3, 20.9; HRMS (ESI-TOF): calculated for *m/z* 658.3789 [M+H$^+$], found 658.3783; HPLC: t_R= 11.89 min, 96.4%.

Boc-Arg-His(4-methylphenyl)-Arg-OMe (10c): Yield: 44%; ^{1}H NMR (CD_3OD): δ 7.83 (d, *J* = 6.02 Hz, 2H), 7.46 (d, *J* = 8.03 Hz, 2H), 7.41 (s, 1H), 4.78 (br.s., 1H), 4.44 (dd, *J* = 5.02, 8.53 Hz, 1H), 4.04 (d, *J* = 7.03 Hz, 1H), 3.69 (s, 3H), 3.18-3.24 (m, 6H), 2.45 (s, 3H), 1.91-1.94 (m, 1H), 1.71-1.76 (m, 1H), 1.63-1.67 (m, 6H), 1.40 (s, 9H); ^{13}C NMR (CD_3OD): δ 175.3, 174.0, 172.2, 158.8, 144.8, 131.6, 131.0, 127.6, 121.4, 119.4, 81.4, 55.7, 53.6, 53.2, 42.1, 41.8, 30.0, 29.4, 28.7, 26.4, 21.6; HRMS (ESI-TOF): calculated for *m/z* 672.3945 [M+H$^+$], found 672.3945; HPLC: t_R= 14.76 min, 95.5%.

Boc-Arg-His(4-methoxyphenyl)-Arg-OMe (10d): Yield: 44%; ^{1}H NMR (CD_3OD): δ 7.82 (d, *J* = 8.8 Hz, 2H), 6.98-7.03 (m, 2H), 6.94 (s, 1H), 4.65-4.70 (m, 1H), 4.43 (dd, *J* = 4.9, 9.2 Hz, 1H), 4.01-4.07 (m, 1H), 3.84 (s, 3H), 3.71 (s, 3H), 3.06-3.27 (m, 5H), 2.94 (br.s., 1H), 1.87-1.99 (m, 1H), 1.74-1.82 (m, 2H), 1.59-1.70 (m, 5H), 1.31 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.8, 174.1, 173.5, 161.7, 158.6, 148.1, 127.9, 124.2, 115.3, 81.0, 55.9, 54.9, 53.3, 52.9, 42.0, 41.9, 40.4, 30.2, 29.5, 28.7, 26.2; HRMS (ESI-TOF): calculated for *m/z* 688.3894 [M+H$^+$], found 688.3894; HPLC: t_R= 13.59 min, 97.1%.

Boc-Arg-His(4-*t*-butylphenyl)-Arg-OMe (10e): Yield: 45%; ^{1}H NMR (CD_3OD): δ 7.79-7.84 (m, 2H), 7.51 (d, *J* = 8.53 Hz, 2H), 6.98 (s, 1H), 4.67-4.70 (m, 1H), 4.42-4.45 (m, 1H), 4.03-4.05 (m, 1H), 3.71 (s, 3H), 3.10-3.16 (m, 6H), 1.92-1.95 (m, 1H), 1.73-1.80 (m, 1H), 1.52-1.64 (m, 5H), 1.46(s, 18H); ^{13}C NMR (CD_3OD): δ 174.8, 174.1, 173.5, 161.7, 158.6, 148.1, 127.9, 124.2, 115.3, 81.0, 55.9, 54.9, 53.3, 52.9, 42.0, 41.9, 40.4, 30.2, 29.5, 28.7, 26.2; HRMS (ESI-TOF): calculated for *m/z* 714.4415 [M+H$^+$], found 714.4115; HPLC: t_R= 20.91 min, 97.3%.

Boc-Arg-His(biphenyl)-Arg-OMe (10f): Yield: 45%; ^{1}H NMR (CD_3OD): δ 7.95-8.05 (m, 2H), 7.73 (d, *J* = 8.28 Hz, 2H), 7.64-7.70 (m, 2H), 7.47 (t, *J* = 7.65 Hz, 2H), 7.34-7.41 (m, 1H), 7.04 (d, *J* = 9.54 Hz, 1H), 4.42-4.49 (m, 2H), 4.06 (d, *J* = 6.53 Hz, 1H), 3.69 (s, 3H), 3.15-3.25 (m, 5H), 2.93 (t, *J* = 6.53 Hz, 1H), 1.91-2.00 (m, 1H), 1.77-1.86 (m, 1H), 1.59-1.74 (m, 4H), 1.44-1.46 (m, 2H), 1.31 (s, 9H); ^{13}C NMR (CD_3OD): δ 179.3, 174.5, 173.6, 158.6, 157.4, 147.7, 143.1, 141.1, 130.1, 128.8, 127.9, 126.9, 122.9, 119.8, 113.6, 109.9, 98.2, 97.1, 80.8, 55.8, 53.1, 41.9, 32.1, 29.6, 28.7, 26.2, 13.4; HRMS (ESI-TOF): calculated for *m/z* 734.4102 [M+H$^+$], found 734.4115; HPLC: t_R= 18.64 min, 98.7%.

Arg-His-Arg-NHBzl (11a): Yield: 95%; ^{1}H NMR (CD_3OD): δ 8.70 (s, 1H), 7.38 (s, 1H), 7.30-7.35 (m, 5H), 4.73 (t, *J* = 6.02 Hz, 1H), 4.44 (s, 2H), 4.35-4.39 (m, 1H), 4.00-4.07 (m, 1H), 3.18-3.23 (m, 6H), 1.86-1.92 (m, 1H), 1.78-1.82 (m, 2H), 1.63-1.70 (m, 5H); ^{13}C NMR (CD_3OD): δ 175.0, 173.6, 173.1, 172.1, 145.9, 133.5, 131.3, 131.2, 130.9, 130.8, 127.9, 124.4, 119.6, 61.6, 55.7, 53.6, 53.4, 53.0, 42.0, 41.8, 30.0, 29.4, 26.3, 20.9; HRMS (ESI-TOF): calculated for *m/z* 557.3424 [M+H$^+$], found 557.3429; HPLC: t_R= 12.58 min, 96.3%.

Arg-His(phenyl)-Arg-NHBzl (11b): Yield: 93%; ^{1}H NMR (CD_3OD): δ 7.86-7.88 (m, 2H), 7.34-7.42 (m, 3H), 7.12-7.27 (m, 5H), 6.96 (s, 1H), 4.60-4.63 (m, 1H), 4.35 (d, *J* = 13.55 Hz, 3H), 3.88-3.91 (m, 1H), 3.12-3.21 (m, 4H), 2.82-3.00 (m, 2H), 1.93-2.02 (m, 1H), 1.90 (br.s., 1H), 1.38-1.45 (m, 6H); ^{13}C NMR (CD_3OD): δ 175.4, 173.7, 148.1, 139.6, 131.4, 130.0, 129.8, 129.5, 128.9, 128.5, 128.2, 126.5, 56.4, 55.4, 54.4, 44.1, 42.0, 41.9, 29.9, 26.3, 26.2, 22.9; HRMS (ESI-TOF): calculated for *m/z* 633.3737 [M+H$^+$], found 633.3730; HPLC: t_R= 14.60 min, 98.9%.

Arg-His(4-methylphenyl)-Arg-NHBzl (11c): Yield: 92%; ^{1}H NMR (CD_3OD): δ 7.83 (d, *J* = 6.02 Hz, 2H), 7.34-7.42 (m, 3H), 7.35-7.40 (m, 4H), 7.41 (s, 1H), 4.78-4.81 (m, 1H), 4.60 (s, 2H) 4.44 (dd, *J* = 5.02, 8.53 Hz, 1H), 4.04 (d, *J* = 7.03 Hz, 1H), 3.12-3.26 (m, 6H), 2.45 (s, 3H), 1.91-2.00 (m, 1H), 1.74-1.81 (m, 2H), 1.60-1.72 (m, 5H); ^{13}C NMR (CD_3OD): δ 175.1, 174.5, 172.0, 163.1, 139.8, 133.8, 131.4, 130.7, 129.6, 128.6, 127.9, 121.4, 119.7, 55.7, 54.8,

54.6, 51.9, 44.2, 44.1, 41.9, 31.2, 30.2, 26.4, 26.3, 21.6; HRMS (ESI-TOF): calculated for *m/z* 647.3893 [M+H$^+$], found 647.3893; HPLC: t_R= 15.89 min, 97.7%.

Arg-His(4-methoxyphenyl)-Arg-NHBzl (11d): Yield: 91%; ^{1}H NMR (CD_3OD): δ 7.85-7.92 (m, *J* = 8.03 Hz, 2H), 7.31 (s, 1H), 7.23-7.32 (m, 5H), 7.02-7.09 (m, *J* = 8.53 Hz, 2H), 4.81-4.87 (m, 1H), 4.43-4.46 (m, 1H), 4.35 (s, 2H), 4.06-4.10 (m, 1H), 3.87 (s, 3H), 3.28-3.31 (m, 2H), 3.14-3.25 (m, 4H), 1.89-1.93 (m, 2H), 1.79-1.81 (m, 2H), 1.62-1.72 (m, 4H); ^{13}C NMR (CD_3OD): δ 175.0, 174.2, 172.0, 164.4, 139.5, 130.3, 129.8, 129.6, 128.3, 119.4, 116.1, 56.2, 54.8, 53.3, 44.2, 41.9, 30.2, 30.0, 26.5, 26.4; HRMS (ESI-TOF): calculated for *m/z* 663.3842 [M+H$^+$], found 663.3849; HPLC: t_R= 16.45 min, 96.5%.

Arg-His(4-*t*-butylphenyl)-Arg-NHBzl (11e): Yield: 95%; ^{1}H NMR (CD_3OD): δ 7.87-7.90 (m, 2H), 7.53 (d, *J* = 7.53 Hz, 2H), 7.29-7.31 (m, 5H), 6.93 (s, 1H), 4.91-4.93 (m, 1H), 4.59-4.68 (m, 2H), 4.46 (s, 2H), 3.01-3.03 (m, 5H), 2.61-2.67 (m, 2H), 1.84-1.89 (m, 2H), 1.63-1.67 (m, 5H), 1.29 (s, 9H); ^{13}C NMR (CD_3OD): δ 175.7, 173.7, 153.1, 148.3, 129.5, 128.6, 128.5, 128.2, 126.9, 126.4, 56.5, 55.5, 54.4, 42.0, 37.9, 35.6, 31.7, 29.9, 29.6, 26.3; HRMS (ESI-TOF): calculated for *m/z* 689.4363 [M+H$^+$], found 689.4363; HPLC: t_R= 22.04 min, 95.4%.

Arg-His(biphenyl)-Arg-NHBzl (11f): Yield: 91%; ^{1}H NMR (CD_3OD): δ 8.02 (d, *J* = 7.03 Hz, 1H), 7.93 (d, *J* = 6.53 Hz, 1H), 7.71 (d, *J* = 7.53 Hz, 1H), 7.66 (d, *J* = 7.03 Hz, 1H), 7.44-7.51 (m, 3H), 7.39 (d, *J* = 6.53 Hz, 1H), 7.20-7.32 (m, 6H), 7.02 (d, *J* = 10.54 Hz, 1H), 4.67-4.70 (m, 1H), 4.43-4.46 (m, 2H), 4.37-4.39 (m, 1H), 3.94-3.97 (m, 1H), 3.18-3.26 (m, 5H), 3.00 (d, *J* = 15.56 Hz, 1H), 1.96 (br.s., 1H), 1.73-1.81 (m, 2H), 1.64-1.73 (m, 5H); ^{13}C NMR (CD_3OD): δ 195.9, 176.7, 175.4, 173.6, 163.3, 142.7, 139.6, 130.0, 129.5, 128.7, 128.5, 128.2, 127.9, 127.0, 126.5, 122.9, 117.7, 103.1, 73.9, 60.2, 54.4, 42.0, 26.2, 8.5; HRMS (ESI-TOF): calculated for *m/z* 709.4050 [M+H$^+$], found 709.4059; HPLC: t_R= 21.26 min, 97.8%.

Arg-His-Arg-OMe (12a): Yield: 92%; ^{1}H NMR (CD_3OD): δ 7.69 (s, 1H), 6.93 (s, 1H), 4.51 (q, *J* = 6.6 Hz, 1H), 4.28 (dd, *J* = 4.9, 9.2 Hz, 1H), 3.81-3.92 (m, 1H), 3.63 (s, 3H), 3.02-3.12 (m, 4H), 2.92-3.02 (m, 2H), 1.66-1.82 (m, 1H), 1.51-1.62 (m, 2H), 1.41-1.49 (m, 5H); ^{13}C NMR (CD_3OD): δ 178.1, 174.5, 173.3, 171.6, 156.6, 135.7, 135.4, 132.2, 117.5, 54.8, 53.2, 52.9, 52.2, 40.6, 27.5, 24.3, 22.5; HRMS (ESI-TOF): calculated for *m/z* 482.2951 [M+H$^+$], found 482.2955; HPLC: t_R= 9.45 min, 97.3%.

Arg-His(phenyl)-Arg-OMe (12b): Yield: 90%; ^{1}H NMR (CD_3OD): δ 7.97 (d, *J* = 6.5 Hz, 2H), 7.59-7.76 (m, 3H), 7.48 (s, 1H), 4.82 (t, *J* = 6.5 Hz, 1H), 4.47 (dd, *J* = 4.6, 8.9 Hz, 1H), 4.02-4.08 (m, 1H), 3.67 (s, 3H), 3.16-3.29 (m, 5H), 1.96 (d, *J* = 3.5 Hz, 2H), 1.77 (d, *J* = 9.3

Hz, 2H), 1.59-1.74 (m, 5H); ^{13}C NMR (CD_3OD): δ 175.0, 173.6, 173.1, 172.1, 145.9, 133.5, 131.3, 131.2, 130.9, 130.8, 127.9, 124.4, 119.6, 61.6, 55.7, 53.6, 53.4, 53.0, 42.0, 41.8, 30.0, 29.4, 26.3, 20.9; HRMS (ESI-TOF): calculated for *m/z* 558.3264 [M+H$^+$], found 558.3261; HPLC: t_R= 10.10 min, 95.6%.

Arg-His(4-methylphenyl)-Arg-OMe (12c): Yield: 93%; ^{1}H NMR (CD_3OD): δ 7.83 (d, *J* = 6.02 Hz, 2H), 7.46 (d, *J* = 8.03 Hz, 2H), 7.41 (s, 1H), 4.78-4.81 (m, 1H), 4.44 (dd, *J* = 5.02, 8.53 Hz, 1H), 4.04 (d, *J* = 7.03 Hz, 1H), 3.64 (s, 3H), 3.12-3.26 (m, 6H), 2.45 (s, 3H), 1.91-2.00 (m, 1H), 1.74-1.81 (m, 2H), 1.60-1.72 (m, 5H); ^{13}C NMR (CD_3OD): δ 175.1, 173.7, 172.2, 158.1, 144.7, 131.5, 130.9, 127.8, 121.5, 119.3, 55.7, 53.6, 53.4, 53.0, 42.0, 41.8, 30.0, 29.4, 26.3, 21.6; HRMS (ESI-TOF): calculated for *m/z* 572.3400 [M+H$^+$], found 572.3411; HPLC: t_R= 14.15 min, 96.7%.

Arg-His(4-methoxyphenyl)-Arg-OMe (12d): Yield: 95%; ^{1}H NMR (CD_3OD): δ 7.82 (d, *J* = 8.8 Hz, 2H), 6.98-7.03 (m, 2H), 6.94 (s, 1H), 4.65-4.70 (m, 1H), 4.43 (dd, *J* = 4.9, 9.2 Hz, 1H), 4.01-4.07 (m, 1H), 3.84 (s, 3H), 3.71 (s, 3H), 3.06-3.27 (m, 5H), 2.94 (br.s., 1H), 1.87-1.99 (m, 1H), 1.74-1.82 (m, 2H), 1.59-1.70 (m, 5H); ^{13}C NMR (CD_3OD): δ 174.8, 174.1, 173.5, 161.7, 148.1, 127.9, 124.2, 115.3, 55.9, 54.9, 53.3, 52.9, 42.0, 41.9, 40.4, 30.2, 29.5, 26.2; HRMS (ESI-TOF): calculated for *m/z* 588.3369 [M+H$^+$], found 588.3374; HPLC: t_R= 12.16 min, 98.1%.

Arg-His(4-*t*-butylphenyl)-Arg-OMe (12e): Yield: 95%; ^{1}H NMR (CD_3OD): δ 7.87-7.90 (m, 2H), 7.53 (d, *J* = 8.53 Hz, 2H), 6.90 (s, 1H), 4.60-4.65 (m, 1H), 4.44-4.48 (m, 1H), 4.04-4.10 (m, 1H), 3.61 (s, 3H), 3.10-3.12 (m, 6H), 1.93-1.98 (m, 2H), 1.70-1.75 (m, 4H), 1.44-1.49 (m, 2H), 1.37 (s, 9H); ^{13}C NMR (CD_3OD): δ 174.8, 174.1, 173.5, 161.7, 148.1, 127.9, 124.2, 115.3, 55.9, 54.9, 53.3, 52.9, 42.0, 41.9, 40.4, 30.2, 29.5, 26.2; HRMS (ESI-TOF): calculated for *m/z* 614.8435 [M+H$^+$], found 614.8415; HPLC: t_R= 15.62 min, 95.6%.

Arg-His(biphenyl)-Arg-OMe (12f): Yield: 92%; ^{1}H NMR (CD_3OD): δ 7.95-8.05 (m, 2H), 7.73 (d, *J* = 8.28 Hz, 2H), 7.64-7.70 (m, 2H), 7.47 (t, *J* = 7.65 Hz, 2H), 7.34-7.41 (m, 1H), 7.04 (d, *J* = 9.54 Hz, 1H), 4.42-4.49 (m, 2H), 4.06 (d, *J* = 6.53 Hz, 1H), 3.69 (s, 3H), 3.15-3.25 (m, 5H), 2.93 (t, *J* = 6.53 Hz, 1H), 1.91-2.00 (m, 1H), 1.77-1.86 (m, 1H), 1.59-1.74 (m, 4H), 1.44-1.46 (m, 2H); ^{13}C NMR (CD_3OD): δ 179.3, 174.5, 173.6, 157.4, 147.7, 143.1, 141.1, 130.1, 128.8, 127.9, 126.9, 122.9, 119.8, 113.6, 109.9, 98.2, 97.1, 55.8, 53.1, 41.9, 32.1, 29.6, 26.2, 13.4; HRMS (ESI-TOF): calculated for *m/z* 634.4102 [M+H$^+$], found 634.4115; HPLC: t_R= 15.18 min, 95.1%.

4. Biological Activity

4.1. Assay for in vitro antimicrobial activity

The antibacterial activities of synthesized peptides were evaluated against *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 33591 (MRSA), *Mycobacterium intracellulare* ATCC 23068, *Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853. Susceptibility of *S. aureus*, MRSA, *E. coli* and *P. aeruginosa* to test compounds was determined using CLSI methods.[3] Susceptibility of *M. intracellulare* was determined using the modified Alamar Blue procedure of Franzblau *et al*. [4-5]

The antifungal activities of the target compounds against *Cryptococcus neoformans* ATCC 90113 were determined according to modified CLSI methods. [6]

Test Procedure: All organisms were obtained from ATCC, Manassas, VA. Samples were serially diluted in 20% DMSO/saline and transferred in duplicate to 96-well flat-bottomed microplates. Inocula were prepared by correcting the OD_{630} of microbe suspensions in incubation broth [Sabouraud Dextrose (Difco) for *C. neoformans*, cation adjusted Mueller-Hinton (Difco) at pH 7.3 for non-mycobacterial bacteria, and 5% Alamar Blue™ (BioSource International, Camarillo, CA) in Middlebrook 7H9 broth with OADC enrichment, pH 7.3 for *M. intracellulare*] to afford: *C. neoformans* 1.5 x 10^3, non-mycobaterial bacteria 5 x 10^5, and *M. intracellulare* 2.0 x 10^6 CFU/mL. Drug controls [Ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and Amphotericin B (ICN Biomedicals, Ohio) for *C. neoformans*] were included in each assay. All organisms were read at either 530 nm using the Biotek Power wave XS plate reader (Bio-Tek Instruments, Vermont) or 544ex/590em (*M. intracellulare*) using the Polarstar Galaxy Plate Reader (BMG Lab Technologies, Germany) prior to and after incubation: *MRSA*/*E. coli*/*P. aeruginosa* at 35 °C for 16-20 h, *C. neoformans* at 35 °C for 70-74 h, and *M. intracellulare* at 37 °C and 10% CO_2 for 70-74 h. The MIC is defined as the lowest test concentration that allows no detectable growth (for *M. intracellulare* no color change from blue to pink). Minimum fungicidal or bactericidal concentrations were determined by removing 5 μL from each clear (or blue) well, transferring to agar and incubating as previously mentioned. The MFC/MBC is defined as the lowest test concentration that kills the organism (allows no growth on agar).

4.2. Cytotoxicity assay

All 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 non-cancerous

mammalian kidney cells (VERO and LLC-PK_1). All cells were obtained from ATCC (American Type Culture Collection).

Test Procedure: The assay was performed in 96-well tissue culture-treated microplates and compounds were tested up to a highest concentration of 10µg/mL. Briefly, cells (25,000 cells/well) were seeded to the wells of the plate and incubated for 24 h for confluency. Samples were added and plates were again incubated for 48 h. The number of viable cells was determined according to a modified version of neutral red uptake assay.[7] Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

None of the tested compounds was found to be cytotoxic up to a concentration of 10.0 µg/mL.

Table 1. Cytotoxicity of peptides

Peptide	IC_{50} (µg/mL)					
	SK-Mel	KB	BT-549	SK-OV-3	VERO	LLC-PK1
9a	NC	NC	NC	NC	NC	NC
9b	NC	NC	NC	NC	NC	NC
9c	NC	NC	NC	NC	NC	NC
9d	NC	NC	NC	NC	NC	NC
9e	NC	NC	NC	NC	NC	NC
9f	NC	NC	NC	NC	NC	NC
10a	NC	NC	NC	NC	NC	NC
10b	NC	NC	NC	NC	NC	NC
10c	NC	NC	NC	NC	NC	NC
10d	NC	NC	NC	NC	NC	NC
10e	NC	NC	NC	NC	NC	NC
10f	NC	NC	NC	NC	NC	NC
11a	NC	NC	NC	NC	NC	NC
11b	NC	NC	NC	NC	NC	NC
11c	NC	NC	NC	NC	NC	NC
11d	NC	NC	NC	NC	NC	NC
11e	NC	NC	NC	NC	NC	NC
11f	NC	NC	NC	NC	NC	NC
12a	NC	NC	NC	NC	NC	NC

12b	NC	NC	NC	NC	NC	NC
12c	NC	NC	NC	NC	NC	NC
12d	NC	NC	NC	NC	NC	NC
12e	NC	NC	NC	NC	NC	NC
12f	NC	NC	NC	NC	NC	NC
DOX	0.75	0.85	0.95	0.85	>5	0.95

NC, non-cytotoxic

5. Correlation of lipophilicity and activity of peptides using RP-HPLC and LogP

Table 2. Biological activity of peptides **12a-f** and **11a-f** and retention time (t_R), logP

Peptide	HPLC analysis t_R(min)	LogP	C. *neoformans* IC_{50} (µg/mL)
12a	9.45	-3.41	19.01
12b	10.10	-1.37	NA
12c	14.15	-0.91	19.72
12d	12.16	-1.21	3.93
12e	15.62	0.31	0.61
12f	15.18	0.27	0.59
11a	12.58	-2.68	NA
11b	14.60	-0.64	0.73
11c	15.89	-0.18	0.35
11d	16.45	-0.48	0.43
11e	22.04	1.05	0.07
11f	21.26	1.00	0.10

Retention times are given for elution of the respective peptide on a reversed phase analytical HPLC system using a C-18 column. NA, not active. Method: C-18 column (25 cm × 4.6 mm, 5 µM) run for 40 min with a flow of 1 mL/min, using a gradient of 85-5%, where buffer A was 0.1% TFA in H_2O and buffer B was 0.1% TFA in CH_3CN and detection at 220 nm.

6. HPLC chromatograms of representative peptides

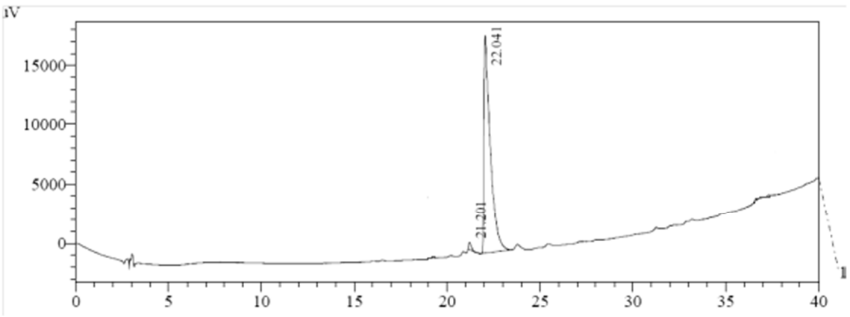


Figure 1. HPLC chromatogram of **11e**

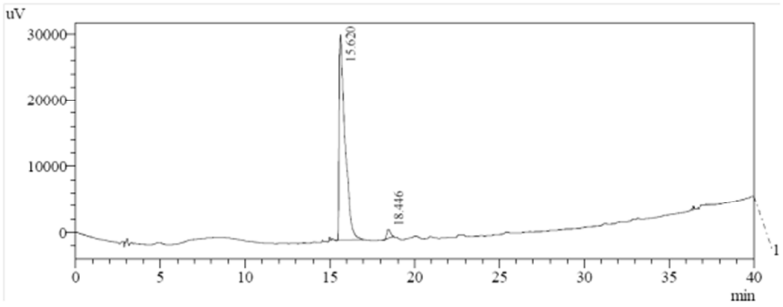
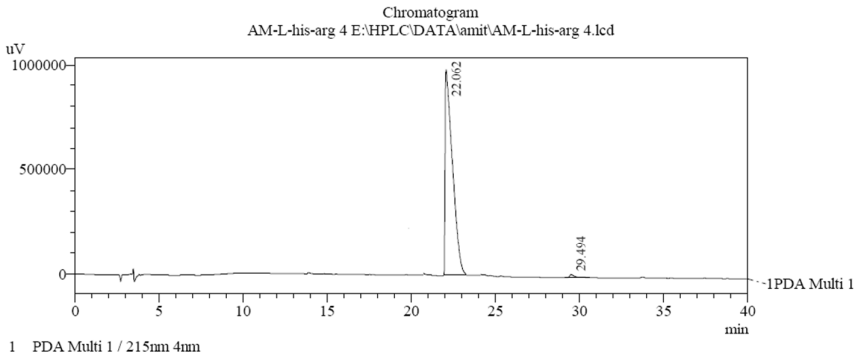


Figure 2. HPLC chromatogram of **12e**

6.1. HPLC chromatographic study to prove the non-racemization of peptides during synthesis



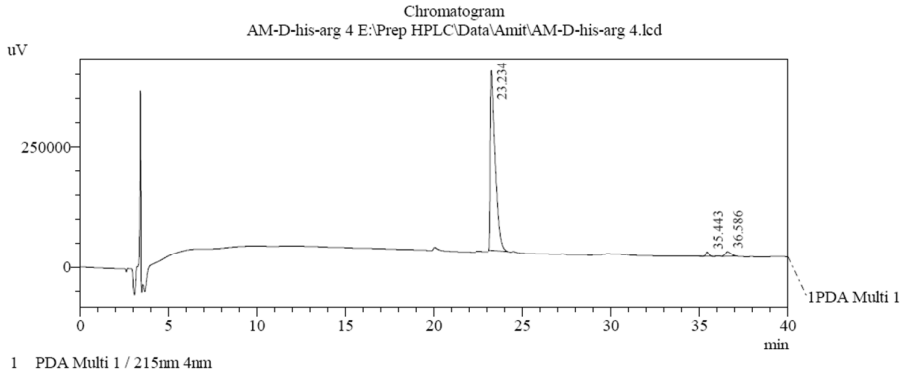
1 PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	22.062	29861067	982167	98.952
2	29.494	316228	13683	1.048
Total		30177295	995850	100.000

Figure 3. HPLC chromatogram of **Boc-L-His-Arg-OMe**



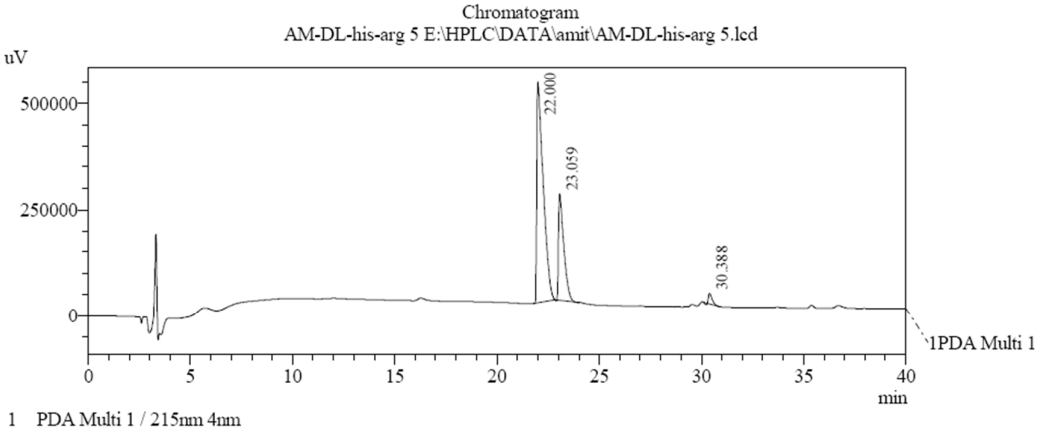
1 PDA Multi 1 / 215nm 4nm

PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	23.234	7107062	370068	95.436
2	35.443	109113	9809	1.465
3	36.586	230779	10572	3.099
Total		7446954	390449	100.000

Figure 4. HPLC chromatogram of **Boc-D-His-Arg-OMe**



PeakTable

PDA Ch1 215nm 4nm

Peak#	Ret. Time	Area	Height	Area %
1	22.000	11429317	518934	70.360
2	23.059	4527633	250085	27.873
3	30.388	287043	24627	1.767
Total		16243993	793646	100.000

Figure 5. HPLC chromatogram of Boc-DL-His-Arg-OMe

7. ^{1}H NMR of Representative Peptides

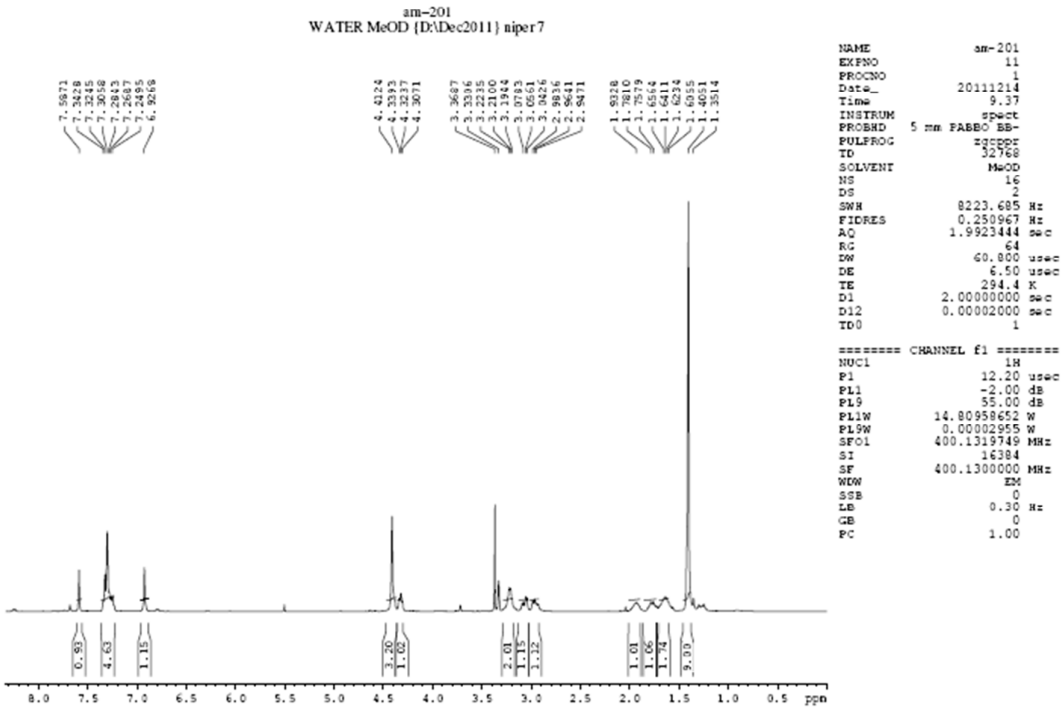


Figure 6. ^{1}H NMR of **7a**

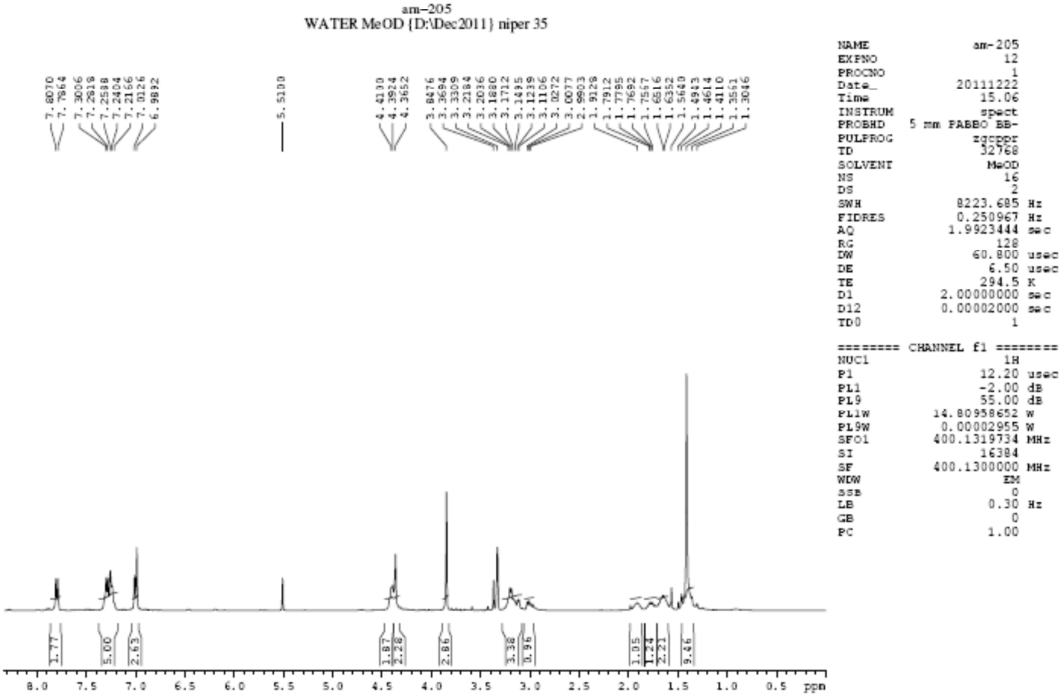


Figure 7. ^{1}H NMR of **7d**

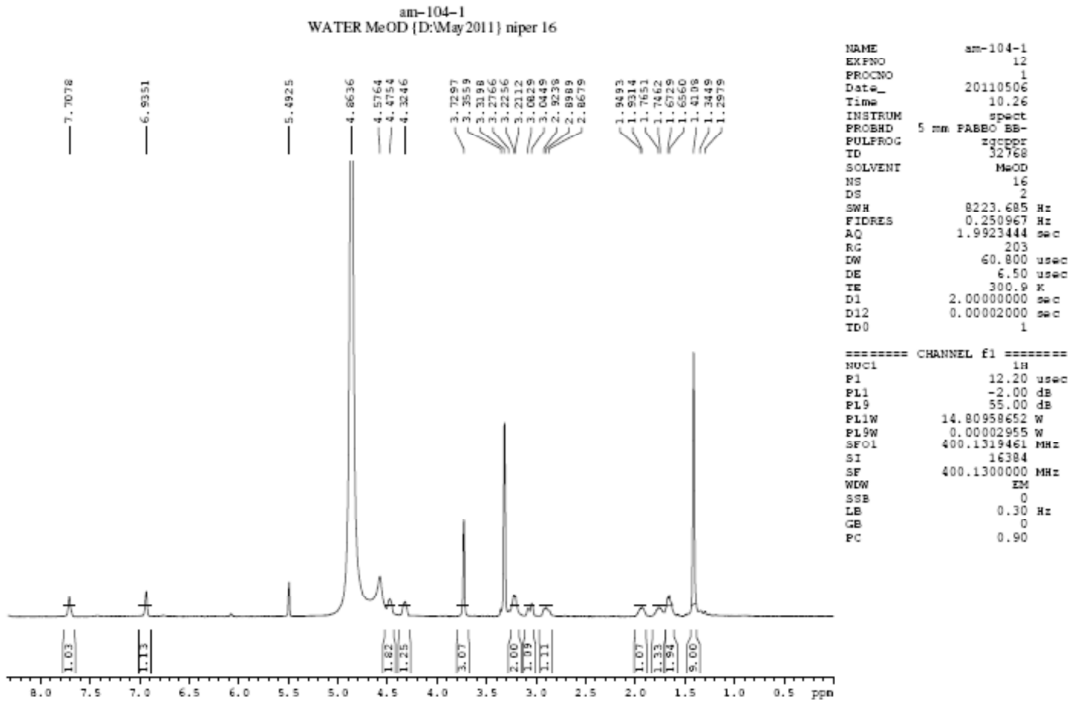


Figure 8. ^{1}H NMR of **8a**

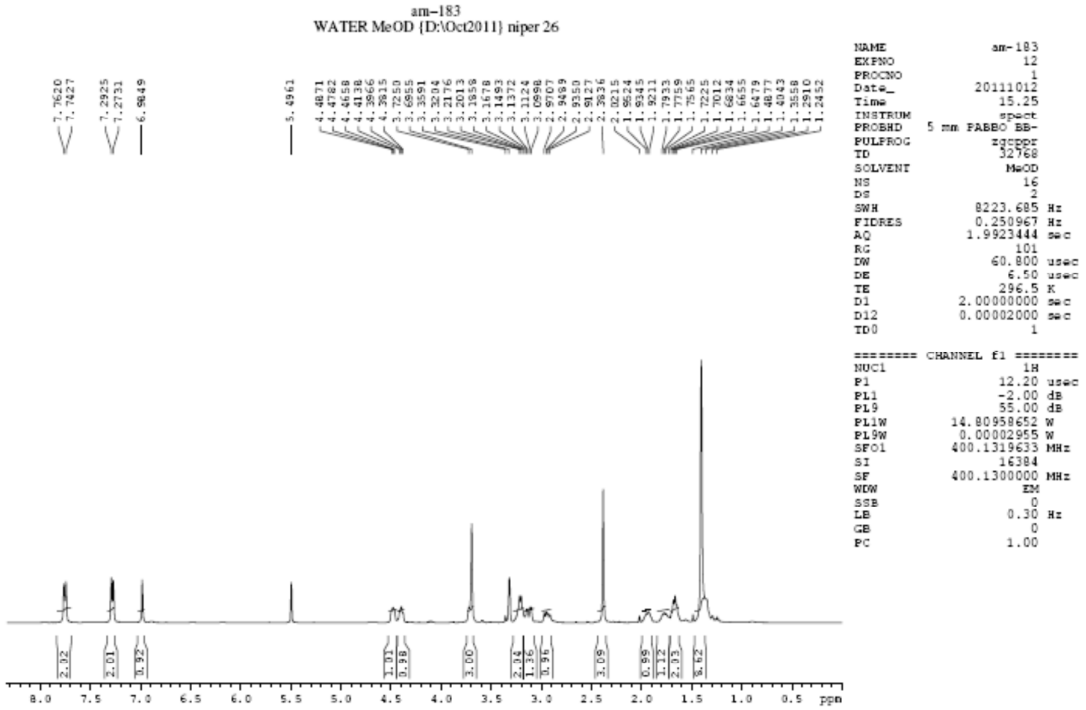


Figure 9. ^{1}H NMR of **8c**

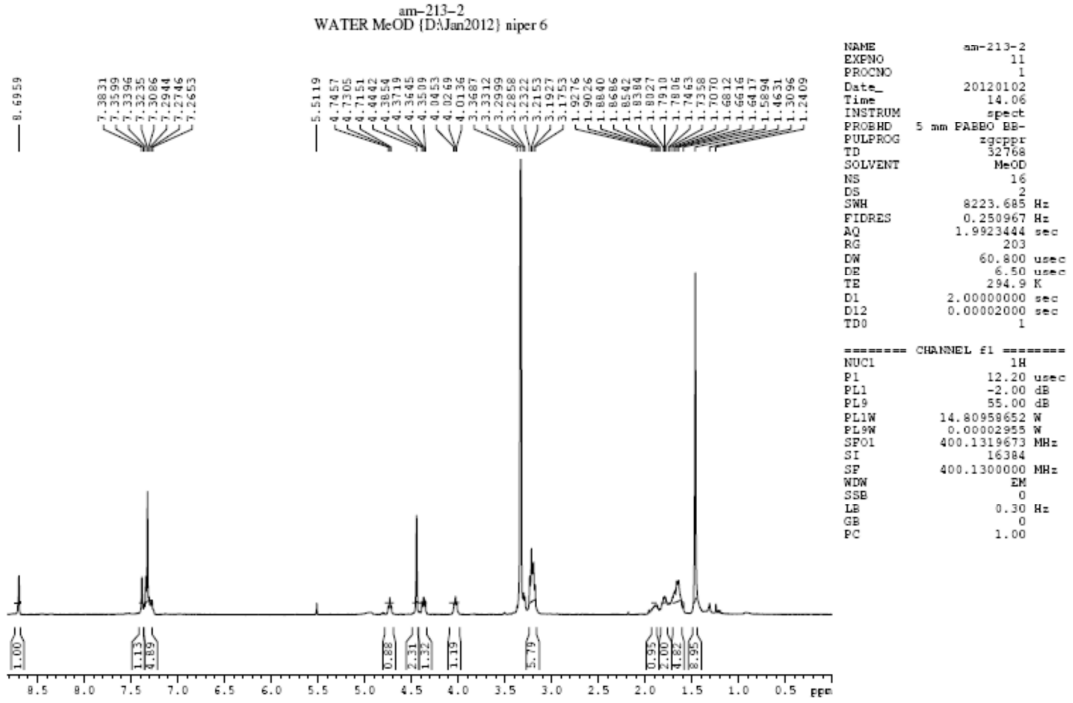


Figure 10 ^{1}H NMR of **9a**

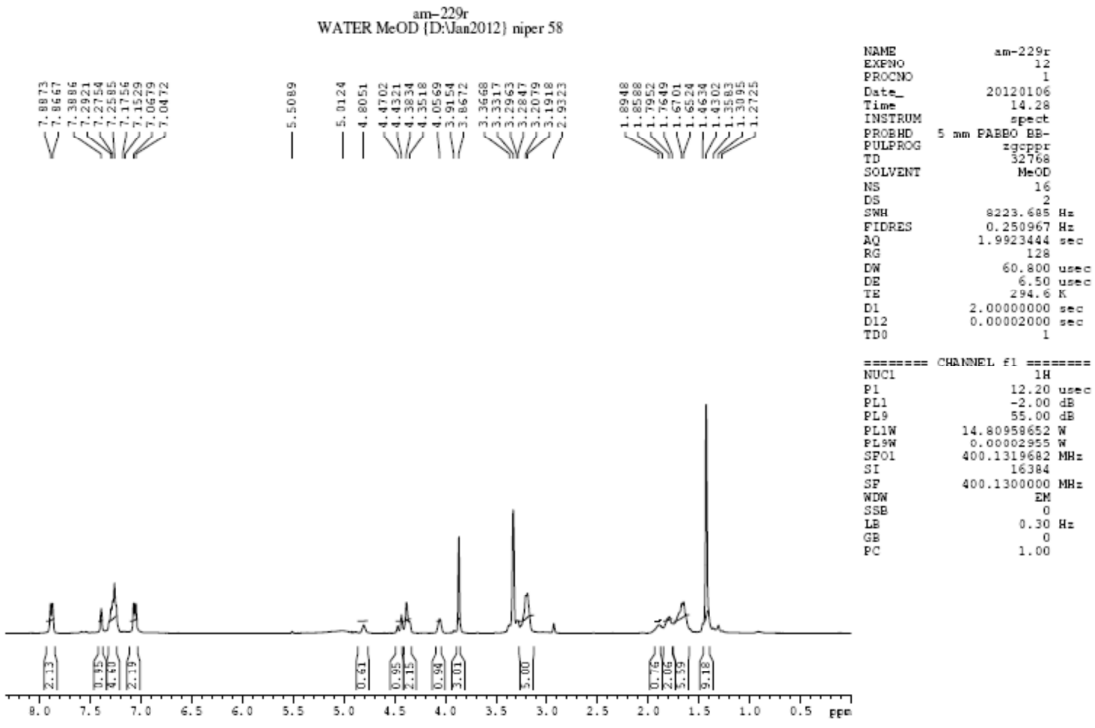


Figure 11. ^{1}H NMR of **9d**

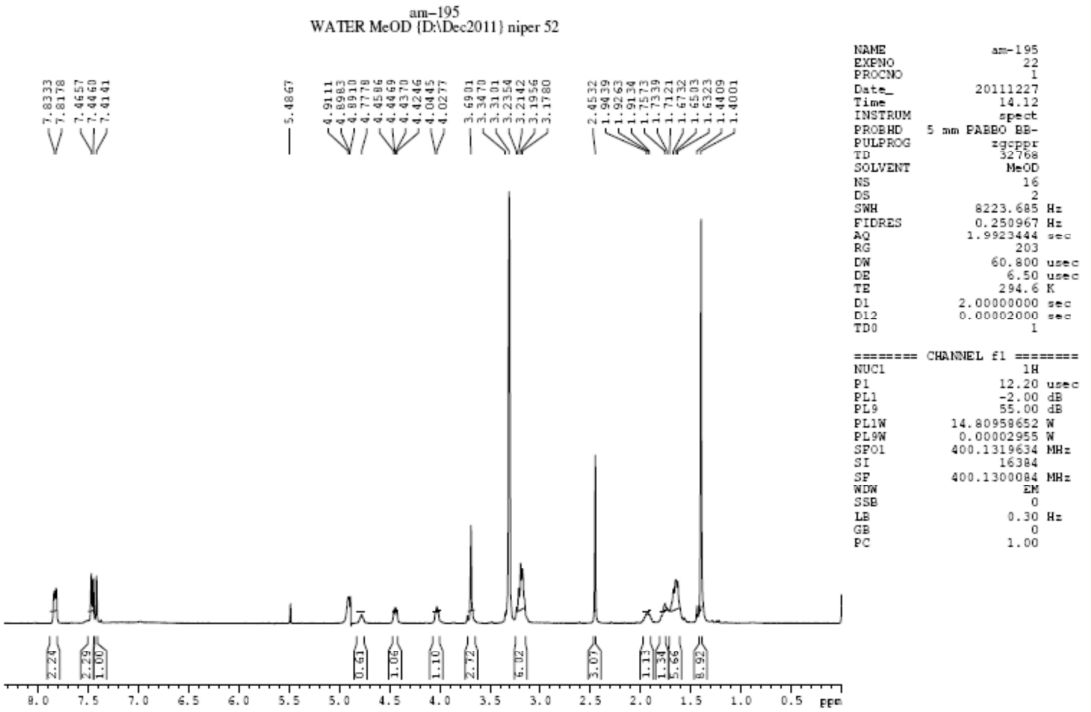


Figure 12. ^{1}H NMR of **10c**

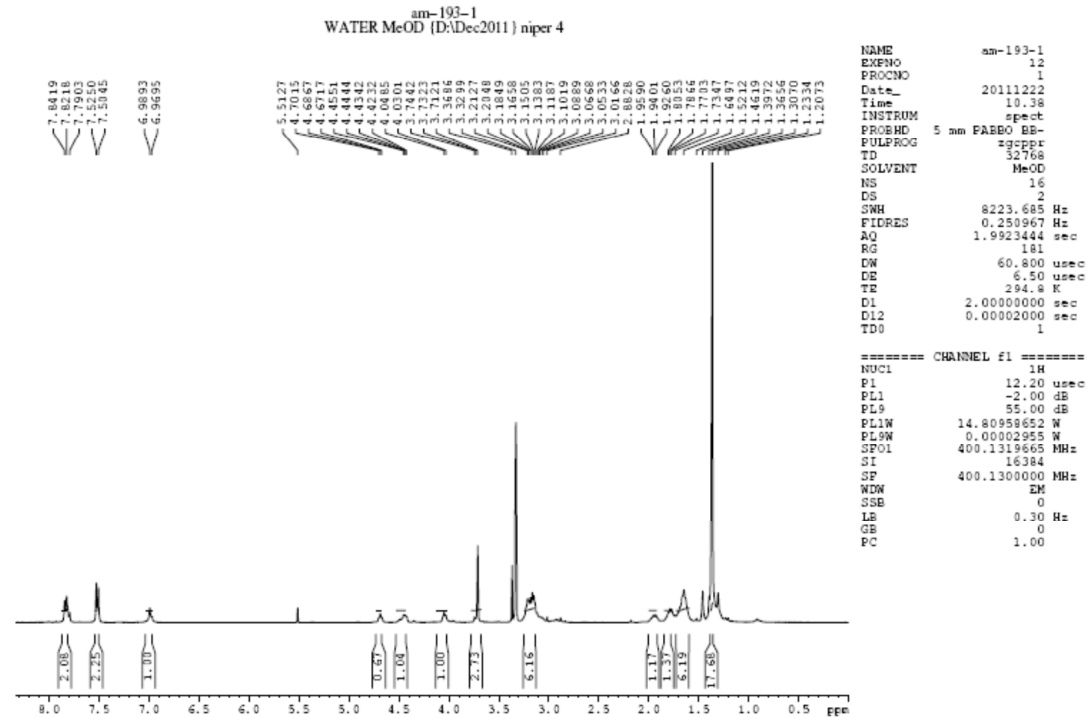


Figure 13 ^{1}H NMR of **10e**

7.1. ^{13}C NMR of Representative Peptides

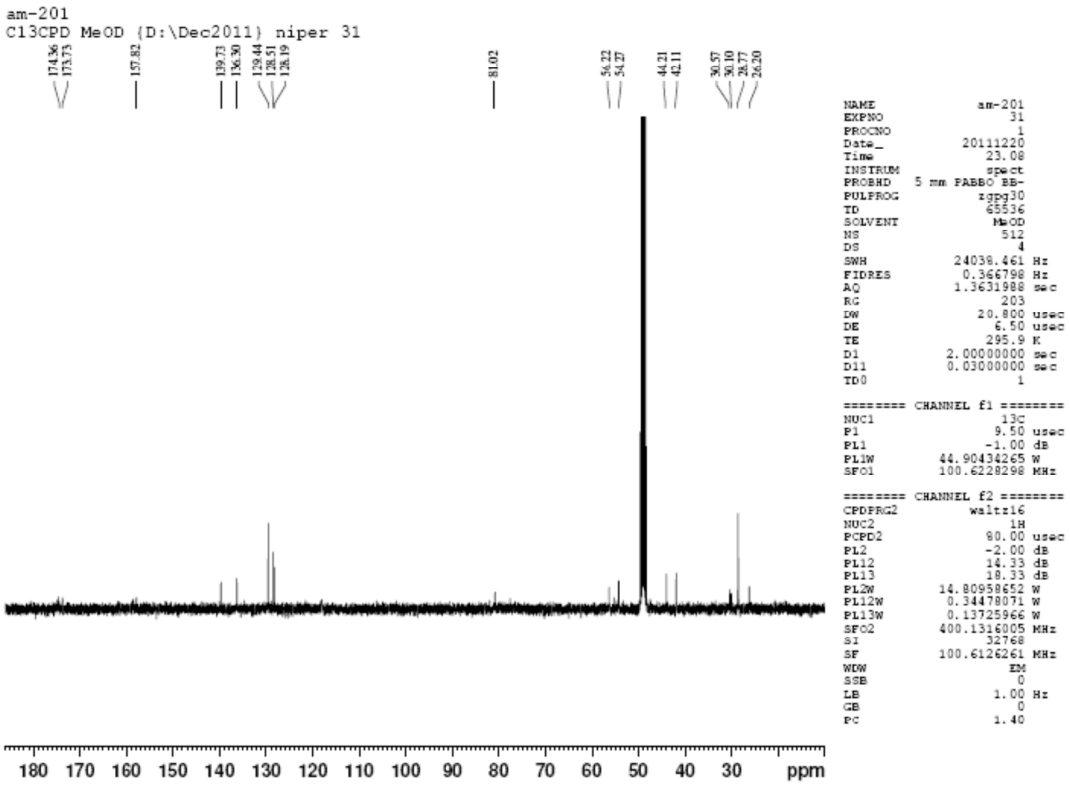


Figure 14 ^{13}C NMR of **7a**

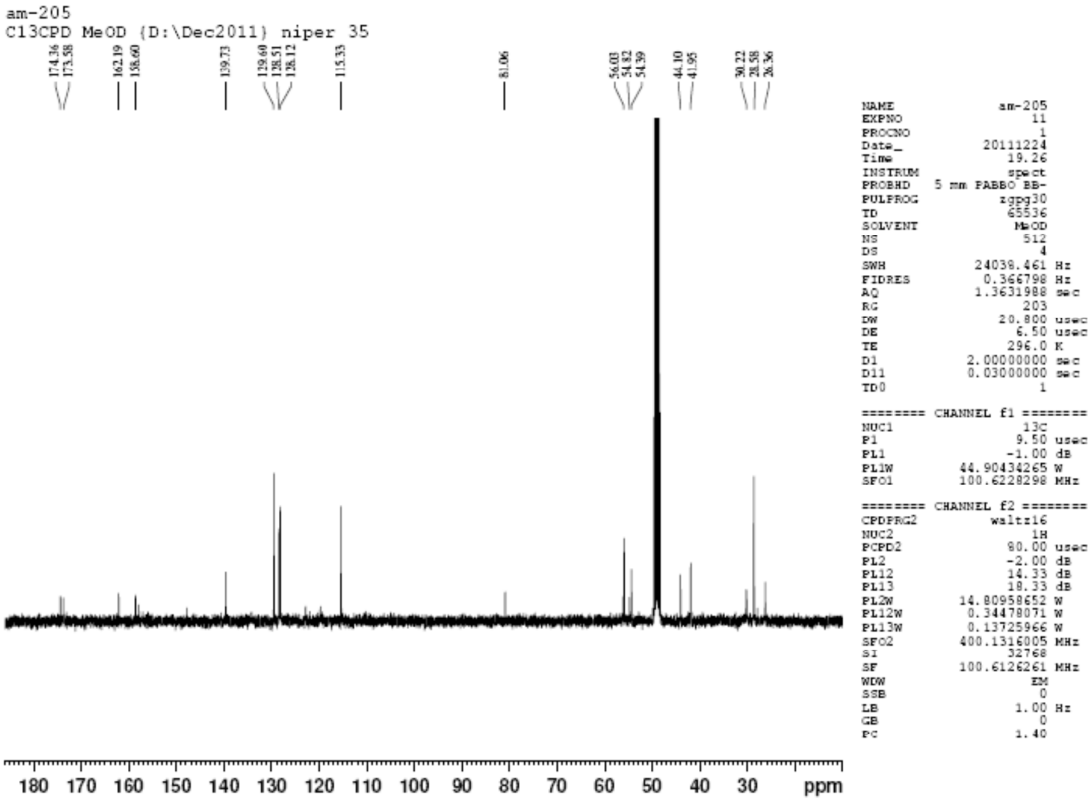


Figure 15 ^{13}C NMR of **7d**

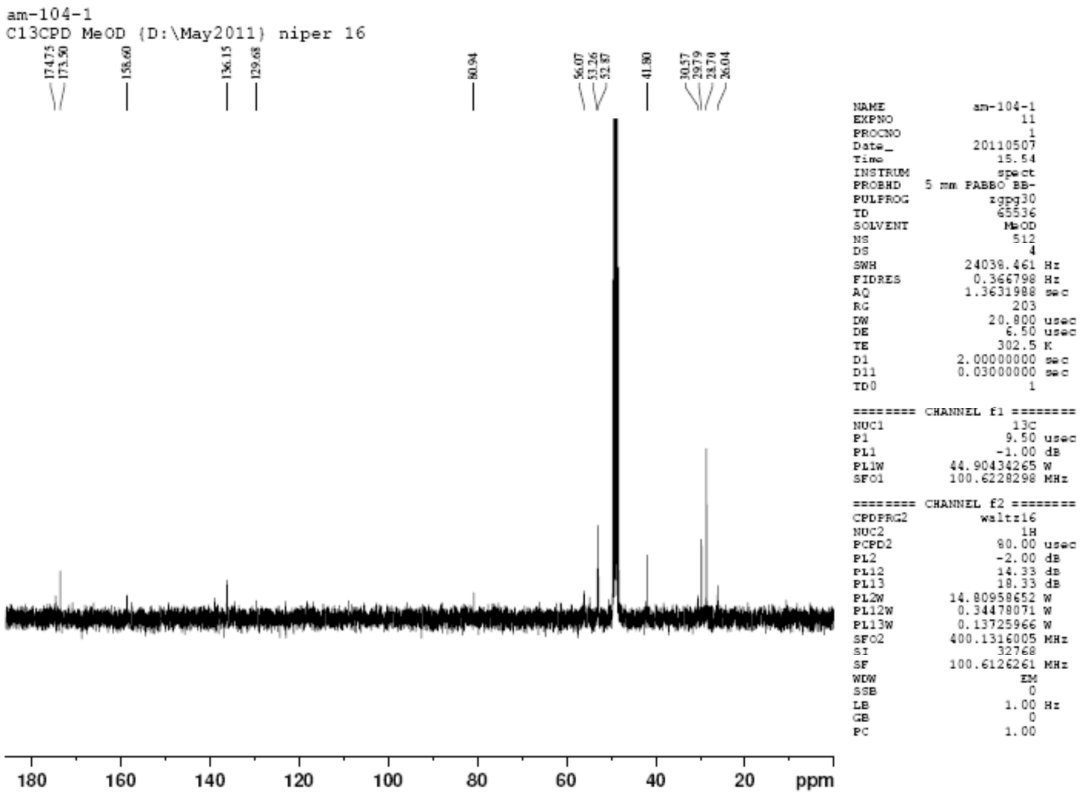


Figure 16 ^{13}C NMR of **8a**

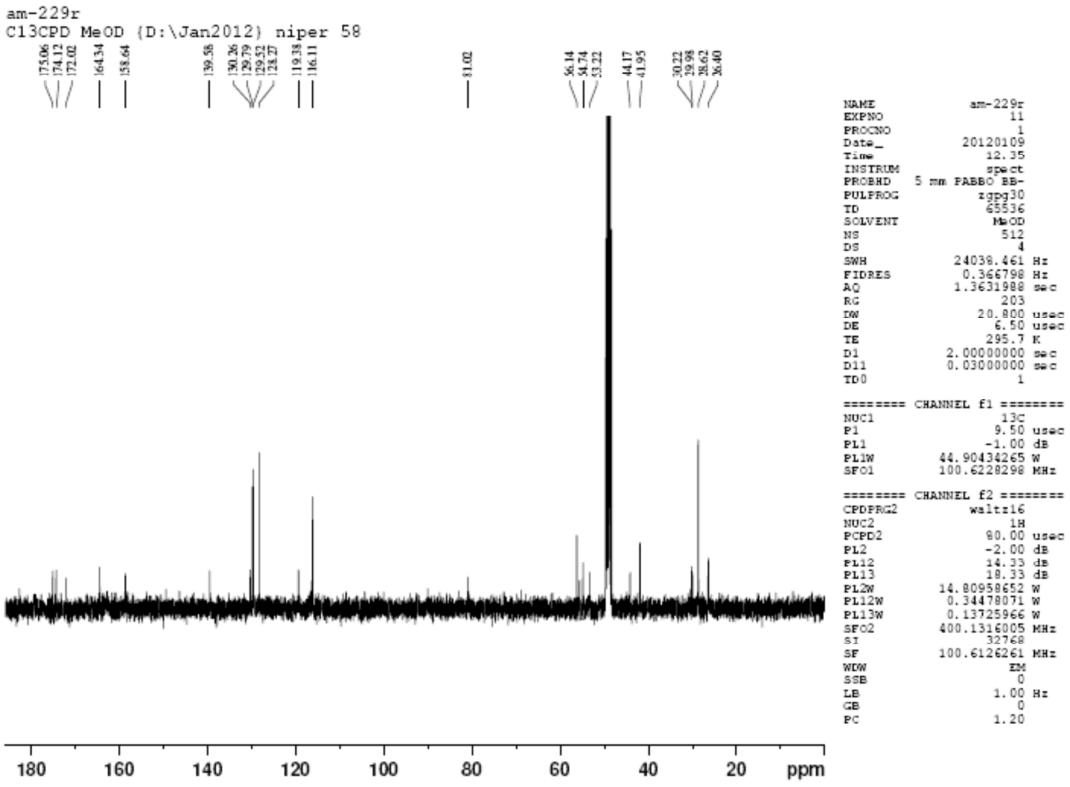


Figure 17 ^{13}C NMR of **9d**

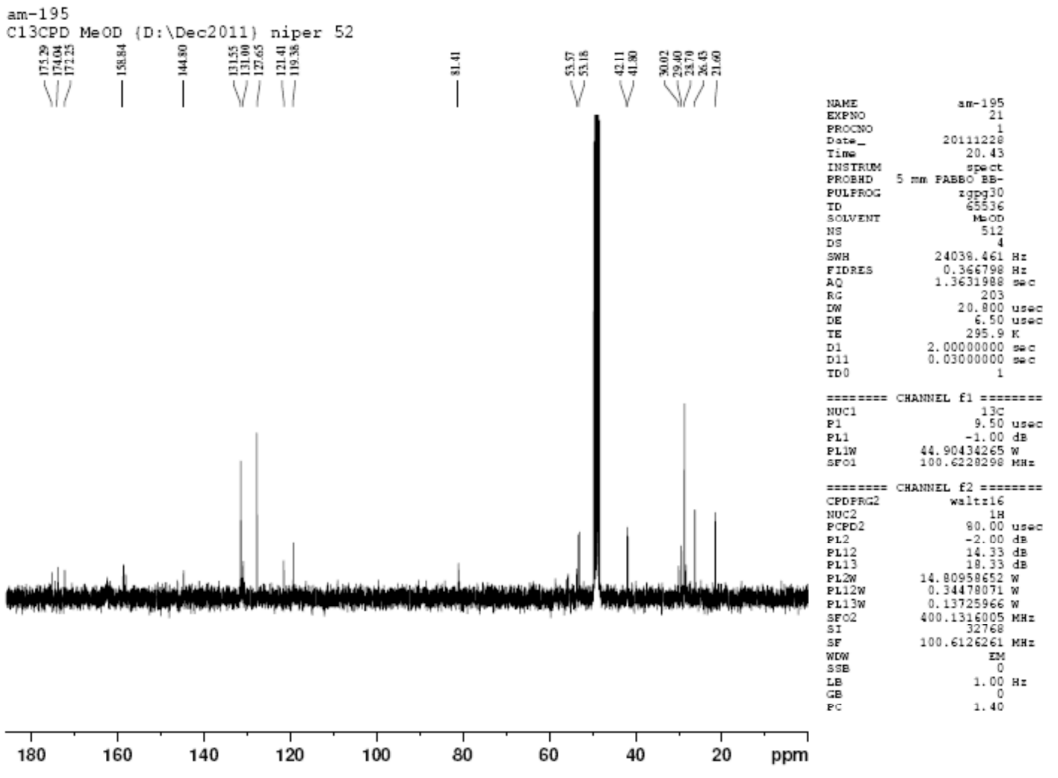


Figure 18 ^{13}C NMR of **10c**

8. References

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