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Optimization Studies of the Important Drug Lead Kahalalide F which Acts on a Novel Cancer Target RPS25

Kully Lynn Woodruff

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by

Kully Lynn Woodruff

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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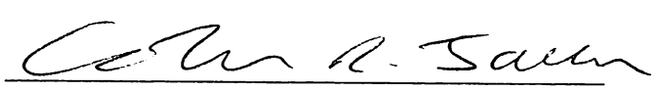
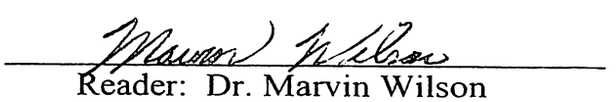
Approved by

Advisor: Dr. Mark T. Hamann



Reader: Dr. Colin Jackson



Reader: Dr. Marvin Wilson

DEDICATION

This manuscript is dedicated to my grandmother, Quana F. Woodruff, whose ever-present love and support is a constant source of strength.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my advisor and director of research, Dr. Mark T. Hamann, for allowing me the opportunity to be involved in such promising research. I would like to thank Dr. Abbas Gholipour Shilabin for including my work in his latest publication. I am ever grateful for his patience and guidance. I would also like to thank Dr. Colin Jackson and Dr. Marvin Wilson for their revisions to this manuscript. I would like to express my profound gratitude to Dr. John Samonds, Associate Dean of the Sally McDonnell Barksdale Honors College, whose support and encouragement has given me strength and inspiration during my four years at the University of Mississippi. Finally, I would like to thank Mr. and Mrs. Jimmy D. Woodruff, Joshua D. Woodruff, Leness E. Woodruff, and Dr. Kimberly K. Shackelford for their constant love, support, and encouragement.

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ABSTRACT

KULLY LYNN WOODRUFF: Optimization Studies of the Important Drug Lead Kahalalide F which Acts on a Novel Cancer Target RPS25

(Under the direction of Dr. Mark T. Hamann)

Kahalalide F (KF, **1**) is the largest and most biologically active cyclic peptide of the naturally occurring kahalalide family of depsipeptides. Previous evaluations of the compound have shown significant *in vitro* and *in vivo* activity against various forms of cancer. KF has been investigated in phase I clinical trials and is being evaluated in phase II clinical studies. The objective of this experiment was to prepare analogues of KF through semisynthetic modification of the parent compound to optimize activity against a slate of pathogens, as well as cancerous cells. Data revealed that KF and analogues were not active against malaria, leishmania, or bacteria. However, the series was active against various strains of fungi, including *Fusarium*, a genus known for causing opportunistic infections in plants, as well as humans. KF and analogues were also evaluated for *in vitro* activity against 60 human cancer cell lines. Analogue **5** exhibited greater activity than **1** against several cell lines, including leukemia, colon, ovarian, renal, and prostate cell lines. Through the evaluations of antiprotozoal, antibacterial, and antifungal activity, as well as the activity against a slate of human cancer cell lines, it was revealed that semisynthetic modification of the parent compound has an affect on activity. Further studies involving semisynthetic modification of KF could lead to vast improvements in current drug therapies.

PREFACE

The research reported in this manuscript has been incorporated into the larger work "Lysosome and HER3 (ErbB3) Selective Anticancer Agent Kahalalide F: Semisynthetic Modifications and Antifungal Lead-Exploration Studies" by Abbas Glolipour Shilabin, Noer Kasanah, David E. Wedge, and Mark T. Hamann, which was published in the *Journal of Medicinal Chemistry*.*

* Shilabin, A. G..; Kasanah, K..; Wedge, D. E.; Hamann, M. T. Lysosome and HER3 (ErbB3) selective anticancer agent kahalalide F: semisynthetic modifications and antifungal lead-exploration studies. *J. Med. Chem.* **2007**, *50*, 4340-4350.

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LIST OF ABBREVIATIONS

advanced malignant melanoma ..... AMM

Centers for Disease Control and Prevention ..... CDC

central nervous system ..... CNS

fungicidal ..... FC

fungistatic ..... FS

hepatocarcinoma ..... HC

kahalalide D ..... KD

kahalalide F ..... KF

kahalalide G ..... KG

microplate Alamar blue assay ..... MABA

minimum bactericidal concentration ..... MBC

minimum fungicidal concentration ..... MFC

minimum inhibitory concentration ..... MIC

National Cancer Institute ..... NCI

non-small-cell lung cancer ..... NSCLC

structure-activity relationship ..... SAR

total growth inhibition ..... TGI

LIST OF ABBREVIATIONS cont.

1..... KF

2.....KG

3..... Monoacetylated-KF

4..... Oxo-KF

5..... 4-Fluorobenzylamino-KF

6..... Bis(4-fluorbenzyl)amino-KF

7.....4-pyridinylmethylamino-KF

8..... 2-Thienylmethylamino-KF

9.....Bis(2-thienylmethyl)amino-KF

10.....*n*-Hexylamino-KF

11.....Di-*n*-hexylamino-KF

16..... DEAC-KF-amide

I. INTRODUCTION

A. BACKGROUND

There is no question that natural products are a source of promise in the areas of drug discovery and development. According to the Natural Products Branch (NPB) of the National Cancer Institute (NCI), "There are good precedents for looking to nature for drug discovery." The NCI states that approximately twenty-five percent of modern medicines come from plants that were used previously in a more traditional form. Drugs, including aspirin, morphine and many antibiotics, were originally derived from natural products, including plants, bacteria, fungi and marine invertebrates. Many of the current chemotherapy drugs were also derived from natural products. For instance, Taxol, a common drug used today in the treatment of breast and ovarian cancer, was derived from the bark of the Pacific yew, a tree native to the Pacific Northwest.[21] Gordon Cragg, chief of the NPB, said, "Nature produces these molecules for a reason." The compounds that have proven useful medicinally often play a critical role in the life of the organism from which they come. The NCI cites examples such as bacteria that produce antibiotics to kill off other invading bacteria and marine organisms that produce toxins for protection from other organisms.[1-2]

The National Center for Natural Products Research (NCNPR) at the University of Mississippi is also participating in the area of drug discovery and development.[3] The research reported in this manuscript was completed under Dr. Mark T. Hamann, research professor with NCNPR. Much of Hamann's research focuses on the development of

marine natural product drug leads via marine derived secondary metabolites. Reported research focuses on the kahalalides.

B. KAHALALIDE F

The kahalalide family consists of a series of natural depsipeptides. They were first isolated from the Hawaiian herbivorous marine sacoglossan mollusk *Elysia rufescens* (Plakobranchidea). *E. rufescens* is a small, soft-bodied sacoglossan (Figure 1). Later isolation was achieved from *Bryopsis pennata* (Bryopsidaceae), the green algal diet of *E. rufescens*.[4-8] The kahalalides range in size and composition from a C31 tripeptide to a C75 tridecapeptide. The largest and most biologically active cyclic peptide of the kahalalide family is the tridecapeptide kahalalide F (KF, **1**). KF has molecular formula $C_{75}H_{124}N_{14}O_{16}$ and mass 1477.9408 $[M + H]^+$.[4,9] Previous evaluations of the parent compound KF have shown significant *in vitro* and *in vivo* activity against various forms of cancer, including colon, breast, non-small-cell lung, and prostate. KF has been investigated in phase I clinical trials for androgen-refractory prostate cancer[10] and in phase II clinical trials for liver cancer, non-small-cell lung cancer (NSCLC), and melanoma.[11] A number of patients with advanced NSCLC, hepatocarcinoma (HC), and advanced malignant melanoma (AMM) have responded positively to treatment with KF.[12] However, structure-activity relationship (SAR) studies involving KF have not been reported due to its limited availability as a natural product.[13,14]

The significant activity of KF against serious forms of cancer has prompted research to investigate the mechanism of action of the compound. It is known that KF

acts on cell lysosomes, causing targeted cells to swell, leading to oncosis, or cellular death by swelling.[15,16] Studies have also shown that inhibition of the receptor tyrosine kinase ErbB3 (HER3) has an affect on the mechanism of action.[23] A recent study by Piggott and Karuso employed chemical proteomics with the goal of isolating and identifying the actual cellular receptor of KF. The study cited human ribosomal protein S25 (RPS25) as a binding partner for the compound. RPS25 is located at the surface of the 40S ribosomal subunit in eukaryotic cells and may aid in stability of the complex. It is likely that RPS25 is only one step in the series of events that eventually leads to disruption of lysosomes and cell death by oncosis.[16]

KF is covered by U.S. patent number 6,274,551 and has been assigned to the pharmaceutical company PharmaMar S.A. in Madrid, Spain for further development.

Figure 1. *Elysia rufescens*

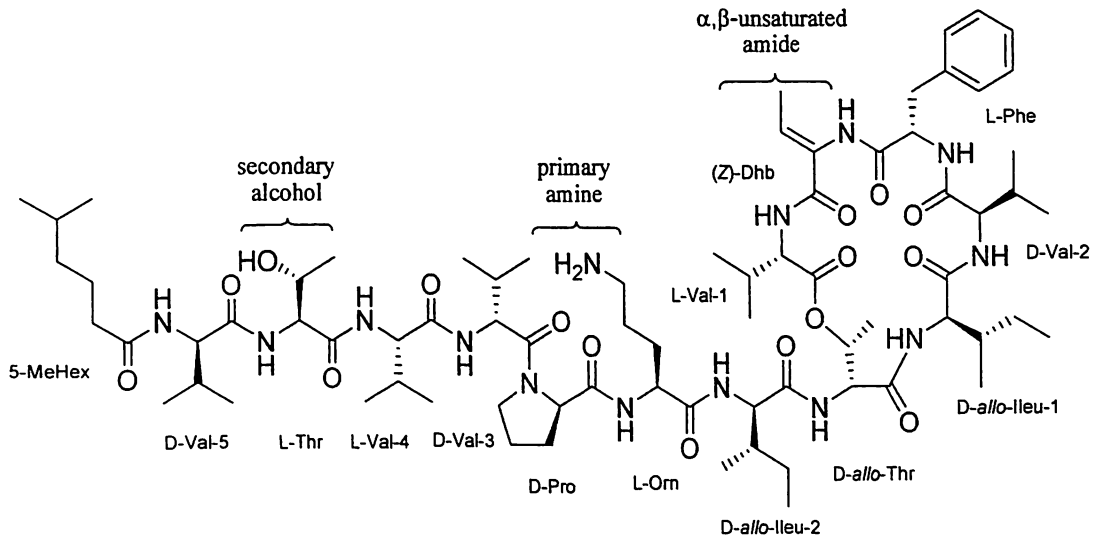

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Figure 2. Kahalalide F and key functional groups

C. TARGETS

1. MICROORGANISMS

The Department of Health and Human Services Centers of Disease Control and Prevention (CDC) cites the development of drug resistance by microorganisms as a pressing public health concern. As these organisms develop resistance to current therapies, research devoted to the discovery of new treatment methods is critical.[17] For this reason, the activity of KF and its derivatives were evaluated for activity against several key organisms, including the fungi *Fusarium* spp., *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus;* the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, Methicillin resistant *Staphylococcus aureus*, *Mycobacterium tuberculosis,* and *Mycobacterium intracellulare*; and the protozoa *Plasmodium falciparum* and *Leishmania donovani.*

Fusarium, a large genus of the Phylum Ascomycota, is a filamentous fungus common to tropical and subtropical areas. The genus *Fusarium* contains over twenty species of fungi, including *Fusarium solani* and *Fusarium oxysporum.* It is commonly found associated with such commodities as rice and soybeans.[18] Along with being a well known plant pathogen, *Fusarium* spp. can also cause various infections in humans, collectively referred to as fusariosis. Fusariosis is commonly found in immunocompromised patients who have undergone transplants or chemotherapy treatment. It is also developing as an opportunistic infection in AIDS patients. The treatment options for patients infected with *Fusarium* spp. are limited due to its intense drug resistance. Because of the difficulty in treating the disease, fusariosis is often fatal in its more invasive form.[19] The activity of KF and its derivatives was also evaluated for activity against the fungi *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*. Like *Fusarium* spp., these microorganisms cause opportunistic infections in immunocompromised patients.[20]

Activity of KF and analogues was also tested against the parasites *Plasmodium falciparum* and *Leishmania donovani. Plasmodium falciparum* is the cause of the most severe form of malaria. The CDC reports that 350-500 million cases of malaria occur worldwide each year. Over one million cases are fatal, with *P. falciparum* being the most deadly. The parasite is spread by certain infected mosquitoes of the genus *Anopheles*. The parasite *Leishmania donovani* causes visceral leishmaniasis. Visceral leishmaniasis affects internal organs, including the spleen, liver, and bone marrow. The CDC reports that over 500,000 new cases of visceral leishmaniasis are diagnosed each year. Major

countries affected include India, Sudan, and Brazil. The parasite is spread by infected sand flies.[17]

The activity of KF and its derivatives was also evaluated against the bacteria *Mycobacterium tuberculosis, Escherichia coli, Pseudomonas aeruginosa,* Methicillin resistant *Staphylococcus aureus,* and *Mycobacterium intracellulare. Mycobacterium tuberculosis* is the bacterium that causes tuberculosis (TB). *M. tuberculosis* usually attacks the lungs, but can also affect the spine, kidney, and brain. The bacteria spread through the air, making the disease extremely contagious. Many cases of TB develop in patients with compromised immune systems, such as those with AIDS and HIV. TB is a leading cause of death worldwide, surpassing malaria and AIDS.[17] In many countries the Bacillus Calmette-Guerin (BCG) vaccine is administered. However, many clinical trials have placed little value on the vaccine. Often a cocktail of drugs is used in treatment. Developing drug resistance is a critical concern with TB, because of the current widespread nature of the disease. The bacteria *Escherichia coli, Pseudomonas aeruginosa,* Methicillin resistant *Staphylococcus aureus,* and *Mycobacterium intracellulare,* which cause opportunistic infections in immunocompromised patients, were also tested. With each of these organisms, developing drug resistance is a critical concern.[20]

2. CANCER

As previously stated, evaluations of KF have shown significant activity against various forms of cancer, including colon, breast, non-small-cell lung, and prostate. KF has been investigated in phase I and phase II clinical trials for androgen-refractory

prostate cancer[10] and for liver cancer, NSCLC, and melanoma, respectively.[11] Patients with advanced NSCLC, HC, and AMM have also responded positively to treatment with KF.[12] Because KF has already shown activity against several serious forms of cancer, the activity of the parent compound and analogues was evaluated against a slate of 60 human cancer cell lines. The cell lines tested included lung, leukemia, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate, and breast. The activity of KF against each of these cell lines was compared with the activity of the current chemotherapy drug Paclitaxel against the slate of cells. Paclitaxel is marketed under the U.S. brand name Taxol. As previously discussed, the compound was derived from the bark of the Pacific yew, a tree native to the Pacific Northwest. It is commonly used today in the treatment of breast and ovarian cancer.[21] Many other chemotherapy drugs were also derived from natural products. For instance, vincristine is an alkaloid isolated from the Madagascar periwinkle (Vinca rosea). It is marketed in the U.S. under the name Oncovin and is commonly used in the treatment of non-Hodgkin's lymphoma.[21] The drugs derived from each of these natural products are currently being used in chemotherapy treatments. This precedent provides promise for the continued potential of KF.

Because of the significant activity of KF against various forms of cancer, unlocking the mechanism of action of the compound has become a critical area of study. As previously discussed, studies have shown that inhibition of the receptor tyrosine kinase ErbB3 (HER3) has an affect on the mechanism of action of the compound. The activity of KF against several cell types has been linked with protein expression levels of ErbB3, which suggests that ErbB3 could play a role in determining the effectiveness of KF in the treatment of individual patients.[11,23] Like HER3, HER1 and HER2 are

members of the human epidermal growth factor receptor family of receptor tyrosine kinases. There are currently drugs on the market that act on HER1 and HER2 receptors. For example, Herceptin (trastuzumab), a monoclonal antibody, acts by inducing cytotoxicity against cells overexpressing HER2. Herceptin has been used in the treatment of breast cancer caused by this overexpression. HER1 inhibiting drugs are also available. Examples include Erbitux (cetuximab), Iressa (gefitinib), and Tarceva (erlotinib hydrocloride).[21] Unlike available drugs for HER1 and HER2 related cancers, there are currently no HER3 inhibiting drugs. Therefore, KF and analogues serve as promising candidates for inhibition of HER3 receptors in tumor cells.

II. METHODS

General Experimental Procedures. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 and MeOD on a Bruker DRX NRM spectrometer operating at 400 MHz for ^{1}H and 100 mHz for ^{13}C NMR. Chemical shift (δ) values are expressed in parts per million (ppm) and are referenced to the residual solvent signals of DMSO-d_6 and MeOD at δ_H/ δ_C 2.50/39.5 and 3.31,4.78/49.1, respectively. UV and IR spectra were respectively obtained using a Perkin-Elmer Lambda 3B UV/Vis spectrophotometer and an AATI Mattson Genesis series FTIR. Optical rotations where measured with a JASCO DIP-310 digital polarimeter. The High Resolution ESI-MS spectra were measured using a Bruker Daltonic (GmbH, Germany) microTOF series with electrospray ionization. TLC analysis was carried out on precoated silica gel G_{254} aluminum plates.

CHEMICALS. Kahalalide F was prepared according to the previously reported methods with some modifications.[4] The animals (*Elysia rufescens*) were collected by snorkeling at low tide near Black Point, Oahu in Hawaii. The ethanolic extraction of freeze-dried animals was subjected to flash chromatography on silica gel (EtOAc/MeOH). Preparative HPLC using Phenomenex 100 mm RP C8 column (250 mm × 100 mm) and a gradient MeCN (0.05% TFA)/H2O, followed by further HPLC purification on amino column (250 mm × 22 mm) using gradient EtOAc/MeOH afforded KF (**1**) as a white amorphous powder. All reagents and solvents were obtained from commercial vendors and were utilized without further purification.

Kahalalide G (2). A solution of kahalalide F free base (30 mg, 20 μmol) and potassium carbonate (0.2 g) in H_2O/MeOH (1:2) (10 mL) was stirred at room temperature for 8 h. The mixture was portioned between $CHCl_3$/IPA (2:1) and water. The organic layer was dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. Reversed-phase HPLC using gradient MeCN (0.05% TFA)/water afforded 28.4 mg (95%) of KG (**2**) as a white powder.[5] Yield 95.0%; $[\alpha]^{25}_D$ – 12.3° (c = 0.20, MeOH); UV λ_{max} (MeOH) 195 nm; IR neat (NaCl) 3281 (s, br), 2966 (s), 2937 (s), 1732 (s), 1636 (s), 1541 (s), 1456 (s), 1204 (s), 1139 (s) cm^{-1}; HRESIMS *m/z* calcd for $C_{75}H_{127}N_{14}O_{17}$ $[M + H]^+$ 1519.9498, found 1519.9466.

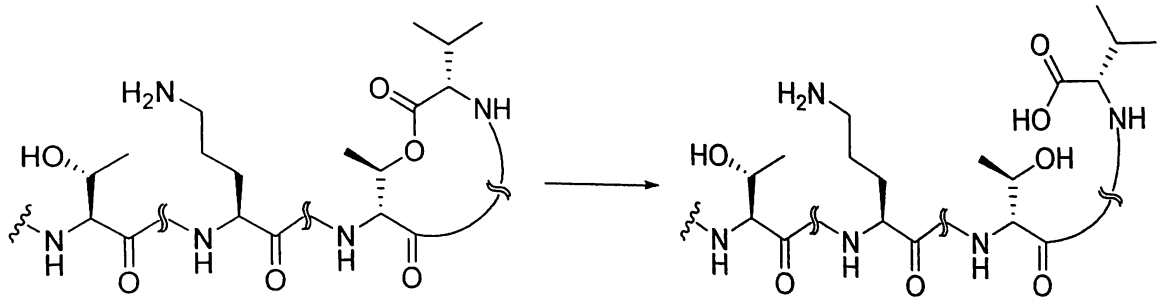


Figure 3. Conversion of kahalalide F (**1**) to kahalalide G (**2**)

Monoacetylated-KF (3). To a suspension of kahalalide F free base (30 mg, 20 μmol) in anhydrous dichloromethane (5 mL) were added acetic anhydride (0.4 mL) and boron trifluoride-diethyl etherate (0.2 mL) at room temperature under a nitrogen atmosphere. The mixture was stirred for 5 min, poured into a cool saturated $NaHCO_3$ solution (20 mL), and extracted with $CHCl_3$/IPA (2:1)(2 × 10 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The purification was carried out with HPLC using a Phenomenex Luna RP C8 column (250 mm × 22 mm) eluted with a gradient water/MeCN (0.05% TFA) to give 20.7 mg (68%)

of monoacetyl KF (**3**). Yield 68.0%; $[\alpha]^{25}_{D}$ – 10.5° (*c* = 0.25, MeOH); UV λ_{max} (MeOH) 194, 222 nm; IR neat (NaCl) 3283 (s, br), 2967 (s), 2936 (s), 1735 (s), 1654 (s), 1458 (s), 1389 (s), 1140 (s) cm^{-1}; HRESIMS *m/z* calcd for $C_{75}H_{127}N_{14}O_{17}$ $[M + H]^+$ 1519.9498, found 1519.9478.

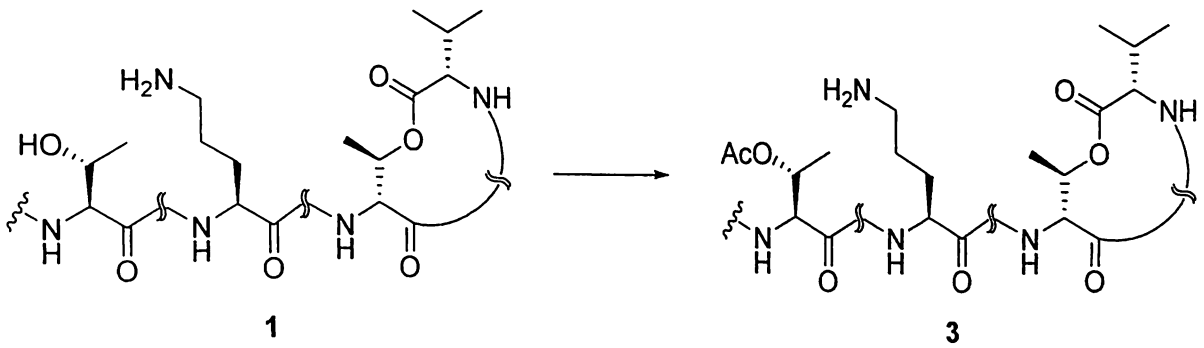


Figure 4. Conversion of **1** to monoacetylated-KF (**3**)

Oxo-KF (4). To a solution of Dess-Martin periodinane (22 mg, 48 μmol) in anhydrous acetonitrile (10 mL) was added kahalalide F (60 mg, 40 μmol) in one portion and stirred at room temperature for 1 h under nitrogen atmosphere. The reaction mixture was quenched with 10% $Na_2S_2O_3$/saturated aqueous $NaHCO_3$ (v/v 1:1, 20 mL) and extracted with $CHCl_3$/IPA (2:1) (2 × 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were evaporated under reduced pressure. The residue was subjected to preparative HPLC using a Phenomenex Luna RP C8 column (250 mm × 22 mm) eluted with gradient water/MeCN (0.05% TFA) to afford 44.2 mg (75%) of pure oxidized compound **4** as a white powder. Yield 75.0%; $[\alpha]^{25}_{D}$ – 5.4° (*c* 0.10, MeOH); UV λ_{max} (MeOH) 195 nm; IR neat (NaCl) 3319 (s, br), 2961 (s), 2925 (s), 1731 (s), 1645 (s), 1531 (s), 1455 (s), 1392 (s) cm^{-1}; HRESIMS *m/z* calcd for $C_{75}H_{123}N_{14}O_{16}$ $[M + H]^+$ 1475.9236, found 1475.9237.

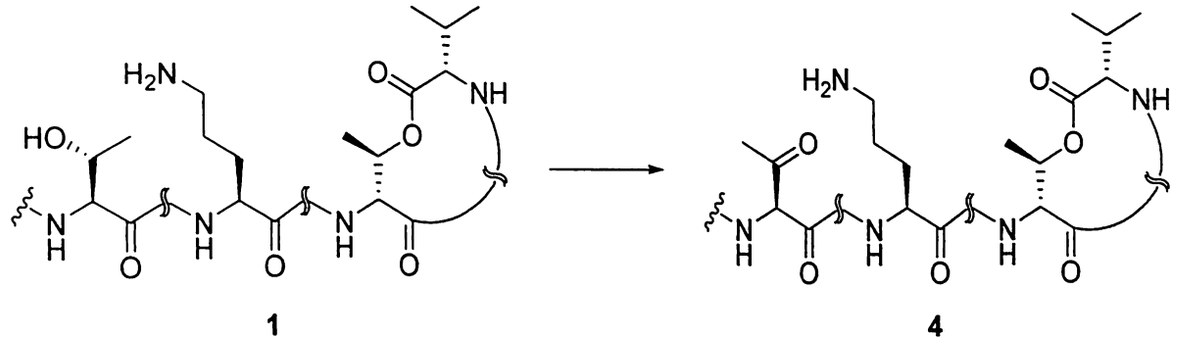


Figure 5. Conversion of **1** to Oxo-KF (**4**)

General Preparation of Compounds 5-11. To a solution of kahalalide F (29.5 mg, 20 μmol) and aldehyde in anhydrous methanol (5 mL) was added via 3 Å molecular sieve (2 g) and stirred for 30 min at room temperature under argon followed by portionwise addition of sodium triacetoxyborohydride (8.5 mg, 40 μmol) over a 20 min period. The reaction mixture was stirred for the above mentioned time at the same condition, quenched with water (20 mL) and extracted with IPA/$CHCl_3$ (1:2) (2 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The resulting residue was purified with preparative HPLC using Phenomenex RP C8 column (250 mm × 22 mm) and eluted with gradient CH3CN (0.05 TFA)/water to yield corresponding monoalkyl-KF (major) and dialkyl-KF (minor) products as colorless powders.

4-Fluorobenzylamino-KF (5). Starting material 4-fluorobenzaldehyde (10.6 μL, 100 μmol) was used and the reaction mixture was stirred for 18 h. Yield 64.5%; $[\alpha]^{25}_D$ − 11.9° (*c* 0.25, MeOH); UV λ_{max} (MeOH) 196 nm; IR neat (NaCl) 3281 (s, br), 2966 (s), 2935 (s), 1774 (s), 1655 (s), 1583 (s), 1450 (s), 1204 (s) cm^{-1}; HRESIMS *m/z* calcd for $C_{82}H_{130}FN_{14}O_{16}$ $[M + H]^+$ 1585.9768, found 1585.9769.

Bis(4-fluorbenzyl)amino-KF (6). Yield 3.1%; HRESIMS *m/z* calcd for $C_{89}H_{134}F_2N_{14}O_{16}$ $[M + H]^+$ 194.0148, found 1694.0141.

4-pyridinylmethylamino-KF (7). Starting material 4-pyridinecarboxaldehyde (9.5 μL, 100 μmol) was used and the reaction mixture was stirred for 2 d. The preliminary stirring of the reaction mixture for 30 min was not completed. Yield 53.1%; $[\alpha]^{25}_D$ – 9.1° (*c* 0.19, MeOH); UV λ_{max} (MeOH) 193 nm; IR neat (NaCl) 3281 (s, br), 2965 (s), 2923 (s), 1741 (s), 1647 (s), 1541 (s), 1523 (s), 1457 (s), 1203 (s), 1139 (s) cm^{-1}; HRESIMS *m/z* calcd for $C_{81}H_{130}N_{15}O_{16}$ $[M + H]^+$ 1568.9815, found 1568.9814.

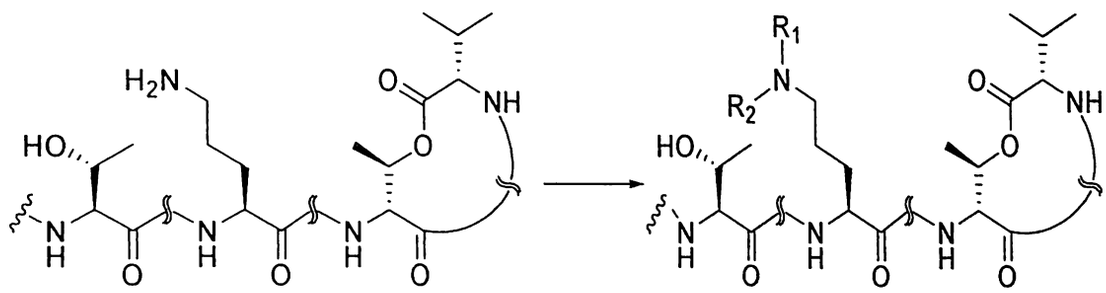
2-Thienylmethylamino-KF (8). Starting material 2-thiophenecarboxaldehyde (9.2 μL, 100 μmol) was used, and the reaction mixture was stirred for 3 d. Yield 52.3%; $[\alpha]^{25}_D$ – 8.0° (*c* 0.25, MeOH); UV λ_{max} (MeOH) 193 nm; IR neat (NaCl) 3283 (s, br), 2966 (s), 2936 (s), 1734 (s), 1648 (s), 1541 (s), 1524 (s), 1458 (s), 1203 (s) cm^{-1}; HRESIMS *m/z* calcd for $C_{80}H_{129}N_{14}O_{16}S$ $[M + H]^+$ 1585.9768, found 1585.9769.

Bis(2-thienylmethyl)amino-KF (9). Yield 12.6%; HRESIMS *m/z* calcd for $C_{85}H_{133}N_{14}O_{16}S_2$ $[M + H]^+$ 1669.9465, found 1669.9461.

***n*-Hexylamino-KF (10).** Starting material *n*-hexanal (12.3 μL, 100 μmol) was used and the reaction mixture was stirred for 1 h. Yield 45.1%; $[\alpha]^{25}_D$ – 7.2° (*c* = 0.10, MeOH); UV λ_{max} (MeOH) 195 nm; IR neat (NaCl) 3280 (s, br), 2964 (s), 2934 (s), 1732 (s), 1651

(s), 1539 (s), 1456 (s), 1203 (s) cm^{-1}; HRESIMS *m/z* calcd for $C_{81}H_{137}N_{14}O_{16}$ $[M + H]^+$ 1562.0331, found 1562.0334.

Di-*n*-hexylamino-KF (11). Yield 25.3%; $[\alpha]^{25}_D$ – 18.2° (*c* = 0.25, MeOH); UV λ_{max} (MeOH) 195 nm; IR neat (NaCl) 3282 (s, br), 2964 (s), 2935 (s), 1732 (s), 1646 (s), 1540 (s), 1457 (s), 1204 (s), 1137 (s) cm^{-1}; HRESIMS *m/z* calcd for $C_{87}H_{149}N_{14}O_{16}$ $[M + H]^+$ 1646.1270, found 1646.1271.



1 **5-11**

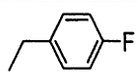
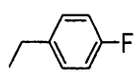
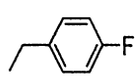
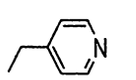
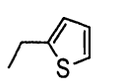
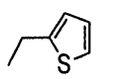
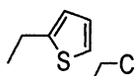
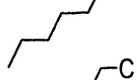
Compound	R_1	R_2	Yield (%)
4-Fluro-benzylamino-KF (**5**)	H		64.5
Bis(4-fluro-benzyl)amino-KF (**6**)			3.1
4-Pyridinylmethylamino-KF (**7**)	H		53.1
2-Thienylmethylamino-KF (**8**)	H		52.3
Bis(2-thienylmethyl)amino-KF (**9**)			12.6
n-Hexylamino-KF (**10**)	H		45.1
Bis(*n*-hexyl)amino-KF (**11**)			25.3

Figure 6. Conversion of **1** to analogous **5-11**

DEAC-KF-amide (16). 7-Diethylaminocoumarin-3-carboxylic acid (5.3 mg, 20.3 μmol), HBTU [*O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate] (9.2 mg, 24.4 μmol), and EDC [*N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide] (4.3 μL, 24.4 μmol) were dissolved in anhydrous DMF (5 mL). A solution of kahalalide F (30.0 mg, 20.3 μmol) in anhydrous DMF (2 mL) was added portionwise over 10 min at 0°C, followed by stirring for 1 h at room temperature. The reaction was quenched by adding water (10 mL) and extracted with IPA/$CHCl_3$ (1:2) (2 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The resulting residue was purified with HPLC using an Phenomenex Luna RP C8 column (250 mm × 22 mm) and eluted with gradient Me_3CN (0.05 TFA)/water to afford peptide **19** as white powders. Yield 84.9%; $[\alpha]^{25}_D$ – 9.1° (*c* = 0.20, MeOH); UV λ_{max} (MeOH) 201, 422 nm; IR neat (NaCl) 3319 (s, br), 2959 (s), 2924 (s), 1731 (s), 1644 (s), 1514 (s), 1455 (s), 1233 (s), 1135 (s) cm^{-1}; HRESIMS *m/z* calcd for $C_{89}H_{138}N_{15}O_{19}$ $[M+H]^+$ 1721.0287, found 1721.0282.

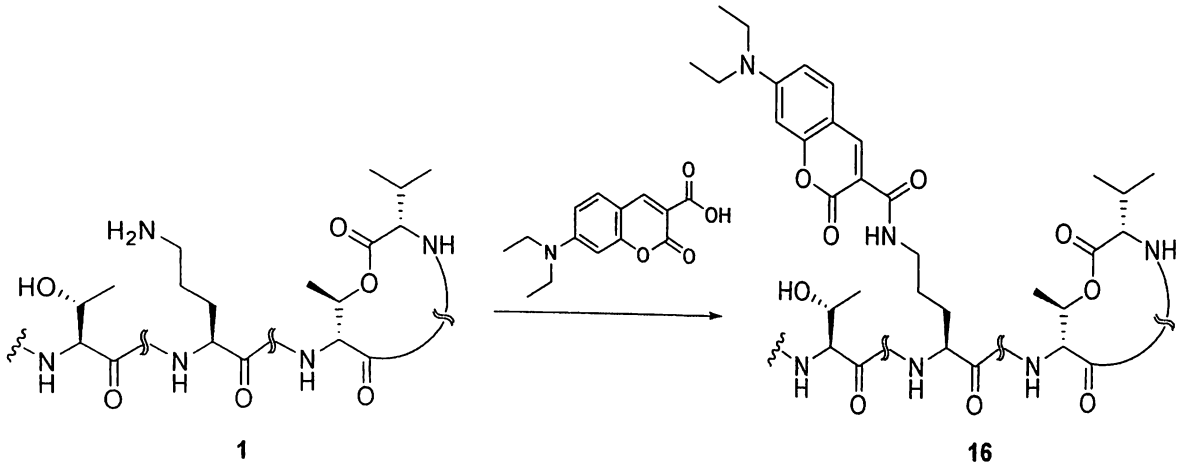


Figure 7. Conversion of **1** to DEAC-KF-amide (**16**)

Pathogen Production of *Fusarium*. *F. solani* (F-0007), *F. oxysporium* (F-0001), and *F. proliferatum* (F0029-1) were obtained from USDA-ARS Laboratory, Natural Products Utilization Research Unit, National Center for Natural Products Research, The University of Mississippi. The isolates were isolated from orchid species by D. E. Wedge and identified by Dr. W. H. Elmer, Connecticut Agricultural Experiment Station. The cultures were stored on silica gel in the USDA-NPURU repository. The isolates were found to be pathogenic to orchid species. *Fusarium* spp. Cultures were initiated on potato dextrose agar (PDA, Difco, Detroit, MI) from spores. Conidia were harvested from 7 day old culture by flooding plates with 10 mL of sterile distilled water and dislodging using an L-shape glass rod. Conidial suspensions were filtered through sterile Miracloth (Calbiochem-Novabiochem Corp., La Jolla, CA) to remove mycelia. Concentration of conidia was determined photometrically at 625 nm from a standard curve. The concentration of conidia was 3×10^5 conidia/mL. The conidia suspension was used for one-dimensional direct bioautography and other experiments.

Bioautography Assay. Each compound was dissolved in methanol at 1 mg/mL. An amount of 100 μL of compounds was spotted on a glass thin layer chromatography plate (TLC plate, silica gel GF, 250 μm, 10 cm × 20 cm, Uniplate, Analtech, Inc., Neward, DE) and allowed to dry. The initial concentration of each compound tested was 100 μg/mL. After drying, the plates were sprayed with spore suspensions of *Fusarium* spp. Conidial solution was prepared with potato dextrose broth 12 g/500 mL, 0.1% bacto agar, 0.1% Tween-80 and contained 3×10^5 conidia/mL of each *Fusarium* spp. Plates were placed in a moisture chamber (Pioneer Plastic, Inc., Dixon, KY) and incubated for 3 days

at 26 °C. After incubation, plates were removed from the moisture chamber, dried at room temperature, and exhaustively sprayed with 0.25% MTT (3-(4,5-dimethylthiazl-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) prepared in phosphate buffer. The plates were placed back into a moisture chamber and incubated at 26 °C for 1 day and allowed to develop. The active compounds were visualized by clear zones of fungal growth inhibition on TLC plates with purple background. Amphotericin B, captan, benomyl, and azoxystrobin were used as positive controls to evaluate the activity of **1** and its analogues.

Microtiter Assay. **1** and its analogues were evaluated for a dose response effect against fungi using a 96-well microdilution broth assay (Nunc, Micro Well, Roskilde, Denmark) at concentrations of 0.3, 3, and 30 μM against *F. proliferatum*, *F. solani*, and *F. oxysporium* according to the procedure published by Wedge et al.[22] Microtiter plates were covered with plastic lids and incubated in a growth chamber at 24 ± 1 °C and 12 h photoperiod under 60 ± 5 $\mu mol/m^2\ s^1$. Fungal growth was monitored photometrically by measuring absorbance at 620 nm at 24, 48, and 72 h. Mean absorbance values and standard error were used to evaluate fungal growth.

Fungicidal and Fungistatic Testing. The fungicidal or fungistatic assay was conducted to determine the action of each compound at 30 μM. After 72 h of incubation in a microtiter assay, 20 μL of solution of each well was cultured onto a 90 mm Petri plate containing 10 mL of potato dextrose agar free of **1** and its analogues. The plates were incubated at 28 °C for 3 days. The fungicidal effect was defined in no fungal growth was

observed on the subcultured plate free of compounds, and the fungistatic activity was defined if fungal growth was observed on the subcultured plate.

Mycobacterium Tuberculosis Assay. In vitro evaluation of antimycobacterial activity was conducted with Mycobacterium tuberculosis H37Rv ATCC 27294 in BACTEC 12B medium using mircoplate Alamar blue assay (MABA). Primary screening is evaluated at 16 μg/mL. Compounds that showed inhibition of greater than 90% are retested at a lower concentration to determine the MIC values.[22]

III. RESULTS AND DISCUSSION

1. Semisynthesis of Kahalalide F Analogues. Methodology for the semisynthesis of kahalalide F (KF, **1**) analogues is presented in the corresponding section along with validation of each structure. Data regarding structural validation is also presented in Table 1.

Table 1. Selected ^{1}H NMR and DEPT 135° Data for **1** and Its N-Alkylated KF Analogues in DMSO-d_6[a]

Compound	HRESIMS $[M+H]^+$	^{1}H-NMR			^{13}C-NMR		
		L-Ornithine		Derivative (R) residue	L-Ornithine		Derivative (R) Residue
		NH*R*	CH_2(δ)		CH_2(δ)	$NHCH_2R$	
1	1477.9408	7.62 (m, 2H, NH_2)	2.74 (m)	7.19-7.28 (m, 5H, Ph)	38.7	-	126.8 (Ph), 128.5 (2C, Ph), 129.5 (Ph), 130.1 (Ph)
5	1585.9769	7.60 (m)	2.91 (m)	7.18-7.28 (m, 5H, Ph), 7.49-7.52 (m, 2H, Ph), 8.77-8.80 (m, 2H, Ph)	38.5	46.6	115.9 (Ph), 116.1 (Ph), 127.0 (Ph), 128.6 (2C, Ph), 129.7 (Ph), 130.3 (Ph), 132.7 (Ph), 132.8 (Ph)
7	1568.9814	7.63 (m)	2.93 (m)	7.21-7.28 (m, 5H, Ph), 7.49 (m, 2H, Py), 8.66 (s, 2H, Py)	38.5	47.6	124.7 (2C, Py), 126.9 (Ph), 128.7 (2C, Ph), 129.7 (Ph), 130.3 (Ph), 150.3 (2C, Py)
8	1573.9429	7.63 (m)	2.92 (m)	7.22-7.29 (m, 5H, Ph), 7.10 (m, Th), 7.21 (m, Th), 7.57 (m, Th)	38.5	47.6	126.9 (Ph), 127.8 (Th), 128.6 (2C, Ph), 128.7 (Th), 129.7 (Ph), 130.2 (Ph), 130.9 (Th)
10	1562.0334	7.59 (m)	2.85 (bs)	7.21-7.27 (m, 5H, Ph), 0.61-1.55 (m, 13H, aliphatic)	38.5	47.6	14.3 (CH_3), 22.3 (CH_2), 25.8 (CH_2), 26.0 (CH_2), 31.1 (CH_2), 126.9 (Ph), 128.6 (2C, Ph), 129.7 (Ph), 130.2 (Ph)
11	1646.1271	-	2.95 (bs)	7.18-7.25 (m, 5H, Ph), 0.61-1.55 (m, 26H, aliphatic)	38.4	47.6	14.3 (CH_3), 22.4 (CH_2), 23.4 (CH_2), 23.5 (CH_2), 31.2 (CH_2), 127.0 (Ph), 128.6 (2C, Ph), 129.7 (Ph), 130.2 (Ph)

Abbreviations: Ph = phenyl, Py = pyridine, Th = thienyl; Aliph = aliphatic

2. Biological Evaluation of Products.

2.A. Activity in Opportunistic Infections. Kahalalide D (KD), kahalalide G (KG, **2**), **1** and its analogues were evaluated *in vitro* for their activity against several microorganisms. These microorganisms included *Escherichia coli*, *Pseudomonas aeruginosa*, Methicillin resistant *Staphylococcus aureus*, *Candida albicans*, *Cryptococcus neoformans*, *Mycobacterium intracellulare* and *Aspergillus fumigatus*, all of which cause opportunistic infections in immunocompromised individuals. KD, **2**, **1** and its analogues showed no activity against *E. coli*, *P. aeruginosa*, or Methicillin resistant *S. aureus*. Only **3** and **10** showed activity against *M. intracellulare*. Results of this evaluation revealed that semisynthetic modification of **1** alters antibacterial activity. **1** and its analogues also exhibited activity against *C. albicans* (fungus type yeast), *C. neoformans* (dimorphic fungus), *A. fumigatus* (filamentous fungus) and *Fusarium* spp. (filamentous fungus).[20] Results of this evaluation are presented in Table 2. The concentration that yields 50% inhibition of growth (IC_{50}), the lowest test concentration that yields detectable growth (minimum inhibitory concentration, MIC), and the lowest test concentration that kills 100% of the organism (minimum fungicidal/bactericidal concentration, MFC/MBC) were determined. Amphotericin B, a common antifungal drug, served as a control. **1** and analogues **3**, **8**, and **10** exhibited activity against *C. albicans*, *C. neoformans*, and *A. fumigatus*. **4** and **7** were active against *C. albicans* and *C. neoformans*. **7** was also active against *A. fumigatus*. **5** and **11** were active against C. neoformans. KD, **2**, **6**, and **16** showed no activity and were not subjected to further testing.

Table 2. In Vitro Data of Antimicrobial[a]

compd	*C. albicans* ATCC 90028 (μM)			*C. neoformans* ATCC 90113 (μM)			*A. fumigatus* ATCC 90906 (μM)			*M. intracellulare* ATCC 23068 (μM)		
	IC_{50}	MIC	MFC	IC_{50}	MIC	MFC	IC_{50}	MIC	MFC	IC_{50}	MIC	MFC
1	3.02 ± 0.04	10	20	1.53 ± 0.38	5	5	3.21 ± 0.05	10	20	> 20	> 20	> 20
2	> 13.38	NT	NT	> 13.38	NT	NT	> 13.38	NT	NT	> 13.38	NT	NT
KD	> 33.57	NT	NT	> 33.57	NT	NT	> 33.57	NT	NT	> 33.57	NT	NT
3	3.28 ± 0.02	10	20	1.89 ± 0.01	5	5	3.38 ± 0.09	10	10	14.32 ± 0.33	> 20	> 20
4	12.44 ± 0.07	> 20	> 20	4.34 ± 0.44	10	20	> 13.6	> 20	> 20	> 20	> 20	> 20
5	> 12.6	> 20	> 20	3.77 ± 0.09	10	10	> 12.6	> 20	> 20	> 20	> 20	> 20
6	> 11.81	NT	NT	> 11.81	NT	NT	> 11.81	NT	NT	> 11.81	NT	NT
7	4.19 ± 0.11	> 20	> 20	2.12 ± 0.37	5	5	4.26 ± 0.1	10	20	> 20	> 20	> 20
8	3.81 ± 0.14	10	20	1.91 ± 0.23	5	10	3.22 ± 0.06	10	20	11.98 ± 0.57	> 20	> 20
10	5.69 ± 0.15	20	20	1.93 ± 0.07	5	10	3.12 ± 0.02	10	20	> 20	> 20	> 20
11	> 12.2	> 20	> 20	6.71 ± 0.02	> 20	> 20	> 12.2	> 20	> 20	> 20	> 20	> 20
16	> 11.62	NT	NT	> 11.62	NT	NT	>11.62	NT	NT	> 11.62	NT	NT
amphotercin B	0.25 ± 0.04	0.625	1.25	0.79 ± 0.05	0.016	0.06	1.32 ± 0.06	2.5	2.5	NT	NT	NT
ciprofloxacin	NT	NT	NT	NT	NT	NT	NT	NT	NT	0.42 ± 0.07	1.0	> 1

[a] NT = not tested. Amphotericin B (a polyene antifungal drug) and ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid) are used as positive antifungal and antibacterial controls, respectively. The IC_{50} is the concentration that affords 50% inhibition of growth. MIC (minimum inhibitory concentration) is the lowest test concentration that yields detectable growth. MFC/MBC (minimum fungicidal/bactericidal concentration) is the lowest test concentration that kills 100% of the organism.

2.B. Antiparasitic Activity. **1** and its analogues were tested against *Plasmodium falciparum*, which causes one of the deadliest forms of malaria, and *Leishmania donovani*, which causes visceral leishmaniasis, as previously discussed.[17] **1** and analogues **8** and **10** were active against *L. donovani*. **1** and its analogues did not show activity against *P. falciparum*.

2.C. Bioactivity against *Mycobacterium tuberculosis*. **1** and its analogues were tested against *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis.[17] Rifampicin, a standard drug used in the treatment of *Mycobacterium* infections, served as the standard.[21] Compounds were evaluated at 16 μg/mL. The minimum inhibitory concentration (MIC), the lowest test concentration that yields detectable growth, of compounds with greater than 90 percent inhibition at 16 μg/mL was determined (Table 3). **3**, **8**, and **11** showed greater activity than **1** (67% inhibition), with inhibitions ranging from 91-94%. These values were comparable to the standard rifampicin, which showed inhibition of 93%. Of the three, **11** showed better potency against *M. tuberculosis*. The minimum inhibitory concentration of the other analogues was not determined, because as previously stated, percent inhibition was less than 90%.

Table 3. Minimum Inhibitory Concentration (MIC) of **1** Analogues versus *Mycobacterium tuberculosis* H37Rv Strains[a]

compd	% inhibition at 16 μg/mL	MIC (μg/mL)
RMP	93	0.08
1	67	> 16
KD	49	NT
2	29	NT
3	94	15.4
5	77	NT
7	24	NT
8	92	15.5
10	88	> 16
11	91	9.4
16	46	NT

[a] NT = not tested. RMP = rifampicin.

2.D. Bioactivity against *Fusarium* spp. The treatment options for patients infected with *Fusarium* spp. are limited due to its intense drug resistance. For this reason, the activity of KF and its derivatives were evaluated for antifungal activity, specifically focusing on *Fusarium* spp.

2.D.1. Bioautography Assay. Bioautography was used to detect antifungal activity against Fusarium spp. Results showed that semisynthetic modification of **1** did not have an effect on antifungal activity. **1** and its analogues were active against *F. proliferatum.* Only **4** was active against *F. solani* and *F. oxysporium.* Analogue **4**, which was prepared by oxidizing the secondary alcohol, could be a key in broadening the spectrum of activity against *Fusarium* spp.

2.D.2. Microtiter Assay. In contrast to the bioautography assay, which revealed that **1** and its analogues were active only against *F. proliferatum*, the microtiter assay revealed that **1** and its analogues were active against *F. oxysporium*, *F. proliferatum*, and *F. solani* (Table 4). Activity was present at the highest concentration, 30 μM. Table 4 shows the percent inhibition of *Fusarium* spp. after treatment with **1** and its analogues at three different concentrations (0.3, 3.0, and 30 μM) for 48 and 72 hours. Standards tested were Azoxystrobin (fungicide), Captan (fungicide), and Amphotericin B (antifungal drug). At the lowest concentration (0.3 μM), only **4** showed activity against *Fusarium* spp. This activity was lost after 72 hours. Azoxystrobin showed activity against *F. oxysporum* and *F. solani* after 48 and 72 hours of exposure. At 3.0 μM, **7**, **10**, and **16** showed no activity against *Fusarium* spp. **1** showed activity against *F. solani* and **4** showed activity against *F. oxysporum* and *F. proliferatum* after 48 hours exposure. Both lost activity after 72 hours. Captan and Amphotericin B showed activity against Fusarium spp. after 48 hours but lost activity after 72 hours, while Azoxystrobin retained activity after 72 hours. All compounds showed no activity after 72 hours, except for **8**, which retained activity against *F. solani*, and Azoxystrobin, which retained activity against *Fusarium* spp. At the highest concentration (30 μM), only compound **16** did not exhibit activity against *Fusarium* spp. **1** and all other analogues displaced activity after 48 and 72 hours of exposure. These compounds showed greater activity that the standard Azoxystrobin. Results of this evaluation revealed that semisynthetic modification of **1** alters the activity against *Fusarium* spp.

Table 4. Microtiter Analysis of Kahalalide Analogues at Different Concentrations (48 and 72 h)

compd	% inhibition[a] (0.3 μM)			% inhibition[a] (3.0 μM)			% inhibition[a] (30 μM)		
	Fusarium oxysporum	*Fusarium proliferatum*	*Fusarium solani*	*Fusarium oxysporum*	*Fusarium proliferatum*	*Fusarium solani*	*Fusarium oxysporum*	*Fusarium proliferatum*	*Fusarium solani*
1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	9.9 (0)	100 (99.6)	99.8 (100)	98.9 (99.9)
3	0 (0)	0 (0.3)	0 (0)	0 (0)	0 (0.3)	0 (0)	78.5 (74.4)	70.8 (34.7)	80.9 (96.7)
4	1.8 (0)	1.8 (0)	0 (0)	1.8 (0)	5.0 (0)	0 (0)	91.9 (82.2)	92.7 (73.2)	90.6 (85.5)
5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16.5 (0)	96.6 (94.3)	97.5 (96.1)	97.3 (97.3)
7	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	98.2 (91.2)	99.1 (95.3)	99.2 (97.5)
8	0 (0)	0 (0)	0 (0)	13.7 (0)	4.8 (0)	47.0 (32.9)	98.8 (99.2)	98.7 (99.0)	99.0 (99.3)
10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	93.6 (89.0)	95.3 (92.8)	94.3 (94.7)
16	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
azoxystrobin	22.7 (15.3)	0 (0)	16.6 (11.7)	32.3 (7.1)	21.7 (14.8)	25.3 (15.0)	77.2 (32.9)	53.0 (40.7)	62.7 (51.7)
captan	0.2 (0)	6.0 (3.0)	0 (0)	7.6 (0)	12.0 (0)	5.1 (0)	99.5 (98.1)	100 (100)	90.7 (47.9)
amphotericin B	0 (0)	0 (0)	0 (0)	12.5 (0)	7.9 (0)	18.1 (0)	99.7 (87.1)	99.8 (79.0)	100 (98.1)

[a] Sample results only indicate inhibition. Zero (0) does not indicate the degree of stimulation, only that there was no inhibition. Numbers above are 48 h results. Numbers enclosed in parentheses are 72 h results.

2.D.3. Fungistatic and Fungicidal Activity. The fungistatic (FS) and fungicidal (FC) activity of **1** and its analogues was examined at 30 μM against *Fusarium* spp. (Table 5). Analysis was completed after 72 hours. The standards Amphotericin B, Captan, and Azoxystrobin were tested and provided expected results. Amphotericin B is fungicidal and Captan, and Azoxystrobin are fungistatic against *Fusarium* spp. **1** was fungicidal against all three species of *Fusarium* tested. The analogues were consistently fungicidal against *Fusarium solani* but varied in effect against *Fusarium oxysporum* and *Fusarium proliferatum*. **16**, prepared through amidation of KF with coumarin, showed no activity against *Fusarium* spp. Results of this evaluation revealed that semisynthetic modification of KF alters the activity against *Fusarium* spp. In particular, amidation with coumarin results in elimination of activity.

Table 5. Fungistatic (FS) and Fungicidal (FC) Activity of **1** and Its Analogues at 30 μM against *Fusarium* spp.

Compound	Antifungal activity at 30 μM		
	Fusarium oxysporum	Fusarium proliferatum	Fusarium solani
1	FC	FC	FC
3	FS	FC	FC
4	FC	FS	FC
5	FS	FS	FC
7	FS	FC	FC
8	FC	FS	FC
10	FS	FS	FC
16	NA	NA	NA
azoxystrobin	FS	FS	FS
captan	FS	FS	FS
amphotericin B	FC	FC	FC

2.E. Cytotoxicity. Analogues **5**, **7**, **8**, and **16** were evaluated for *in vitro* anticancer drug screening in 60 human cancer cell lines. The cell lines tested included lung, leukemia, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate, and breast. The concentration needed to reduce the growth of treated cells to half that of untreated cells (GI_{50} – 50% inhibition of cell growth) and the concentration required to completely halt the growth of treated cells (TGI – total growth inhibition) was determined using each analogue with each cell line. These values were compared with the values of the current chemotherapy drug, Paclitaxel. The TGI of the analogues exhibited higher cytotoxic potency than Paclitaxel against most of the cell lines. The analogues exhibited significant activity against non-small-cell lung (NSCL), colon, ovarian, breast, and prostate cancer (Table 6). **5** showed better activity than **1** against the cell lines RPMI-8226 (leukemia), HCC-2998 (colon), HT29 (colon), SK-OV-3 (ovarian), ACHN (renal), and PC-3 (prostate). **7**, **8**, and **16** showed better activity that **1** against NCI-H322M (NSCL). **7** and **8** showed better activity than **1** against HCC-2998 (colon). **5** showed increased activity against most of the tested cell lines, while **16** showed loss of activity in most cases. Results of this evaluation revealed that improvements against selected cancer cell lines are possible from semisynthetic modification of **1**.

Table. 6. In Vitro Activity Data of Compounds **5**, **7**, **8**, and **16** (μM) against Various Cell Lines[a]

cancer	cell line	Paclitaxel[b]		**1**[b]		**5**		**7**		**8**		**16**	
		GI50	TGI	GI50	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
NSCL	A549/ATCC	0.004	25.11	0.135	0.302	0.161	0.340	0.189	0.425	0.166	–	13.70	>100
	NCI-H322M	0.013	6.310	0.191	0.372	0.203	0.429	0.131	0.238	0.167	0.304	0.150	0.490
leukemia	RPMI-8226	0.002	5.012	1.738	6.918	0.475	1.780	0.638	1.890	1.270	2.760	40.10	>100
colon	COLO 205	0.003	0.316	–	–	0.156	0.315	0.618	–	0.243	–	4.520	>100
	HCC-2998	0.003	0.126	0.288	0.616	0.201	0.517	0.236	0.732	0.255	0.971	2.270	11.40
	HCT-15	0.158	15.85	0.269	0.741	0.151	0.289	0.492	1.560	0.416	1.360	0.964	17.70
	HT29	0.002	0.251	0.162	0.316	0.151	0.288	0.279	–	0.187	–	2.390	>100
	KM12	0.004	15.85	0.182	0.363	0.179	0.379	0.152	–	0.226	0.503	0.413	3.070
CNS	SNB-75	0.004	0.126	0.224	1.905	0.265	0.655	1.290	2.610	1.010	2.190	2.110	6.870
melanoma	UACC-257	0.040	25.12	1.023	2.818	1.250	0.249	1.310	2.430	1.440	2.820	>100	>100
ovarian	SK–OV-3	0.008	15.85	0.191	0.355	0.183	0.387	0.374	1.360	0.223	0.485	2.550	20.20
renal	ACHN	0.398	12.59	1.659	3.236	1.080	2.06	1.280	2.320	1.320	2.370	26.40	69.80
prostate	PC-3	0.004	10.00	0.170	0.324	0.142	0.287	0.267	0.973	0.228	0.665	3.700	21.80
	DU-145	0.005	0.794	–	–	0.112	0.230	0.124	0.266	0.144	0.292	0.330	1.830
breast	T-47D	–	–	–	–	0.161	0.333	–	–	0.191	0.394	0.518	2.830
	HS 578T	0.003	0.100	0.162	0.479	0.212	0.695	0.162	0.444	0.427	2.130	3.030	>100

[a] Dash (–) = no data. GI_{50} (50% inhibition of cell growth): the concentration needed to reduce the growth of treated cells to half that of untreated (i.e., control) cells. TGI (100% (total) growth inhibition): the concentration required to completely halt the growth of treated cells. [b] Developmental Thrapeutics Program NCI/NIH (http://dtp.nci.nih.gov)

IV. CONCLUSION

It is quite evident that the diverse array of life housed within our world's ecosystems has proved itself in providing key elements in the treatment of various forms of cancer and other diseases. And yet, after years of study, the NCI feels that we have only scratched the surface on what is really out there. However, in an ever changing world, research in the area of natural products for drug development "becomes a race against time". As species housed within our ecosystems continue to disappear and as pathogens evolve and become resistant to current drug therapies, drug discovery is critical.[1,2] For these reasons, continued research with the kahalalide family of compounds is crucial. As previously stated, evaluations of KF have shown significant activity against various forms of cancer, including colon, breast, non-small-cell lung, and prostate. KF has been investigated in phase I and phase II clinical trials for androgen-refractory prostate cancer[10] and for liver cancer, NSCLC, and melanoma, respectively.[11] Patients with advanced NSCLC, HC, and AMM have also responded positively to treatment with KF.[12] This study has confirmed that semisynthetic modifications of KF can positively affect antifungal activity, as well as the activity against a slate of human cancer cell lines.

As previously discussed, the significant activity of KF against serious forms of cancer, as well as fungi, has prompted research to investigate the mechanism of action of the compound. It is thought that human ribosomal protein S25 (RPS25) is a binding partner for KF. Research shows that this may only be one step in a series of events leading to disruption of lysosomes and eventual cell death by oncosis. Further exploration into the interaction between KF and RPS25 could lead to a better

understanding of the signaling pathway and mechanism of action of the parent compound.[16] Research has also shown that inhibition of the receptor tyrosine kinase ErbB3 (HER3) has an affect on the mechanism of action. Because there are currently no ErbB3 (HER3) inhibiting drugs, KF would serve as an excellent candidate for inhibition of these receptors in tumor cells.[16,23] Further semisynthetic modifications and lead-exploration studies could reveal other uses for the compound and continue to enhance its therapeutic possibilities.

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