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(54) **KAHALALIDE F AND COMPOSITIONS AND USES THEREOF**

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(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

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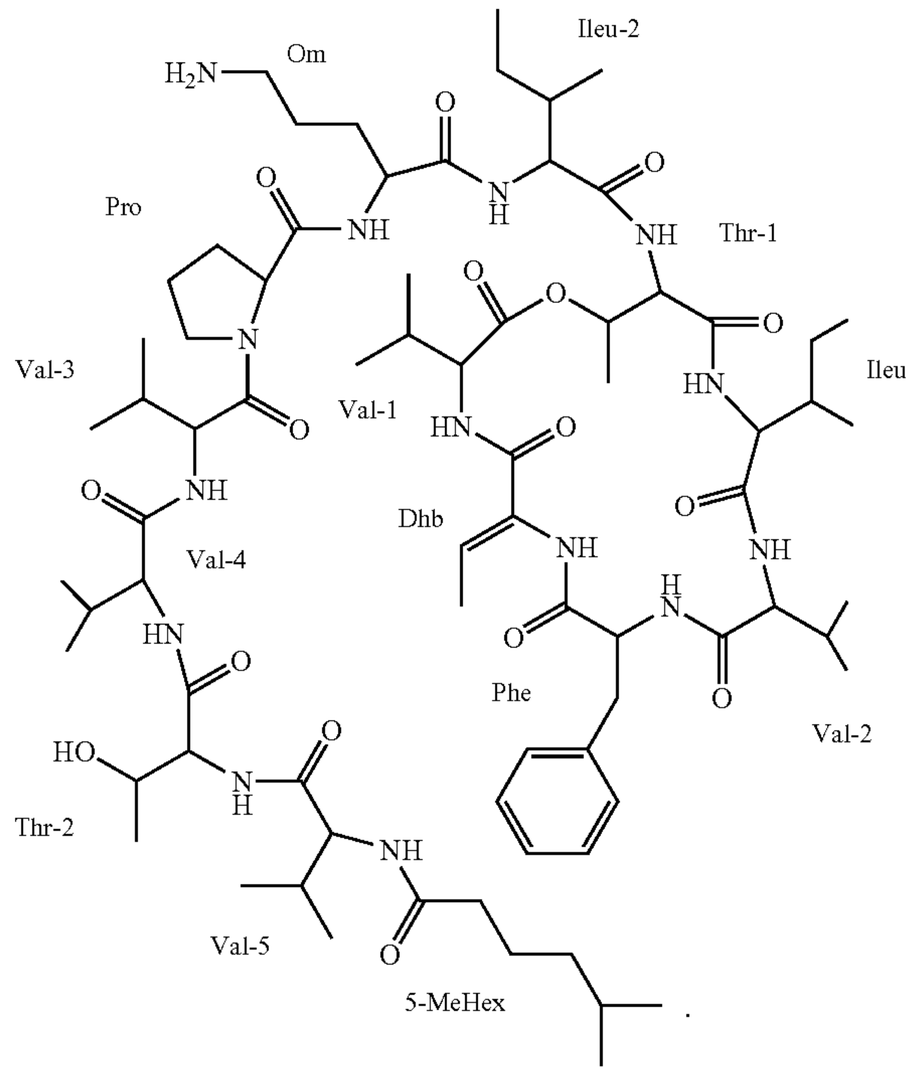
(57) **ABSTRACT**

[Kalahide] *Kahalalide* F, of formula I below, may be isolated from a sacoglossan. The compound may be used in the manufacture of pharmaceutical compositions or in the treatment of tumors or viral conditions

Formula I



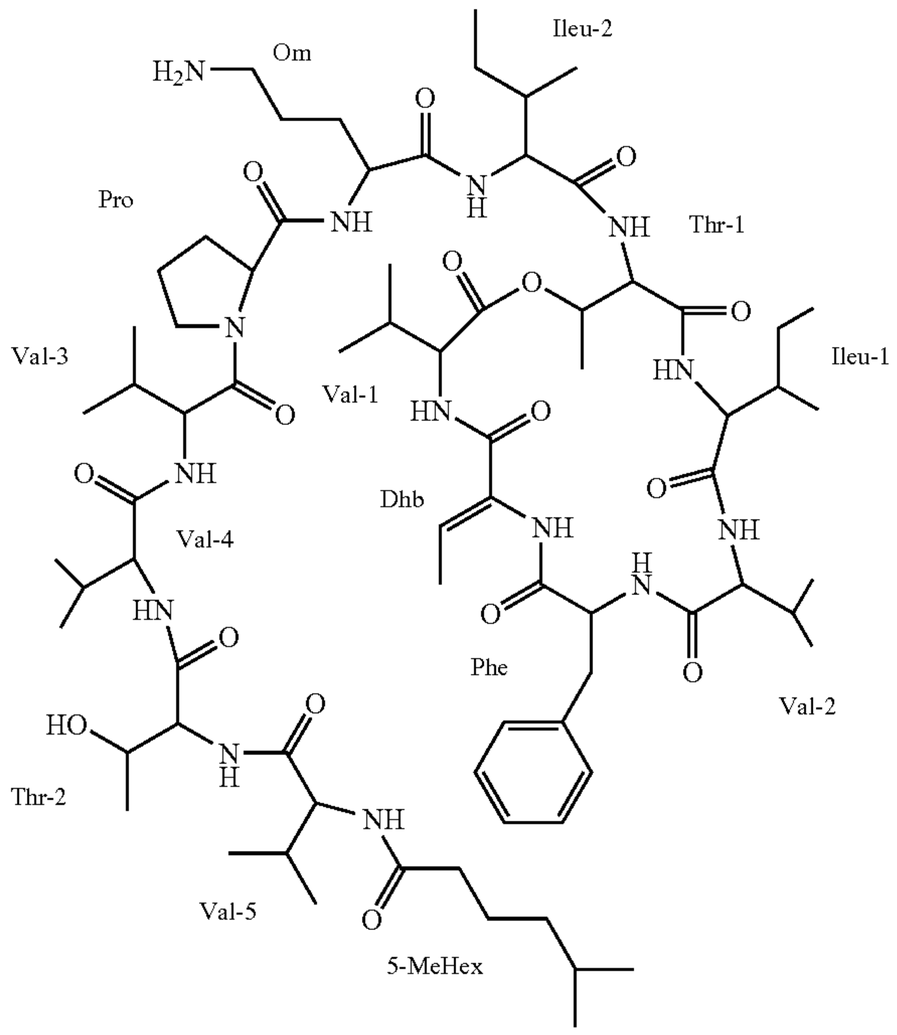
10 Claims, No Drawings

KAHALALIDE F AND COMPOSITIONS AND USES THEREOF

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

This invention is concerned with a cytotoxic and antiviral compound isolated from the sacoglossan, Elysia [rafescens] *rufescens*.

According to the invention there is provided[, a] *the* new compound, [the peptide,] Kahalalide F, of [the] formula *I*:



The antitumor activities of this compound has been determined "in vitro" in cell cultures of human lung carcinoma A-549 and human colon carcinoma HT-29. The procedure was carried out using the [metnhodology] *methodology* described by Raymond J. Bergeron et al. Biochem. Bioph. Res. Comm. 1984, 121(3), 848–854 and by Alan C. Schroeder et al. J. Med. Chem. 1981, 24 1078–1083.

The antiviral activities of [this compound] *the compound Kahalalide F* have also been determined "in vitro" against HSV (Herpes simplex virus) and VSV (Vesicular stomatitis virus). The methodology used to carry out this determination is described by Raymond J. Bergeron et al. Biochem. Bioph. Res. Comm. 1984, 121(3), 848–854 and by Alan C. Schroeder et al. J. Med. Chem. 1981, 24 1078–1083.

Therefore, the present invention also provides a method of treating any mammal affected by a malignant tumor sensitive to compounds above described, which comprises administering to the affected individual a therapeutically effective amount of these compounds or a pharmaceutical composition thereof; and a method of treating viral infections in mammals, comprising administering to a patient in need of such treatment, an antiviral effective amount of the compounds described in the present invention.

The present invention also relates to pharmaceutical preparations which contain as active ingredient these compounds, or a pharmaceutically acceptable acid addition salt thereof, as well as the process for its preparation.

Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) suitable composition for oral, topical or parenteral administration, and they may contain [the] pure compound or in combination with any carrier or other pharmacologically active compounds. These compositions may need to be sterile when administered parenterally.

The correct dosage of a pharmaceutical composition of [these compounds] *this compound* will vary according to the particular formulation, the mode of application and particular site, host and tumor being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of disease shall be taken in account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

Kahalalide F was isolated from the sacoglossan, [Elysia rufescens] *Elysia rufescens* (family Plakobranchidae, order Sacoglossa), collected near Black [point] *Point*, Oahu. This animal varies in size between 1 and 4 cm; it is dark red-brown in color with light-colored spots. There is orange fringing of the parapodia, which have very small dark green spots from sequestered chloroplasts. Elysia rufescens feeds on the delicate, feather-like green alga [Bryopsis] *Bryopsis* sp. [1] Kahalalide F can also be isolated from this alga. Two hundred animals were collected over the period of several weeks during *the* spring[,] *of* 1991 and extracted with EtOH. The extracts were then chromatographed by silica gel flash chromatography (hexane, hexane/EtOAc (1:1), EtOAc, [EtOAc (1:1)] *EtOAc/MeOH (1:1)*, MeOH and MeOH/HOAc (98:2)). The peptides were eluted with EtOAc/MeOH (1:1). Final purification was accomplished by repeated HPLC (RP C18) using MeCN/H_2O with 0.1% TFA (70–45% H_2O).

ISOLATION SCHEME

Elysia rufescens

EXTRACTION WITH ETOH
300 g wet weight , 200 animals

1	2	3	4	5	
Hex	Hex/EtOAc 1:1	EtO Ac	EtOAc/MeOH 1:1	MeOH	MeOH/HOAc 98:2

REPEATED HPLC RP C18 CH_3CN/H_2O/TFA
70% H_2O in 45% H_2O

1	2	3	4	5	6
12 mg	40 mg	4 mg	25 mg	25 mg	10 mg
0.004%	0.01%	0.001%	0.008%	0.008%	0.003% from wet u1.
Peptide D	F	C	A	B	E

The structures of the peptides were elucidated by 2D NMR experiments (HMQC, HMBC, TOCSY, COSY and ROESY).

Kahalalide F was isolated as a white amorphous powder in [0.02%] *0.01%* yield. A molecular formula of $C_{75}H_{124}N_{14}O_{16}$ was deduced from detailed analyses of the ^{13}C and ^{1}H NMR spectra and the high resolution FAB mass spectrum. The 14 substructures in this compound arise from five valines, two isoleucines, two threonines, ornithine, dehydroaminobutyric acid[.], proline, [phenilalanine] *phenylalanine* and [5-methythexanoic] *5-methylhexanoic* acid (5-MeHex). Kahalalide F is the largest peptide in this series of compounds.

EXPERIMENTAL

General Considerations

Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Infrared spectra were recorded on a Nicolet MX-5 FTIR spectrometer. Gas chromatography was accomplished using a Hewlett-Packard Model 5890 instrument. Mass spectra were measured on a VG-70SE magnetic sector mass spectrometer. NMR spectra were measured on a General Electric QE-300 or a GN OMEGA 500 instrument. ^{1}H NMR chemical shifts are reported in ppm with the chemical shift of the residual protons of the solvent used as internal standards. ^{13}C NMR chemical shifts are reported in ppm by using the natural abundance ^{13}C of the solvent as an internal standard. Ultraviolet spectra were recorded on a Hewlett-Packard Model 8452A diode array spectrophotometer. All solvents were distilled from glass before use.

Two hundred sacoglossans (Elysia rufescens), were collected at Black Point, O'ahu during April and May [1992] *1991*, and extracted 3 times with EtOH. Spring appears to be the time of year Elysia rufescens is in greatest abundance at Black Point. The combined extracts were then chromatographed using silica gel flash chromatography (hexane, hexane/EtOAc (1:1), EtOAc, EtOAc/MeOH (1:1), MeOH, MeOH/HOAc (98:2)). The depsipeptides were found in the EtOAc/MeOH (1:1) fraction. Repeated HPLC [RP18] *RP C18* MeCN/H_2O/TFA ([55/45/1]*30/70/1*)—MeCN/H_2O/TFA ([(30/70/1]*55/45/1*) yielded [six] *the* new depsipeptides.

[KAHALALDDE F] *KAHALALIDE F*

Final purification was accomplished by HPLC on [RP18] *RP C18* MeCN/H_2O/TFA (55/45/1). Physical data: [[α]D-8°] $[\alpha]_D$-*8°* (c 4.32, MeOH); ^{1}H NMR (500 MHz, TFA/DMF); amino acid unit, δ (carbon position, mult, J): Val-1 4.16 (2, t, J=9.0 Hz), 7.11 (NH on 2, d, J=8.9 Hz), 1.77 (3, m), 0.95 (4, m), 0.95 (5, m); Dhb 9.20 (NH on 2, s), 6.48 (3, q, J=6.9 Hz), 1.43 (4, d, J=6.6 Hz); Phe 4.68 (2, q, J=6.6 Hz), 8.62 (NH on 2, d, J=6.6 Hz), 3.2*3* (3, dd, J=13.7, 7.2 Hz), 3.0*0* (3, dd, J=13.7, 9.0 Hz), 7.32 (5, d, J=7.2 Hz), 7.28 (6, t, J=7.5 Hz), 7.21 (7, t, J=7.2 Hz); Val-2 4.36 (2, m), 7.82 (NH on 2, d, J=6.6 Hz), 2.12 (3, m), 0.85 (4, m), 0.77 (5, d, J=6.6 Hz); Ileu-1 4.53 (2, m), 8.38 (NH on 2, d, J=9.6 Hz), 1.98 (3, m), 0.92 (4, d, J=6.6 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, t, J=7.2 Hz); Thr-1 4.63 (2, t, J=9.3 Hz), 8.12 (NH on 2, d, J=5.7), 5.07 (3, dq, *J*=9.6, 6.0 Hz), 1.18 (4, d, J=6.3 Hz); Ileu-2 4.52 (2, m), 7.72 (NH on 2, d, J=8.4 Hz), 1.88 (3, m), 0.88 (4, d, J=6.3 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, [d] *t*, J=7.2 Hz); Orn 4.48 (2, m), 7.92 (NH on 2, d, J=7.8 Hz), 1.76 (3, m), 1.83 (4, m), 3.10 (5, p, J=5.1 Hz); Pro 4.42 (2, m), 2.12 (3, m), *1.97* (*3, m*), 2.02 (4, m), 1.88 (4, m), 3.75 (5, m), 3.68 (5, m); Val-3 4.41 (2, m), 7.90 (NH on 2, d, J=7.2 Hz), 2.12 (3, m), 0.95 (4, m), 0.85 (5, m); Val-4 4.34 (2, m), 7.68 (NH on 2, d, J=8.1 Hz), 2.17 (3, m), 0.95 (4, m), 0.90 (5, m); Thr-2 4.46 (2, m), 7.77 (NH on 2, d, J=8.1), 4.21 (3, dq, *J*=6.3, 3.6 Hz), 1.12 (4, d, J=6.6 *Hz*); Val-5 4.32 (2, m), 7.85, (NH on 2, d, J=8.1 Hz), [7.82 (NH on (second conformation) d, J=8.1 Hz),] 2.14 (3, m), 0.95 (4, m), 0.90 (5, m); 5-MeHex 2.26 (2, m), 1.60 (3, m), 1.20 (4, m), 1.55 (5, m), 0.87 (6, d, J=7.2 Hz), 0.87 (7, d, J=7.2 Hz); [5-MeHex 2.29 (2, m), 1.65 (3, m), 1.40 (3, m), 1.13 (4, m), 1.35 (5, m), 0.90 (6, m), 0.90 (7, m)]; ^{13}C NMR (125 MHz TFA/DMF): amino acid unit, δ (carbon position); Val-1 [70.40] *170.40* (1), 60.31 (2), 30.75 (3), 19.58 (4), 18.76 (5); Dhb 164.54 (1), 130.30 (2), 131.26 (3), 12.68 (4); Phe 171.31 (1), 56.27 (2), 36.79 (3), 138.23 (4), 129.86 (5), 128.77 (6), 126.98 (7); Val-2 [172–94] *172.94* (1), 58.57 (2), 32.38 (3), 18.92 (4), 17.60 (5); Ileu-1 171.87 (1), 57.48 (2), 38.78 (3), 14.56 (4), 26.78 (5), 11.67 (*6*); Thr-1 169.68 (1), 57.37 (2), 71.05 (3), 17.34 (4); Ileu-2 171.92 (1), 57.29 (2), 38.01 (3), 14.78 (4), 26.55 (5), 11.63 (6); Orn 172.01 (1), 52.87 (2), 29.63 (3), 24.39 (4), 40.05 (5); Pro 172.55 (1), 60.23 (2), 29.58 (3), 25.38 (4), 48.03 (5); Val-3 171.28 (1), 57.57 (2), 30.54 (3), 19.61 (4), 18.80 (5); Val-4 171.83 (1), 59.10 (2), 31.26 (3), 19.45 (4), 18.08 (5); Thr-2 170.97 (1), 58.89 (2), 67.36 (3), 19.66 (4); Val-5 172.67 (1), 59.64 (2), 30.66 (3), 19.61 (4), 18.43 (5), 5-MeHex 173.83 (1), 36.28 (2), 23.99 (3), 38.96 (4), 28.10 (5), 22.54 (6), 22.50 (7); [5-MeHex (second conformation) 174.08 (1), 33.86 (2), 32.84 (3), 29.75 (4), 34.54 (5), 19.51 (6), 11.20 (7)]; IR neat (NaCl): 3287 (s, br), 2964 (s, br), 1646 (s), 1528 (s), 1465 (s), 1388 (m), 1228 (m), cm^{-1}; mass spectrum HRFAB m/z (fragment, %) 1477.9408 (M^{+}+1, 85) (calcd for $C_{75}H_{125}N_{14}O_{16}$: 1477.9398); UV (MeOH): λ_{max} 204 (89, 630) nm.

Amino acid analysis by GC-MS with a Chirasil-Val column indicates that Kahalalide F consists of 2-D-*allo*-Ileu, *L*-Orn, L-Phe, D-Pro, L-Thr, D-Allo-Thr, 3 D-Val and 2 L-Val.

[TABLE II] *TABLE I*

^{1}H and ^{13}C NMR Data for [Kahalalide F (I)] *KF in DMF/TFA*

Amino Acid	Carbon	^{13}C, ppm[a]	Mult.	^{1}H, ppm[b]	Multiplicity
Valine-1	1	170.4	s	(NH) 7.11	d, J=8.9
	2	60.3	d	4.16	t, J=9.0
	3	30.8	d	1.77	m
	4	19.6	q	0.95	m
	5	18.8	q	0.95	m
Dehydroamino butyric acid	1	164.5	s	(NH) 9.20	s
	2	130.3	s		
	3	131.3	d	6.48	q, J=6.9
	4	12.7	q	1.43	d, J=6.6
Phenylalanine	1	171.3	s	(NH) 8.62	d, J=6.6
	2	56.3	d	4.68	q, J=6.6
	3	36.8	t	3.23	dd, J=13.7,
				[3.00]	7.2
				3.00	dd, J=13.7,
					9.0
	4	138.2	s		
	5,5'	129.9	d	7.32	d, J=7.2
	6,6'	128.8	d	7.28	t, J=7.5
	7	127.0	d	7.21	t, J=7.2
Valine-2	1	172.9	s	(NH) 7.82	d, J=6.6
	2	58.6	d	4.36	m
	3	32.4	d	2.12	m
	4	18.0	q	0.85	m
	5	17.6	q	0.77	d, J=6.6
Isoleucine-1	1	171.9	s	(NH) 8.38	d, J=0.6
	2	57.5	d	4.53	m
	3	38.8	d	1.98	m
	4	14.6	q	0.92	d, J=6.6
	5	26.8	t	1.40, 1.13	m, m
	6	11.7	q	0.88	t, J=7.2

[TABLE II]*TABLE I*-continued

^{1}H and ^{13}C NMR Data for [Kahalalide F (I)]*KF in DMF/TFA*

Amino Acid	Carbon	^{13}C, ppm[a]	Mult.	^{1}H, ppm[b]	Multiplicity
Threonine-1	1	169.7	s	(NH) 8.12	d, J=5.7
	2	57.4	d	4.63	t, J=9.3
	3	71.1	d	5.07	dq, J=9.6, 6.0
	4	17.3	q	1.18	d, J=6.3
Isoleucine-2	1	171.9	s	(NH) 7.72	d, J=8.4
	2	57.3	d	4.52	m
	3	38.0	d	1.88	m
	4	14.8	q	0.88	d, J=6.3
	5	26.6	t	1.40, 1.13	m, m
	6	11.6	q	0.88	t, J=7.2
Ornithine	1	172.0	s	(NH) 7.92	d, J=7.8
	2	52.9	d	4.48	m
	3	29.6	t	1.76	m
	4	24.4	t	1.83	m
	5	40.1	t	3.10	p, 5.1
Proline	1	172.6	s		
	2	60.2	d	4.42	m
	3	29.6	t	2.12, 1.97	m, m
	4	25.4	t	2.02, 1.88	m, m
	5	48.0	t	3.75, 3.68	m, m
Valine-3	1	171.3	s	(NH) 7.90	d, J=7.2
	2	57.6	d	4.41	m
	3	30.5	d	2.12	m
	4	19.6	q	0.95	m
	5	18.8	q	0.85	m
Valine-4	1	171.8	s	(NH) 7.68	d, J=8.1
	2	59.1	d	4.34	m
	3	31.3	d	2.17	m
	4	19.5	q	0.95	m
	5	18.1	q	0.90	m
Threonine-2	1	171.0	s	(NH) 7.77	d, J=8.1
	2	58.9	d	4.46	m
	3	67.4	d	4.21	dq, J=6.3, 3.6
	4	19.7	q	1.12	d, J=6.6
Valine-5	1	172.7	s	(NH) *7.85*	d, J=8.1
		[conf. #2		7.85	d, J=7.81]
				[(NH) 7.82]	
	2	59.6	d	4.32	m
	3	30.7	d	2.14	m
	4	19.6	q	0.95	m
	5	18.4	q	0.90	m
5-Methyl-	1	173.8	s		
Hexanoic acid	2	36.3	t	2.26	m
	3	24.0	t	1.60	m
	4	39.0	t	1.20	m
	5	28.1	d	1.55	m
	6	22.5	q	0.87	d, J=7.2
	7	22.5	q	0.87	d, J=7.2
[5-Methyl-	1	174.1	s]		
[Hexanoic acid	2	33.9	t	2.29	m]
[(second	3	32.8	t	1.65, 1.40	m]
[conformation)	4	29.8	t	1.13	m]
[	5	34.5	d	1.35	m]
[	6	19.5	q	0.90	m]
[	7	11.2	q	0.90	m]

[a]at 125 MHz, DMF signal at 35.2 ppm;
[b]at 500 MHz, DMF signal at 2.91 ppm.

[TABLE I]*TABLE II*

In vitro Activity of [Kahalalide F]*KF from Elysia rufescens*
Assay (M.I.C. μg/mL)

Cytoxicity μg/mL (IC50)	
A-549	2.5
HT-29	0.25–0.5
Antiviral μg/mL (% reduction)	
Mv 2 Lu/HSV II	0.5 (95%)
CV-1/HSV-1	>8
BHK/VSV	>8

[TABLE I]*TABLE II*-continued

In vitro Activity of [Kahalalide F]*KF from Elysia rufescens*
Assay (M.I.C. μg/mL)

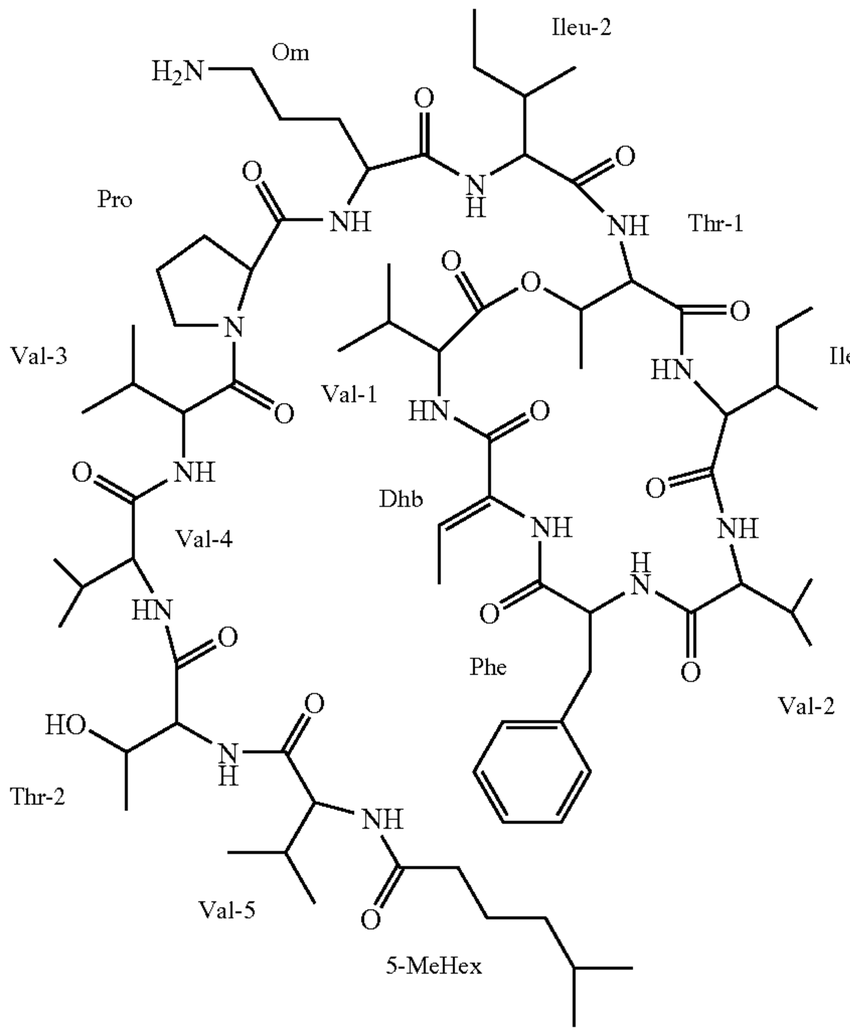
Antifungal 6 mm disk	50 μg/disk
Aspergillus oryzae	19 mm
Penicillium notatum	26 mm
Tricophyton mentagrophy	34 mm
Saccharomyces cerevisiac	neg
Candida albicans	16 mm

We claim:

1. [A substially] *Substantially* pure [compound] Kahalalide F *compound*, said compound [hag] *having* a molecular formula of $C_{75}H_{124}N_{14}O_{16}$, and consisting of five valines, two isoleucines, two threonines, ornithine, [dehydroaminobutynic] *dehydroaminobutyric* acid, proline, phenylalanine and 5-methylhexanoic acid; said compound further exhibiting the following physical and chemical properties: $[\alpha]_D$-8° (c 4.32, MeOH); ^{1}H NMR (500 MHz, TFA/DMF); amino acid unit, δ (carbon position, mult, J): Val-1 4.16 (2, t, J=9.0 Hz), 7.11 (NH on 2, d, J=[8,9]*8.9* Hz), 1.77 (3, m), 0.95 (4, m), 0.95 (5, m), Dhb 9.20 (NH on 2, s), 6.48 (3, q, J=6.9 Hz), 1.43 (4, d, J=6.6 Hz); Phe 4.68 (2, q, J=6.6 Hz), 8.62 (NH on 2, d, J=6.6 Hz), 3.*23* (3, dd, J=13.7, 7.2 Hz), 3.*00* (3, dd, J=13.7, 9.0 Hz), 7.32 (5, d, J=7.2 Hz), 7.28 (6, t, J=7.5 Hz), 7.21 (7, t, J=7.2 Hz); [Vol-2] *Val-2* 4.36 (2, m), 7.82 (NH on 2, d, J=6.6 Hz), 2.12 (3, m), 0.85 (4, m), 0.77 (5, d, J=6.6 Hz); Ileu-1 4.53 (2, m), 8.38 (NH on 2, d, J=9.6 Hz), 1.98 (3, m), 0.92 (4, d, J=6.6 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, t, J=7.2 Hz); Thr-1 4.63 (2, t, J=9.3 Hz), 8.12 ([HN] *NH* on 2, d, J=5.7), 5.07 (3, dq, *J*=9.6, 6.0 Hz), 1.18 (4, d, J=6.3 Hz); Ileu-2 4.52 (2, m), 7.72 (NH on 2, d, J=8.4 Hz), 1.88 (3, m), 0.88 (4, d, J=6.3 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, [d] *t*, J=7.2 Hz) Orn 4.48 (2, m), 7.92 (NH on 2, d, J=7.8 Hz), 1.76 (3, m), 1.83 (4, m), 3.10 (5, p, J=5.1 Hz); Pro 4.42 (2, m), 2.12 (3, m), 1.97 (3, m), 2.02 (4, m), 1.88 (4, m), 3.75 (5, m), 3.68 (5, m); Val-3 4.41 (2, m), 7.90 (NH on 2, d, J=7.2 Hz), 2.12 (3, m), 0.95 (4, m), 0.85 (5, m); Val-4 4.34 (2, m), 7.68 (NH on 2, d, J=8.1 Hz), 2.17 (3, m), 0.95 (4, m), 0.90 (5, m); Thr-2 4.46 (2, m), 7.77 (NH on 2, d, J=8.1 Hz), 4.21 (3, dq, *J*=6.3, 3.6 Hz), 1.12 (4, d, J=6.6 Hz); Val-5 4.32 (2, m), 7.85 ([HN] *NH* on 2, d, J=8.1 Hz), [7.82 (NH on (second conformation), d, J=8.1 Hz),] 2.14 (3, m), 0.95 (4, m), 0.90 (5, m); 5-MeHex 2.26 (2, m), 1.60 (3, m), 1.20 (4, m), 1.55 (5, m), 0.87 (6, d, J=7.2 Hz), 0.87 (7, d, J=[72]*7.2* Hz); [5-MeHex 2.29 (2, m), 1.65 (3, m), 1.40 (3, m), 1.13 (4, m), 1.35 (5, m), 0.90 (6, m), 0.90 (7, m);] ^{13}C NMR (125 MHz TFA/DMF): amino acid unit, δ (carbon position); Val-1 170.40 (1), 60.31 (2), 30.75 (3), 19.58 (4), 18.76 (5); Dhb 164.54 (1), 130.30 (2), 131.26 (3), [126.6] *12.68* (4); Phe 171.31 (1), 56.27 (2), 36.79 (3), 138.23 (4), 129.86 (5), 128.77 (6), 126.98 (7); Val-2 172.94 (1), 58.57 (2), 32.38 (3), 18.92 (4), 17.60 (5); Ileu-1 171.87 (1), 57.48 (2), 38.78 (3), 14.56 (4), 26.78 (5), 11.67 (*6*); Thr-1 169.68 (1), 57.37 (2), 71.05 (3), 17.34 (4); Ileu-2 171.92 (1), 57.29 (2), 38.01 (3), 14.78 (4), 26.55 (5), 11.63 (6); Orn 172.01 (1), 52.87 (2), 29.63 (3), 24.39 (4), 40.05 (5); Pro 172.55 (1), 60.23 (2), 29.58 (3), 25.38 (4) 48.03 (5); Val-3 171.28 (1), 57.57 (2), 30.54 (3), 19.61 (4), 18.80 (5); Val-4 171.83 (1), 59.10 (2), 31.26 (3), 19.45 (4), 18.08 (5); [thr-2] *Thr-2* 170.97 (1), 58.89 (2), 67.36 (3), 19.66 (4); Val-5 172.67 (1), 59.64 (2), 30.66 (3), 19.61 (4), 18.43 (5); 5-MeHex 173.83 (1), 36.28 (2), 23.99 (3), 38.96 (4), 28.10 (5), 22.54 (6), 22.50 (7); [5-MeHex (second conformation) 174.08 (1), 33.86 (2), 32.84 (3), 29.75 (4), 34.54 (5), 19.51 (6), 11.20 (7);] IR neat (NaCl): 3287 (s, br), 2964 (s, br), 1646 (s), 1528

(s), 1464 (s), 1388 (m), 1228 (m), cm^{-1}; mass spectrum HRFAB m/z (fragment, %) 1477.9408 (M^++1, 85); UV (MeOH): λ_{max} 204 (89,630) nm.

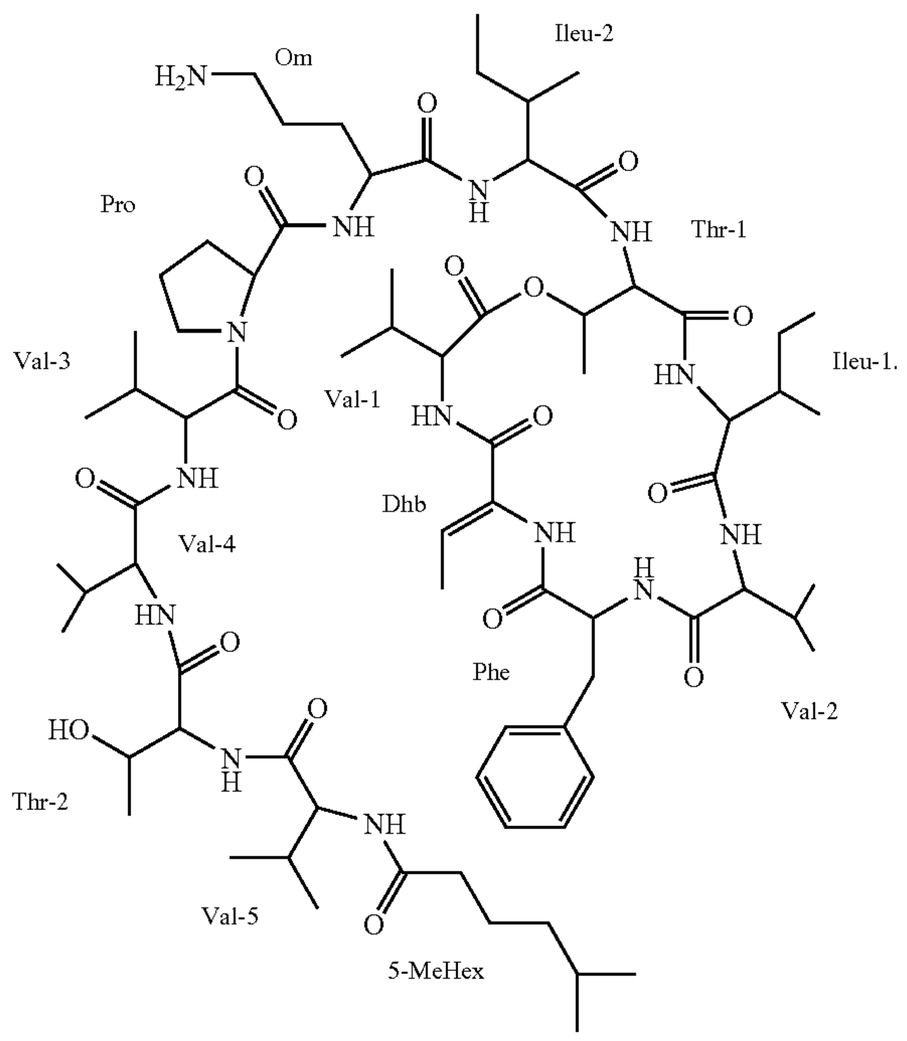
2. The **[**compound**]** Kahalalide F *compound* of claim **1**, which further has the **[**folowing**]** *following* non-stereospecific structure:



3. A pharmaceutical **[**compostion**]** *composition* comprising a pharmaceutical carrier or diluent and **[**the**]** substantially pure **[**compound**]** Kahalalide F**[**, said compoumd**]** *compound* having a molecular formula of $C_{75}H_{124}N_{14}O_{16}$, and **[**conng**]** *consisting* of five valines, two isoleucines, two threonines, ornithine, **[**dehydroamiobutyric**]** *dehydroaminobutyric* acid, proline, phenylalanine and **[**5-methylhexoic**]** *5-methylhexanoic* acid; said compound further exhibiting the following physical and chemical properties:

$[\alpha]_D$-8° (c 4.32, MEOH); ^{1}H NMR (500 MHz, TFA/DMF); amino acid unit, δ (carbon position, mult, J): Val-1 4.16 (2, t, J=9.0 Hz), 7.11 (NH on 2, d, J=**[**8,9**]***8.9* Hz), 1.77 (3, m), 0.95 (4, m), 0.95 (5, **[**ma**]** *m*); Dhb 9.20 (NH on 2, s), 6.48 (3, q, J=6.9 Hz), 1.43 (4, d, J=6.6 Hz); Phe 4.68 (2, q, J=6.6 Hz), 8.62 (NH on 2, d, J=6.6 Hz), 3.2*3* (3, dd, J=13.7, 7.2 Hz), 3.0*0* (3, dd, J=13.7, 9.0 Hz), 7.32 (5, d, J=7.2 Hz), 7.28 (6, t, J=7.5 Hz), 7.21 (7, t, J=7.2 Hz); Val-2 4.36 (2, m), 7.82 (NH on 2, d, J=6.6 Hz), 2.12 (3, m), 0.85 (4, m), 0.77 (5, d, J=6.6 Hz); **[**Ileu-1**]** *Ileu-1* 4.53 (2, m), 8.38 (NH on 2, d, J=9.6 Hz), 1.98 (3, m), 0.92 (4, d, J=6.6 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, t, J=7.2 Hz); Thr-1 4.63 (2, t, J=9.3 Hz), 8.12 (NH on 2, d, J=5.7), 5.07 (3, dq, *J*=9.6, 6.0 Hz), 1.18 (4, d, J=6.3 Hz); Ileu-2 4.52 (2, m), 7.72 (NH on 2, d, J=8.4 Hz), 1.88 (3, m), 0.88 (4, d, J=6.3 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, **[**d**]** *t*, J=7.2 Hz) Orn 4.48 (2, m), 7.92 (NH on 2, d, J=7.8 Hz), 1.76 (3, m), 1.83 (4, m), 3.10 (5, p, J=5.1 Hz); Pro 4.42 (2, m), 2.12 (3, m), 1.97 (3, m), 2.02 (4, m), 1.88 (4, m), 3.75 (5, m), 3.68 (5, m); Val-3 4.41 (2, m), 7.90 (NH on 2, d, J=7.2 Hz), 2.12 (3, m), 0.95 (4, m), 0.85 (5, m); Val-4 4.34 (2, m), 7.68 (NH on 2, d, J=8.1 Hz), 2.17 (3, m), 0.95 (4, m), 0.90 (5, m); Thr-2 4.46 (2, m), 7.77 (NH on 2, d, J=8.1Hz), 4.21 (3, dq, *J*=6.3, 3.6 Hz), 1.12 (4, d, J=6.6 Hz); Val-5 4.32 (2, m), 7.85, (NH on 2, d, J=8.1 Hz), **[**7.82 (NH on (second conformation), d, J=8.1 Hz),**]** 2.14 (3, m), 0.95 (4, m), 0.90 (5, m); 5MeHex 2.26 (2, m), 1.60 (3, m), 1.20 (4, m), 1.55 (5, m), 0.87 (6, d, J=7.2 Hz), 0.87 (7, d, J=7.2 Hz); **[**5-MeHex 2.29 (2, m), 1.65 (3, m), 1.40 (3, m), 1.13 (4, m), 1.35 (5, m), 0.90 (6, m), 0.90 (7, m);**]** ^{13}C NMR (125 MHz TFA/DMF): amino acid unit, δ (carbon position); Val-1 170.40 (1), 60.31 (2), 30.75 (3), 19.58 (4), 18.76 (5); Dhb 164.54 (1), 130.30 (2), 131.26 (3), 12.68 (4); Phe 171.31 (1), **[**56–27**]** *56.27* (2), 36.79 (3), 138.23 (4), 129.86 (5), 128.77 (6), 126.98 (7); Val-2 172.94 (1), 58.27 (2), 32.38 (3), 18.92 (4), 17.60 (5); Ileu-1 171.87 (1), 57.48 (2), 38.78 (3), 14.56 (4), 26.78 (5), 11.67 (*6*); Thr-1 169.68 (1), 57.37 (2), 71.05 (3), 17.34 (4); Ileu-2 171.92 (1), 57.29 (2), 38.01 (3), 14.78 (4), 26.55 (5), 11.63 (6); Orn 172.01 (1), 52.87 (2), 29.63 (3), 24.39 (4), 40.05 (5); Pro **[**172.35**]** *172.55* (1), 60.23 (2), 29.58 (3), 25.38 (4), 48.03 (5); Val-3 171.28 (1), 57.57 (2), 30.54 (3), 19.61 (4), 18.80 (5); Val-4 171.83 (1), 59.10 (2), 31.26 (3), 19.45 (4), 18.08 (5); Thr-2 170.97 (1), 58.89 (2), 67.36 (3), 19.66 (4); Val-5 172.67 (1), 59.64 (2), 30.66 (3), 19.61 (4), 18.43 (5); 5-MeHex 173.83 (1), 36.28 (2), 23.99 (3), 38.96 (4), 28.10 (5), 22.54 (6), 22.50 (7); **[**5-MeHex (second conformation) 174.08 (1), 33.86 (2), 32.84 (3), 29.75 (4), 34.54 (5), 19.51 (6), 11.20 (7);**]** IR neat (NaCl): 3287 (s, br), 2964 (s, br), 1646 (s), 1528 (s), 1464 (s), 1388 (m), 1228 (m), cm^{-1}; mass spectrum HRFAB m/z (fragment, %) 1477.9408 (M^++1, 85); UV (MeOH): λ_{max} 204 (89,630) nm.

4. The pharmaceutical composition of claim **3**, wherein the **[**compound**]** Kahalalide F *compound* further has the **[**folowing non-streospecific**]** *following non-stereospecific* structure



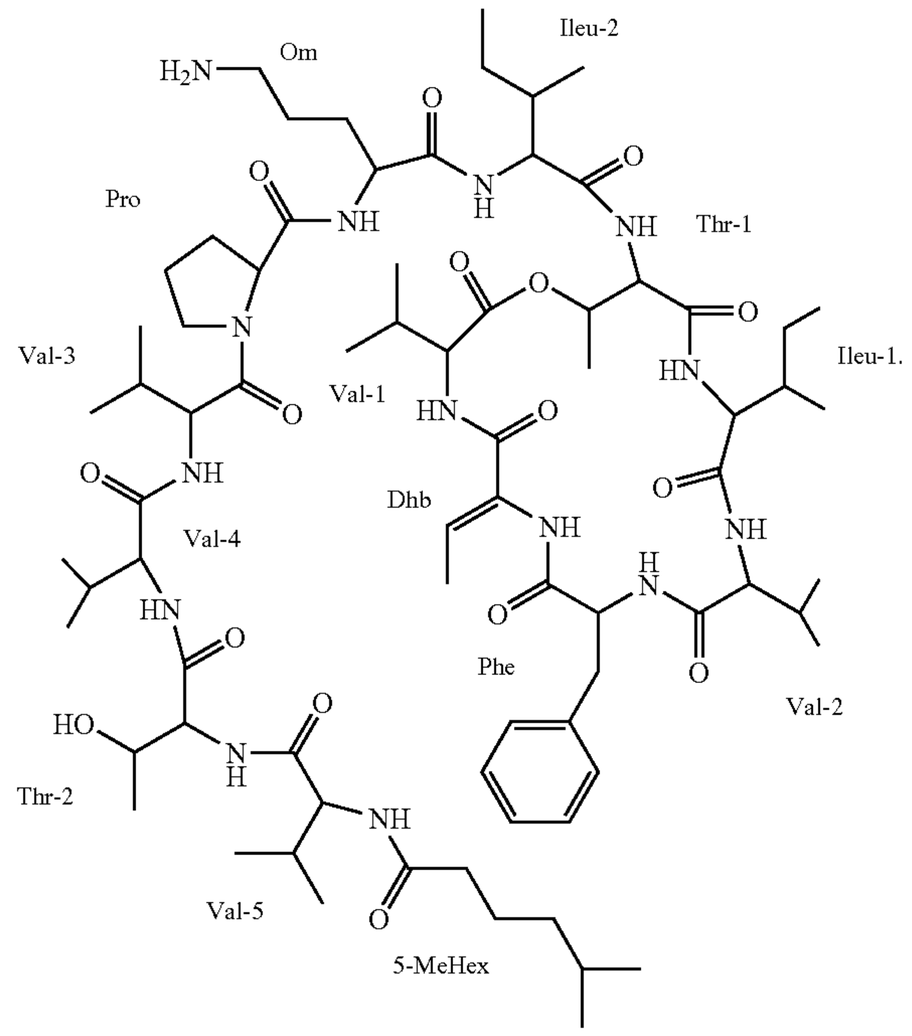
5. A method of treating fungal infections in mammals comprising administering to *a* patient in need of such treatment, an amount of the substantially **[**puxe compound**]** *pure* Kahalalide F *compound* or a pharmaceutically acceptable salt thereof, sufficient to slow or stop the growth of the fungal infection; said compound having a **[**molecula**]**

molecular formula of $C_{75}H_{124}N_{14}O_{16}$, and consisting of five valines, two isoleucines, two [theonines] *threonines*, ornithine, [dehydroaminobutiric] *dehydroaminobutyric* acid, proline, [phenylalanie] *phenylalanine* and 5-methylhexanoic acid; said compound further exhibiting the [folowing] *following* physical and chemical properties:

$[\alpha]_D$-8° (c 4.32, MEOH); ^{1}H NMR (500 MHz, TFA/DMF); amino acid unit, δ (carbon position, mult, J): Val-1 4.16 (2, t, J=9.0 Hz), 7.11 (NH on 2, d, J=[8,9]*8.9* Hz), 1.77 (3, m), 0.95 (4, m), 0.95 (5, [ma] *m*); Dhb 9.20 (NH on 2, s), 6.48 (3, q, J=6.9 Hz), 1.43 (4, d, J=6.6 Hz); Phe 4.68 (2, q, J=6.6 Hz), 8.62 (NH on 2, d, J=6.6 Hz), 3.2*3* (3, dd, J=13.7, 7.2 Hz), 3.0*0* (3, dd, J=13.7, 9.0 Hz), 7.32 (5, d, J=7.2 Hz), 7.28 (6, t, J=7.5 Hz), 7.21 (7, t, J=7.2 Hz); Val-2 4.36 (2, m), 7.82 (NH on 2, d, J=6.6 Hz), 2.12 (3, m), 0.85 (4, m), 0.77 (5, d, J=6.6 Hz); [Ilue-1] *Ileu-1* 4.53 (2, m), 8.38 (NH on 2, d, J=9.6 Hz), 1.98 (3, m), 0.92 (4, d, J=6.6 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, t, J=7.2 Hz); Thr-1 4.63 (2, t, J=9.3 Hz), 8.12 (NH on 2, d, J=5.7), 5.07 (3, dq, *J*=9.6, 6.0 Hz), 1.18 (4, d, J=6.3 Hz); Ileu-2 4.52 (2, m), 7.72 (NH on 2, d, J=8.4 Hz), 1.88 (3, m), 0.88 (4, d, J=6.3 Hz), 1.40 (5, m), 1.13 (5, m), 0.88 (6, [d] *t*, J=7.2 Hz) Orn 4.48 (2, m), 7.92 (NH on 2, d, J=7.8 Hz), 1.76 (3, m), 1.83 (4, m), 3.10 (5, p, J=5.1 Hz); Pro 4.42 (2, m), 2.12 (3, m), 1.97 (3, m), 2.02 (4, m), 1.88 (4, m), 3.75 (5, m), 3.68 (5, m); Val-3 4.41 (2, m), 7.90 (NH on 2, d, J=7.2 Hz), 2.12 (3, m), 0.95 (4, m), 0.85 (5, m); Val-4 4.34 (2, m), 7.68 (NH on 2, d, J=8.1 Hz), 2.17 (3, m), 0.95 (4, m), 0.90 (5, m); Thr-2 4.46 (2, m), 7.77 (NH on 2, d, J=8.1), 4.21 (3, dq, *J*=6.3, 3.6 Hz), 1.12 (4, d, J=6.6 Hz); Val-5 4.32 (2, m), 7.85, (NH on 2, d, J=8.1 Hz), [7.82 (NH on (second conformation), d, J=8.1 Hz),] 2.14 (3, m), 0.95 (4, m), 0.90 (5, m); 5MeHex 2.26 (2, m), 1.60 (3, m), 1.20 (4, m), 1.55 (5, m), 0.87 (6, d, J=7.2 Hz), 0.87 (7, d, J=7.2 Hz); [5MeHex 2.29 (2, m), 1.65 (3, m), 1.40 (3, m) 1.13 (4, m), 1.35 (5, m), 0.90 (6, m), 0.90 (7, m);] ^{13}C NMR (125 MHz, TFA/DMF): amino acid unit, 6 (carbon position); Val-1 170.40 (1), 60.31 (2), 30.75 (3), 19.58 (4), 18.76 (5); Dhb 164.54 (1), 130.30 (2), 131.26 (3), 12.68 (4); Phe 171.31 (1), [56–27] *56.27* (2), 36.79 (3), 138.23 (4), 129.86 (5), 128.77 (6), 126.98 (7); Val-2 172.94 (1), 58.27 (2), 32.38 (3), 18.92 (4), 17.60 (5); Ileu-1 171.87 (1), 57.48 (2), 38.78 (3), 14.56 (4), 26.78 (5), 11.67 (*6*); Thr-1 169.68 (1), 57.37 (2), 71.05 (3), 17.34 (4); Ileu-2 171.92 (1), 57.29 (2), 38.01 (3), 14.78 (4), 26.55 (5), 11.63 (6); Orn 172.01 (1), 52.87 (2), 29.63 (3), 24.39 (4), 40.05 (5); Pro [172.35] *172.55* (1), 60.23 (2), 29.58 (3), 25.38 (4), 48.03 (5); Val-3 171.28 (1), 57.57 (2),

30.54 (3), 19.61 (4), 18.80 (5); Val-4 171.83 (1), 59.10 (2), 31.26 (3), 19.45 (4), 18.08 (5); Thr-2 170.97 (1), 58.89 (2), 67.36 (3), 19.66 (4); Val-5 172.67 (1), 59.64 (2), 30.66 (3), 19.61 (4), 18.43 (5); 5-MeHex 173.83 (1), 36.28 (2), 23.99 (3), 38.96 (4), 28.10 (5), 22.54 (6), 22.50 (7); [5-MeHex (second conformation) 174.08 (1), 33.86 (2), 32.84 (3), 29.75 (4), 34.54 (5), 19.51 (6), 11.20 (7);] IR neat (NaCl): 3287 (s, br), 2964 (s, br), 1646 (s), 1528 (s), 1464 (s), 1388 (m), 1228 (m), cm^{-1}; mass spectrum HRFAB m/z (fragment, %) 1477.9408 (M^++1, 85); UV (MeOH): λ_{max} 204 (89,630) nm.

6. The method of treatment of claim **5**, wherein compound Kahalalide F has the following non-stereospecific structure:



7. The method of claim **5**, wherein the fungal infection is caused by Aspergillus oryzae.

8. The method of claim **5**, wherein the fungal infection is caused by Penicillium notatum.

9. The method of claim **5**, wherein the fungal infection is caused by Trichophyton mentagrophy.

10. The method of claim **5**, wherein the fungal infection is caused by Candida albicans.

* * * * *