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

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 See application file for complete search history.

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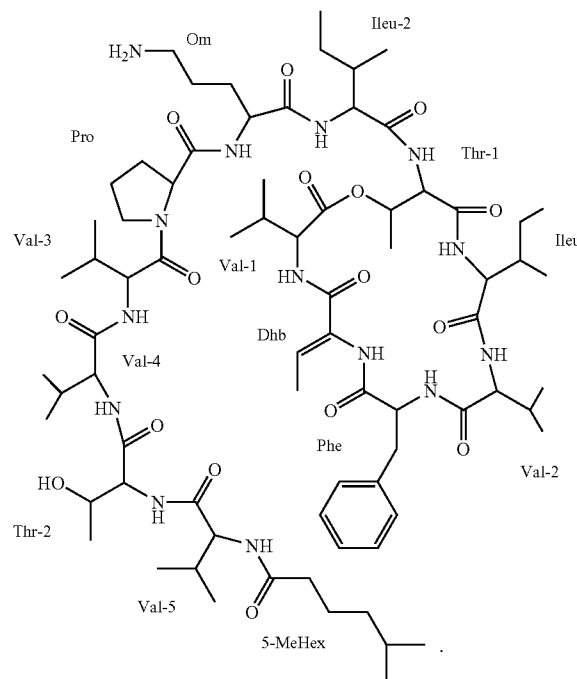
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(57) ABSTRACT

[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

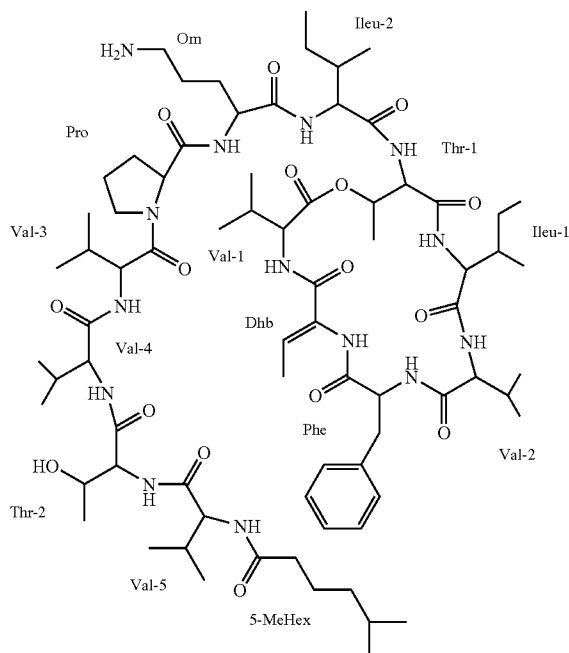
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KAHALALIDE F AND COMPOSITIONS AND
USES THEREOF

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 rufescens* [*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 [methodology] *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.*

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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.

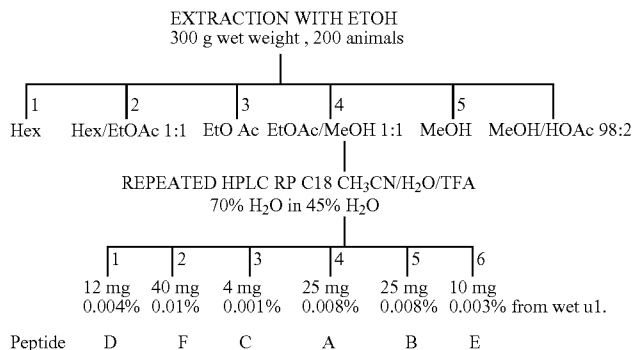
The present invention also relates to pharmaceutical preparations which contain as active ingredient these compounds, or a pharmaceutically acceptable acid addition salt thereof, as well as the process for its preparation.

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. [] 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 (RPC18) using MeCN/H₂O with 0.1% TFA (70-45% H₂O).

ISOLATION SCHEME

Elysia rufescens

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 1H 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, [phenylalanine] *phenylalanine* and [5-methylhexanoic] *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. 1H 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/H₂O/TFA [(55/45/1)30/70/1]—MeCN/H₂O/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/H₂O/TFA (55/45/1). Physical data: [[α]_D]²⁰ [8°] [α]_D²⁰ (c 4.32, MeOH); 1H 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), 3.23 (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); 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

1H and ^{13}C NMR Data for [Kahalalide F (I)] <i>KF</i> in DMF/TFA					
Amino Acid	Carbon	^{13}C , ppm ^a	Mult.	1H , 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
					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
5-Methyl-Hexanoic acid	1	173.8	s		
	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-Hexanoic acid (second conformation)]	1	174.1	s		
	2	33.9	t	2.29	m
	3	32.8	t	1.65, 1.40	m
	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

^aat 125 MHz, DMF signal at 35.2 ppm;^bat 500 MHz, DMF signal at 2.91 ppm.

[TABLE I] TABLE II

In vitro Activity of [Kahalalide F]KF from <i>Elysia rufescens</i> Assay (M.I.C. µg/mL)	
Cytotoxicity 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 <i>Elysia rufescens</i> Assay (M.I.C. µg/mL)	
5	Antifungal 6 mm disk
	50 µg/disk
	<i>Aspergillus oryzae</i>
	19 mm
	<i>Penicillium notatum</i>
	26 mm
	<i>Tricophyton mentagrophy</i>
	34 mm
	<i>Saccharomyces cerevisiac</i>
	neg
10	<i>Candida albicans</i>
	16 mm

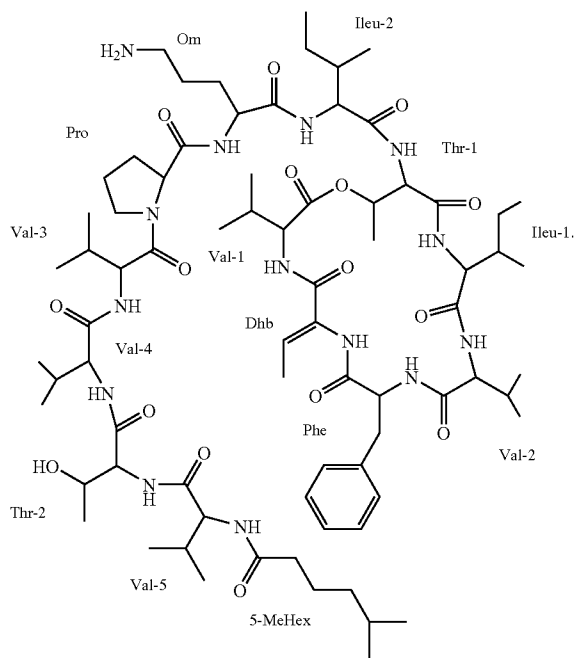
We claim:

1. [A substantially] *Substantially* pure [compound] Kahalalide F *compound*, said compound [hag] *having* a molecular formula of C₇₅H₁₂₄N₁₄O₁₆, and consisting of five valines, two isoleucines, two threonines, ornithine, [dehydroaminobutyric] *dehydroaminobutyric* acid, proline, phenylalanine and 5-methylhexanoic acid; said compound further exhibiting the following physical and chemical properties:
- 20 [α]_D²⁰ -8° (c 4.32, MeOH); ¹H 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).] ¹³C 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

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(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 [following] following non-stereospecific structure:



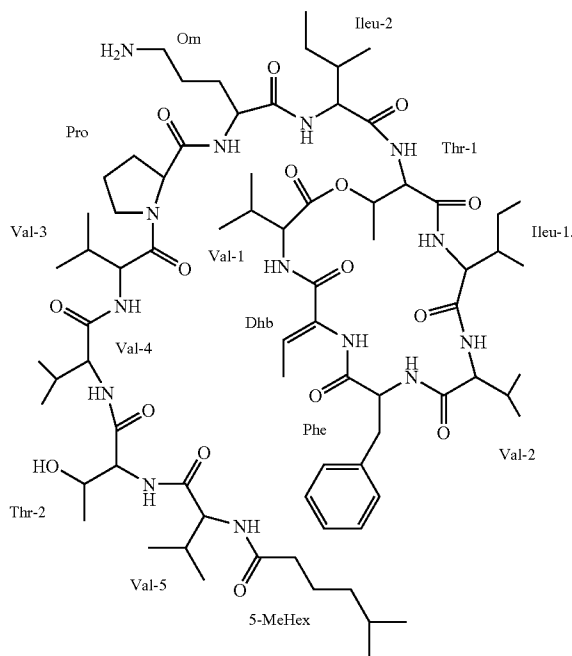
3. A pharmaceutical [composition] composition comprising a pharmaceutical carrier or diluent and [the] substantially pure [compound] Kahalalide F, said compound] compound having a molecular formula of $\text{C}_{75}\text{H}_{124}\text{N}_{14}\text{O}_{16}$, and [conng] consisting of five valines, two 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:

$[\alpha]_{\text{D}}^{25}$ -8° (c 4.32, MeOH); ^1H 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), 3.23 (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); 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, $J=8.1$ Hz), 4.21 (3, dq, $J=6.3, 3.6$ Hz), 1.12 (4,

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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 [following non-stereospecific] 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 [pure 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]

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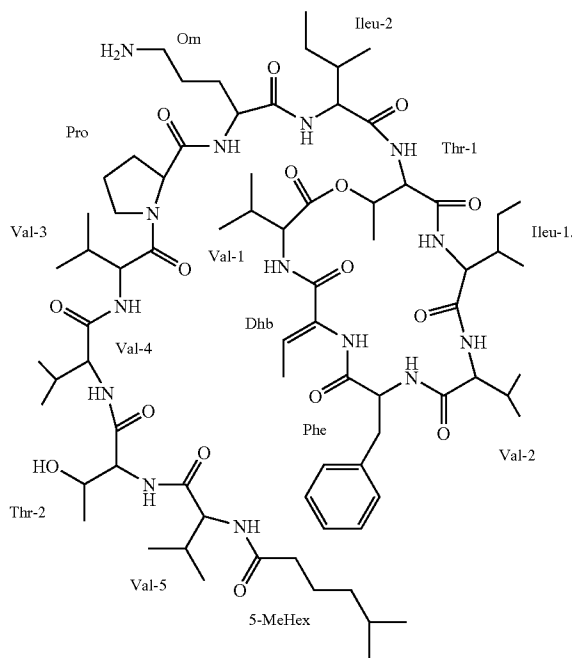
molecular formula of $C_{75}H_{124}N_{14}O_{16}$, and consisting of five valines, two isoleucines, two [theonines] *theonines*, ornithine, [dehydroaminobutyric] *dehydroaminobutyric acid*, proline, [phenylalanine] *phenylalanine* and 5-methylhexanoic acid; said compound further exhibiting the [following]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

$[\alpha]_D^{25}$ -8° (c 4.32, MeOH); 1H 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), 3.23 (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); 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, $J=8.1$), 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, 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),

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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*.

* * * * *