CHEMICAL REVIEWS

REVIEW

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Chemistry and Biology of Kahalalides

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CONTENTS

1. INTRODUCTION

With drug discovery from marine natural products hailing a renaissance over the past 5 years, the use of marine extracts in the search for biologically active natural products continues to be a powerful approach for the identification of lead compounds for chemistry programs involved in drug discovery.[1] Natural products continue to serve as valuable starting points in developing druglike candidates, and the first step in the development of therapeutic agents is the identification of lead compounds that bind to a specific target or receptor of interest.[2] Structure−activity relationships (SARs) of lead compounds are then studied by synthesis or semisynthesis of a number of analogues to define the key recognition elements for maximal activity.[3]

However, the role of natural products in drug discovery became a lower priority in terms of participation by the major pharmaceutical companies by the mid-1990s.[4] Reasons for this decline include the perceived disadvantages of natural products, including difficulties in access and supply, structure elucidation and synthesis because of the complexity of natural products, and concerns about intellectual property rights associated with published structures and international collections. In addition, the availability of large collections of compounds prepared by combinatorial chemistry methods provides inexpensive access to large numbers of molecules for random screening and starting materials for rational design.[5,6]

Nevertheless, the natural products chemistry field has welcomed a renaissance over the past 5 years because of new developments in analytical chemistry, spectroscopy high-throughput screening, and a disappointing number of leads generated through combinatorial chemistry.[1,7−9] Currently, basic scientific research in chemistry and biology of marine natural products that started in the 1970s has finally borne fruit for marine-derived drug discovery. Ziconotide (Prialt; Elan Pharmaceuticals), a peptide originally discovered from a tropical cone snail, was the first marine-derived compound approved in the United States in December 2004 for the treatment of pain. Then, in October 2007, trabectedin (Yondelis; PharmaMar) was approved and became the first marine anticancer drug in the European Union. Collaborations between industrial and academic scientists continue to meet the challenges involved in discovering, understanding, and developing new anticancer drugs.[10] Several other candidate compounds from marine origins are in the pipeline and are being evaluated in phase I−III clinical trials for the treatment of various cancers in the United States and in Europe.[11−13] Here, we review the kahalalides, a family of structurally unrelated depsipeptides isolated from the herbivorous marine mollusk *Elysia rufescens*, *Elysia ornata*, or *Elysia grandifolia* and their algal diet of *Bryopsis pennata*.[14−27] Two of the most active compounds of this family, kahalalide F (KF) (**6**) and isoKF (**22**), have been evaluated in phase II clinical trials in hepatocellular carcinoma, non-small-cell lung cancer (NSCLC), and melanoma. Moreover, KF (**6**) is being evaluated in phase II clinical trials in patients with severe psoriasis.[83−93] Of greatest significance is the fact that KF (**6**) can effectively inhibit receptor

Received: June 16, 2010
Published: April 11, 2011

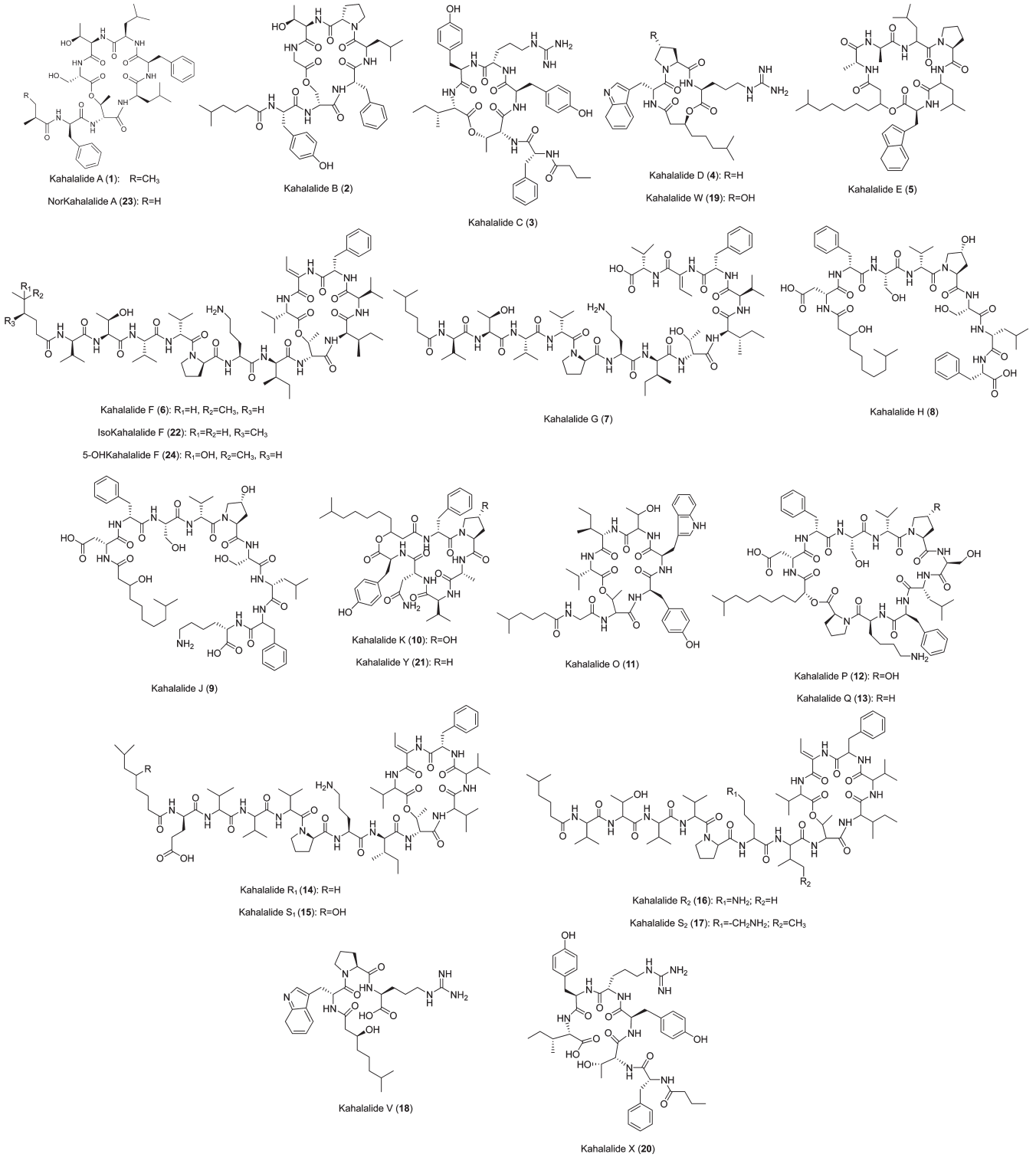


Figure 1. Structures of kahalalide peptides.

tyrosine kinase ErbB3 (HER3) and the phosphatidylinositol 3-kinase-Akt signaling pathways in sensitive cell lines, which suggests that KF (**6**) and isoKF (**22**) are involved in an unknown oncosis signaling pathway, though the mechanism of action has not yet been completely characterized.[103−107] The kahalalides would represent the first anticancer drugs that can inhibit HER3 receptors.

2. BACKGROUND

2.1. Discovery and Isolation of Naturally Occurring Kahalalides

The kahalalides (Figure 1) consist of a series of depsispeptides that were first identified from the herbivorous marine mollusk *E. rufescens*, *E. ornata*, or *E. grandifolia* and later from their algal diet

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Figure 2. Photograph of the sacoglossan mollusk *E. rufescens* and the green alga *B. pennata* collected from Hawaii (photo by M. Huggett).

of *B. pennata* or *Bryopsis plumosa* (Figure 2).[16] The size and composition of this series of peptides are highly variable, ranging from a C31 tripeptide to a C77 tridecapeptide, and each peptide contains a different relatively obscure fatty acid (Table 1). Many review articles and patents on the kahalalides have appeared in the literature.[28−60] The initial discovery of KF (**6**) and its closely related isomer, isoKF (**22**), was first reported by Hamann and Scheuer in 1993.[14,15] In the course of the investigation of natural products from the mollusk *E. rufescens* and the alga *B. pennata*, the constituents were shown to be dominated by amino and fatty acid-derived depsipeptides. Isolation of seven compounds, kahalalides A−E, F/isoKF, and G, was accomplished using silica gel and preparative HPLC. The largest and most active peptide, KF (**6**), and its closely related isomer, isoKF (**22**), exhibited significant activity in vitro against various solid tumor cell lines.

The sea slug *E. rufescens* and the alga *B. pennata* were extracted with ethanol and subjected to silica gel flash chromatography with a stepwise gradient: *n*-hexane, *n*-hexane/EtOAc (1:1), EtOAc, EtOAc/MeOH (1:1), MeOH, and MeOH/H_2O (50:50). The EtOAc/MeOH (1:1) fraction was found to contain the depsipeptides. HPLC using an RP C18 column and a gradient from a H_2O/MeCN/TFA mixture (70:30:0.1) to a H_2O/MeCN/TFA mixture (45:55:0.1) yielded the kahalalides, and final purification was completed using isocratic conditions.[14,15,30−32]

IsoKF (**22**) is the (4*S*)-methylhexanoic isomer of KF and remains inseparable from KF (**6**) under preparative scale conditions but can be resolved using LC-TOF-MS (**6**).[14] This compound is currently obtained via a synthetic process by PharmaMar.[35] The activity of isoKF (**22**) was similar to that of KF (**6**) and showed enhanced efficacy against breast and prostate tumor cell lines. Currently, the compound has entered phase I clinical trials in advanced pretreated solid tumors.

Because KF (**6**) and isoKF (**22**) exhibit significant bioactivity against various solid tumor cell lines, isolation of new kahalalide peptides became of interest for natural products chemists. Since 1997, the structures of 16 new kahalalide derivatives have been elucidated and 14 of them isolated.[16−23] In 1997, Scheuer et al. reported two acyclic kahalalide peptides, kahalalides H (**8**) and J (**9**) from the mollusk *E. rufescens*.[16] Kahalalides H (**8**) and J (**9**) share only four amino acids (leucine, phenylalanine, serine, and valine). They have in common a 3-hydroxy-9-methyldecanoic acid, previously encountered in kahalalide E (**5**). In common with the acyclic constituent of the alga-derived kahalalide G (**7**), kahalalides H (**8**) and J (**9**) exhibited no significant activity. In 1999, a new peptide kahalalide K (**10**) was isolated from the alga *B. pennata* and kahalalide K (**10**) was determined to possess a new array of L-amino acids (Val, Tyr, and Hyp), D-amino acids (Asn, Phe, and Ala), and a 3-hydroxy-9-methyldecanoic acid previously reported in kahalalides E (**5**), H (**8**), and J (**9**).[17] In 2000, one new cyclic peptide, kahalalide O (**11**), was identified from the sacoglossan *E. ornata* and the alga *B. pennata* followed by the isolation of kahalalides P (**12**) and Q (**13**), which also possessed (3*R*)-hydroxy-9-methyldecanoic acid determined by Mosher's ester procedure.[18,19] In 2006, new KF analogues, kahalalides R_1 (**14**) and S_1 (**15**), were isolated from *E. grandifolia*, and kahalalide R_1 (**14**) was found to exert comparable or even higher cytotoxicity than KF (**6**) toward the MCF7 human mammary carcinoma cell line.[20] Kahalalides R_1 (**14**) and S_1 (**15**) differed in only the fatty acid. The fatty acid residues in kahalalides R_1 (**14**) and S_1 (**15**) were 7-methyloctanoic acid (7-Me-Oct) and 5-hydroxy-7-methyloctanoic acid (7-Me-5-Octol), respectively. Kahalalides R_1 (**14**) and S_1 (**15**) were shown to contain six units of valines and one unit each of Phe, Ile, Thr, Orn, Pro, Glu, and dehydroaminobutyric acid. In kahalalides R_1 (**14**) and S_1 (**15**), Val and Glu units replaced Thr and Ile units previously found in KF (**6**). The absolute configuration of amino acids was determined by Marfey's analysis,[61] which identified one unit of D-Glu, D-Pro, L-Orn, D-*allo*-Ile, D-*allo*-Thr, and L-Pro. Absolute configuration of the individual Val units could not be unambiguously determined because Marfey's analysis suggested the presence of D- and L-isomers in these two compounds, and there are three units of Val in both the cyclic and linear fragments. In 2007, two new KF analogues, kahalalides R_2 (**16**) and S_2 (**17**) (their names are identical to the two compounds isolated by Ashour et al. in 2006), were characterized by using tandem mass spectrometry.[21] The amino acid sequences of kahalalides R_2 (**16**) and S_2 (**17**) were proposed by collision-induced dissociation (CID) experiments with singly and doubly charged molecular ions and by comparison with the amino acid sequences of KF (**6**). The absolute configuration of kahalalides R_2 (**16**) and S_2 (**17**) could not be assessed by chemical methods because no pure kahalalide R_2 (**16**) or S_2 (**17**) was isolated from the mollusk *E. grandifolia*. In 2008, four new kahalalide peptides from the mollusk *E. rufescens* were reported.[22] Kahalalide V (**18**) was identified as an acyclic derivative of kahalalide D (**4**), while kahalalide W (**19**) was determined to have a 4-hydroxy-L-Pro residue instead of the proline in kahalalide D (**4**). Kahalalide X (**20**) was an acyclic derivative of kahalalide C (**3**), and kahalalide Y (**21**) was found to have an L-proline residue instead of the hydroxyproline in kahalalide K (**10**). In 2009, our group isolated two new kahalalide peptides, NorKA (**23**) and 5-OHKF (**24**). NorKA (**23**) and KA (**1**) differ in only a methylene group.[23] The former contains an isobutyric acid as the fatty acid moiety and the latter a 2-methylbutyric acid. 5-OHKF (**24**) and KF (**6**) are different in one hydroxyl group. The fatty acid residue of **24** is 5-hydroxy-5-methylhexanoic acid.

2.2. Structure Elucidation of Kahalalides

The gross structure proposed for the kahalalide peptides was deduced from NMR methods and mass spectrometry.[14−23]

Table 1. Comparative Composition of Kahalalides

				amino aicds																			
	kahalalide	mol formula	mol wt	Ala	Arg	Asp	Asn	Dhb	Glu	Gly	Ile	Leu	Lys	Orn	Phe	Pro	Hyp	Ser	Thr	Trp	Tyr	Val	fatty acid	refs
1	A	$C_{46}H_{67}N_7O_{11}$	894.5									D(2)			D(2)			L(1)	L(2)				2-Me-Bu	15
2	B	$C_{45}H_{63}N_7O_{11}$	878.5							1		D(1)			L(1)	L(1)		L(1)	L(1)		L(1)		5-Me-Hex	15
3	C	$C_{47}H_{63}N_9O_{10}$	914.5		L(1)						L(1)				D(1)				L(1)		D(2)		Bu	15
4	D	$C_{31}H_{45}N_7O_5$	595.4		L(1)											L(1)				D(1)			7-Me-3-Octol	15
5	E	$C_{45}H_{69}N_7O_8$	836.5	D(2)								2				L(1)				L(1)			9-Me-3-Decol	15
6	F	$C_{75}H_{124}N_{14}O_{16}$	1477.9					Z(1)			Da(2)[a]			L(1)	L(1)	D(1)			L(1)			D(3)	5-Me-Hex	14, 24−27
																			Da(1)			L(2)		
7	G	$C_{75}H_{126}N_{14}O_{17}$	1495.9					Z(1)			Da(2)				L(1)	D(1)			L(1)			D(3)	5-Me-Hex	15
																			Da(1)			L(2)		
8	H	$C_{55}H_{82}N_8O_{16}$	1110.5			D(1)						D(1)			D(1)		L(1)	L(2)				D(1)	9-Me-3-Decol	16
															L(1)									
9	J	$C_{61}H_{94}N_{10}O_{17}$	1239.7			D(1)						D(1)	L(1)		2		L(1)	L(2)				D(1)	9-Me-3-Decol	16
10	K	$C_{46}H_{66}N_7O_{11}$	892.5	D(1)			D(1)								D(1)		L(1)				L(1)	L(1)	9-Me-3-Decol	17
11	O	$C_{48}H_{68}N_8O_{11}$	933.5							1	L(1)								2	D(1)	D(1)	L(1)	5-Me-Hex	18
12	P	$C_{66}H_{99}N_{11}O_{17}$	1318.7									D(1)	L(1)		D(1)	L(1)	L(1)					D(1)	9-Me-3-Decol	19
															L(1)									
13	Q	$C_{66}H_{100}N_{11}O_{16}$	1302.7			D(1)						D(1)	L(1)		D(1)	L(2)						D(1)	9-Me-3-Decol	19
															L(1)									
14	R_1	$C_{77}H_{127}N_{14}O_{17}$	1519.9					Z(1)	D(1)		Da(1)			L(1)	L(1)	D(1)			Da(1)			6	7-Me-Oct	20
15	S_1	$C_{77}H_{127}N_{14}O_{18}$	1535.9					Z(1)	D(1)		Da(1)			L(1)	L(1)	D(1)			Da(1)			6	7-Me-5-Octol	20
16	R_2	$C_{74}H_{121}N_{14}O_{16}$	1464.0					(1)			(1)			(1)	(1)	(1)			(2)			6	5-Me-Hex	21
17	S_2	$C_{76}H_{126}N_{14}O_{16}$	1492.0					(1)			(1)		1	(1)	(1)	(1)			(2)			5	5-Me-Hex	21
18	V	$C_{31}H_{47}N_7O_6$	614.3		L(1)											L(1)				D(1)			7-Me-3-Octol	22
19	W	$C_{31}H_{45}N_7O_6$	612.3		L(1)												L(1)			D(1)			7-Me-3-Octol	22
20	X	$C_{47}H_{66}N_9O_{11}$	932.5		L(1)						L(1)				D(1)				L(1)		D(2)		Bu	22
21	Y	$C_{46}H_{66}N_7O_{10}$	876.5	D(1)			D(1)								D(1)	L(1)					L(1)	L(1)	9-Me-3-Decol	22
22	isoKF	$C_{75}H_{124}N_{14}O_{16}$	1477.9					Z(1)			Da(2)			L(1)		D(1)			L(1)			D(3)	4(*S*)-Me-Hex	14, 35
																			Da(1)			L(2)		
23	NorKA	$C_{45}H_{65}N_7O_{11}$	880.5									D(2)			D(2)			L(1)	L(2)				IsoBu	23
24	5-OHKF	$C_{75}H_{124}N_{14}O_{17}$	1493.9					Z(1)			Da(2)[a]			L(1)	L(1)	D(1)			L(1)			D(3)	5-OH-5-MeHex	23
																			Da(1)			L(2)		

[a] D-*allo* configuration of amino acids.

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Table 2. Biological Activity Profile of Kahalalides

kahalalide	biological activity	refs
A (**1**)	in vitro activity against *Mycobacterium tuberculosis*: 83% inhibition at 12.5 g/mL	15
E (**5**)	selective activity against herpes simplex II virus (HSV II)	15
F (**6**)	selectivity against solid tumor cell lines: IC_{50} values of 2.5, 0.25, and <1.0 μg/mL against A-549, HT-29, and LOVO, respectively; IC_{50} values of 10 and >10 μg/mL against P-388 and KB, respectively; KF is active against CV-1 cells with an IC_{50} of 0.25 μg/mL	14, 15, 68−70
	antiviral activity: 0.5 μg/mL (95% reduction) with HSV II using mink lung cells	
	antifungal activity: IC_{50} values of 3.02 μM against *Candida albicans*, 1.53 μM against *Candida neoformans*, and 3.21 μM against *Aspergillus fumigatus*	
	immunosuppressive activity: IC_{50} of 3 μg/mL in a mixed lymphocyte reaction assay, IC_{50} of 23 μg/mL with lymphocyte viability (LcV)	
	antileishmanial activity: LC_{50} values of 6.13 μM against *Leishmania donovani* (promastigote), 8.31 μM against *Leishmania pifanoi* (promastigote), 29.53 μM against *L. pifanoi* (amastigotes); in vitro antitumor activity similar to that of KF (**6**)	
IsoKF (**22**)	IsoKF (**22**) has enhanced efficacy against breast and prostate xenografts	35, 70
R_1 (**14**)	IC_{50} of 0.14 mmol/L against the human breast adenocarcinoma MCF-7 cell line	
	IC_{50} of 4.28 mmol/L against the mouse lymphoma L1578Y cell line	20
NorKA (**23**)	100 μM norKA (**2**) inhibited 82% of the specific binding of [^{3}H]NPY to the Y_1 receptor, and it showed no inhibitory activity (only 4%) for [^{3}H]BQ-123 binding to the ET_A receptor	23
5-OHKF (**24**)	in vitro antimalarial activity against D6 and W2 clones of *Plasmodium falciparum* with IC_{50} values of 1.5 and 1.2 μg/mL, respectively	23

Tandem mass spectrometry is also a useful tool that can provide detailed information for characterizing the kahalalide peptides.[21] In most cases, the absolute configuration of each amino acid in the peptide can be determined by Marfey's methods after hydrolysis.[54] L-FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide), Marfey's reagent, reacts by nucleophilic substitution of the aromatic fluorine with the free amino group on an amino acid. When a racemic mixture of amino acids is treated with this reagent, the production of analogous diastereomers occurs. These diastereomers can be separated using reverse phase HPLC. However, it is not possible to determine the sequential position of the DL-antipodal amino acids by Marfey's method.[14,16,18,20,23]

The structure of KF (**6**) and isoKF (**22**) was initially reported to contain a cyclized macrolide region consisting of two Val residues, one D-*allo*-Ile, one Thr, one dehydroaminobutyric acid, and one L-Phe, a linear region including three Val residues, one D-*allo*-Ile, one L-Orn, one Thr, one D-Pro, and a methylhexanoic acid, conjugated with the N-terminus.[14] In the ring, the carboxyl group of the Val was linked to Thr through the hydroxyl group. The proposed structure of KF (**6**) was based upon NMR methods, degradation by acid hydrolysis, mass spectrometry, and GC analysis of the individual amino acid components using Marfey's reagent. Only the Ile, Orn, Pro, and Phe amino acids have one stereoisomer. However, further work was needed to investigate the absolute configuration of the conundrum posed by multiple possible stereoisomers in the molecule including three D-Val and two L-Val isomers together with one D-*allo*-Thr isomer and one L-Thr isomer. The remaining absolute configuration was reported by Goetz and Scheuer,[24,25] and Val-3 and Val-4 were assigned the L- and D-configurations, respectively. The group of Albericio and Giralt at the University of Barcelona[27] synthesized the originally proposed structure and showed the differences in chromatographic and spectroscopic behavior between the synthesized peptide and the natural peptide. Later, Rinehart et al. at the University of Illinois elected to reinvestigate the absolute configuration of KF (**6**) and finally suggested that the absolute configuration of Val-3 and Val-4 should be reversed and played an important role in the activity of KF (**6**) and isoKF (**22**) because the depsipeptide with L-Val-3 and D-Val-4 in its structure was not active, while the molecule with D-Val-3 and L-Val-4 was active.[26] In 2006, the group of Dmitrenok and Nagai used a carboxypeptidase hydrolysis reaction to determine the sequential positions of the DL-Phe in kahalalides P (**12**) and Q (**13**).[19] The absolute configuration of amino acids in 5-OHKF (**24**) was achieved by the combination of chemical hydrolysis and Marfey's method.[23] Amino acid analysis by Marfey's method revealed 12 amino acids: L-Orn, D-*allo*-Ile (two), D-Pro, L-Thr, D-*allo*-Thr, D-Val (three), L-Val (two), and L-Phe. A single Marfey's analysis was not enough because there is more than one valine or threonine that has a different absolute configuration. We hydrolyzed 5-OHKF (**24**) and KF (**6**) partially into smaller units without the fatty acid residues and then assessed their chromatographic properties. So far, the sequential positions of DL-antipodal Leu in kahalalide E (**5**), DL-antipodal Phe in kahalalide J (**9**), L-Thr and D-*allo*-Thr in kahalalide O (**11**), and DL-antipodal valine in kahalalides R_1 (**14**) and S_1 (**15**) remain unassigned.[16,18,20]

2.3. Biological Activity Profiles

2.3.1. Cytotoxicity and Antitumor Activity. Among the kahalalides, only kahalalides A (**1**), E (**5**), F (**6**)/isoKF (**22**), R_1 (**14**), isoKA (**23**), and 5-OHKF (**24**) exhibited significant biological activity (Table 2). In 2001, Becerro et al. studied the ecological role of KF (**6**).[62] The results showed that KF (**6**) protected both *B. pennata* and *E. rufescens* from fish predation. *E. rufescens* is a chemically defended species. In fact, it is possible that *E. rufescens* has evolved defensive mechanisms to reduce its chances of predation. *B. pennata* is a chemically defended alga that may provide the sacoglossan an associational refuge. By feeding on *B. pennata*, *E. refescens* sequesters algal chloroplasts

and makes itself highly cryptic.[63−67] However, the risk of predation may still be high for cryptic organisms, so the acquisition of other defensive strategies may expand the benefits of crypsis. *E. rufescens* sequesters the antipredatory compound KF (**6**) from *B. pennata*, accumulating it a concentration several times the concentration in the alga, and uses KF (**6**) to chemically defend itself. Moreover, *E. rufescens* generates KF (**6**) the mucus. Therefore, KF (**6**) is responsible for the deterrent properties of the mollusk. It is hypothesized that the KF-producing *Vibro* sp. from the surface of *B. pennata* are acquired by *E. rufescens*, which maintains the bacteria as symbionts.[39,43]

Early preclinical data showed that KF (**6**) exhibited a potent new chemical entity with significant cytotoxicity against solid tumor cell lines.[110] Preliminary in vitro screening studies indicated micromolar activity of KF (**6**) against selected cell lines, in particular NSCL, colon, ovarian, and breast cancers and especially prostate cancer (Table 2). In vitro cell culture studies indicated that 10 μM KF (**6**) could produce cytotoxicity to central nervous system neurons but not astrocytesa or sensory and motor neurons.[80]

A human tumor colony-forming unit (TCFU) assay from surgically derived tumors showed that KF (**6**) completely inhibits breast, colon, kidney, NSCLC, ovary, prostate, stomach, and uterine tumor specimens. An IC_{50} of <10 nM in a limited number of specimens has been identified, and prostate and stomach tumor specimens are the most sensitive.[71]

In vitro antitumor activity of isoKF (**22**) is similar to that of KF (**6**). However, isoKF (**22**) has enhanced efficacy against breast and prostate xenografts.[35,70]

Kahalalides R_1 (**14**) and KF (**6**) were tested and found to be comparably cytotoxic toward MCF-7 cell lines with IC_{50} values of 0.14 and 0.22 μM, respectively. Furthermore, kahalalide R_1 was cytotoxic toward the mouse lymphoma L1578 Y cell line with an IC_{50} of 4.26 nM, almost identical to that of KF (**6**).[20]

2.3.2. Antimicrobial Activity. In 1996, Hamann et al. reported that KA (**1**) was shown to inhibit 83% of the growth of *M. tuberculosis* at 12.5 μg/mL. KF (**6**) exhibited antifungal activity with IC_{50} values of 3.02 μM against *C. albicans*, 1.53 μM against *C. neoformans*, and 3.21 μM against *A. fumigates*.[69] In an agar diffusion assay, KF (**6**) exhibited strong antifungal activity at a level of 5 μg/disk against the plant pathogens *Cladosporium herbarum* and *Cladosporium cucumerinum* with inhibition zones of 17 and 24 mm, respectively.[20] Kahalalide R_1 (**14**) also exhibited significant antifungal activity against the two species with inhibition zones of 16 and 24 mm, respectively.[20]

KF (**6**) exhibited antiviral activity at 0.5 μg/mL (95% reduction) with herpes simplex II virus (HSV II) using mink lung cells. Furthermore, KF (**6**) exhibited selective activity against some of the AIDS opportunistic infections.[14,15]

KE (**5**) also exhibited selective activity against herpes simplex II virus (HSV II).[15]

2.3.3. Antileishmanial Activity. KF (**6**) was tested for its activity against promastigote and amastigote stages of *Leishmania*. Their respective LC_{50} (concentration at which the proliferation of the parasites was inhibited by 50%) values are listed in Table 2.

2.3.4. Immunosuppressive Activity. KF (**6**) exhibited slight immunosuppressive activity in a mixed lymphocyte reaction assay (MLR) with an IC_{50} of 3 μg/mL, and with a lymphocyte viability (LcV) IC_{50} of 23 μg/mL.[14]

3. METABOLISM AND PHARMACOKINETICS OF KF

3.1. Metabolism of KF

An analytical method using HPLC with positive ion turbo-ion spray tandem MS has been applied to the study of KF (**6**) in human plasma. Ammonium acetate was chosen to replace trifluoroacetic acid to enhance sensitivity in the positive ion mode.[72−74] A lower-limit quantitation of 1 ng/mL using a 500 μL sample volume and a linear dynamic range extending to 1000 ng/mL were obtained, and KF (**6**) was stable in the biomatrix for a period of 9 months at −20 °C and 24 h at room temperature. The interassay accuracy was −15.1% at the lower limit of quantitation and between −2.68 and −9.05% for quality control solutions ranging in concentration from 2.24 to 715 ng/mL.[72] The analyte was stable in plasma for 16 h after reconstitution of plasma extracts for liquid chromatography analysis at room temperature.

High-performance liquid chromatography with ultraviolet detection was used to study the chemical degradation of KF (**6**) under acid, neutral, and alkaline conditions, and the results showed that the half-lives ($t_{1/2}$) of KF (**6**) at 80 °C were 1.1, 20, and 8.6 h at pH 0, 1, and 7, respectively.[75] The half-life of KF (**6**) at 26 °C and pH 11 was 1.65 h. Kahalalide G (**7**), the only product of KF (**6**), was produced at pH 7 and 11. In addition, metabolic conversion of KF (**6**) was conducted using three different enzyme systems, including pooled human microsomes, pooled human plasma, and uridine 5′-diphosphoglucuronyl transferase. Biotransformation was not observed during these in vitro studies, so KF (**6**) was metabolically stable.

Furthermore, infrared (IR) spectroscopy and differential scanning calorimetry (DSC) were applied to investigate stabilities of **6**-lyophilized products containing crystalline (mannitol) or amorphous (sucrose) bulking agents at 5 and 30 °C with or without 60% relative humidity (RH) in the dark. A stable lyophilized formulation was created, and it contained 100 μg of KF (**6**), 100 mg of sucrose, 2 mg of polysorbate 80, and 2.1 mg of citric acid monohydrate to be reconstituted with a vehicle composed of 5%/5%/90% (v/v/v) CEW and to be diluted further using normal saline.[76] Lyophilized products became less stable when polysorbate 80 and citric acid monohydrate concentrations were increased. Sorption to contact surfaces with an infusion container composed of low-density polyethylene could lead to loss of KF (**6**).[77,78] Therefore, KF (**6**) must be administered in a 3 h infusion at concentrations of 0.5−14.7 μg/mL, and an administration set consisting of a glass container and a low-extrables, DEHP-free extension set must be used. An in vitro biocompatibility study was performed, and the results showed that no significant hemolysis due to the KF (**6**) formulation as well as the CE vehicle was found using a static or dynamic test model.[79]

IsoKF (**22**) was very stable in dog plasma, and no significant changes in concentration were observed after incubation for up to 4 h in a water bath at 37 °C.[94]

3.2. Pharmacokinetics of KF

An HPLC−MS assay method was utilized to determine the pharmacokinetics of KF (**6**) (Table 3).

4. CLINICAL STATUS

Relevant preclinical experiments have shown that fractionation of a lethal or MTD dose of KF (**6**) by daily administration for 5 days reduces drug-induced toxicity and appears to be a viable

Table 3. Pharmacokinetic Profile of KF

model used	results	refs
KF studied first in female mice for both intravenous and intraperitoneal administration of the drug (280 g/kg)	Upon intravenous administration to mice, there was no accumulation of the drug after repeated intravenous administration at 24 h intervals and plasma levels declined from a peak concentration of 1.0 μM with a $t_{1/2}$ of 35 min When the drug was administered intraperitoneally at the same dose, the peak concentration was 0.3 μM approximately 1 h after dosing.	81, 82
phase I study in patients with androgen-refractory prostate cancer in which KF (20−930 mg/m^2) was administered as a daily 1 h intravenous infusion for 5 days every 3 weeks	A linear relationship between dose and AUC over a dose range of 20−560 g/m^2/day. At doses of >560 g/m^2/day, the AUC increased in a non-dose-proportional manner. On day 1, the total plasma clearance was 11.02 ± 4.54 L/h and the terminal $t_{1/2}$ value of intravenous KF in these patients was 0.54 ± 0.14 h.	83, 84, 111
phase I study in patients with various solid tumors in which KF was administered as a continuous weekly 1 h intravenous infusion at doses ranging from 266 to 1200 g/m^2	A linear relationship between dose and AUC over a dose range of 266−800 mg/m^2/week.	85
phase I study in patients with advanced solid tumors in which KF was administered weekly as a 1 h intravenous infusion at a starting dose of 266 g/m^2/day	This schedule was characterized by linear kinetics for C_{max} and AUC values, a short terminal half-time (0.52 h vs 0.47 h), and a narrow volume of distribution (5.5 L at the recommended dose with the once-weekly schedule vs 7.16 L at the recommended dose with the daily schedule). The volume of distribution and clearance increased with body size and were best predicted with body surface area and height, respectively.	86, 109
phase II study in patients with advanced malignant melanoma (AMM) in which KF was administered as a weekly 1 h intravenous infusion with a dose of 650 g/m^2	Means (SD) of half-life, clearance, and volume of distribution at steady state were 0.49 h (0.15), 5.60 L h^{-1} m^{-2} (1.26), and 4.00 L/m^2 (1.00), respectively. The pharmacokinetic profile of KF in phase II studies did not differ significantly from those found in phase I studies.	87, 88

option for the clinical evaluation of KF (**6**) for the treatment of cancer.[113,114] The activity of KF (**6**) has been investigated in phase I clinical trials for androgen-refractory prostate cancer solid tumors and phase II clinical studies with patients having liver, non-small-lung cancer, melanoma, and psoriasis.[83−92] Some results about clinical trials of KF (**6**) are listed in Table 4.

IsoKF (**22**) has been selected for clinical development on the basis of its in vivo activity in xenografted human tumors, as well as an acceptable nonclinical toxicology profile. The compound is in phase I clinical trials in patients with advanced malignant solid tumors.[92−94]

5. TOTAL SYNTHESIS

As a result of the biological activity profile of the kahalalides, they, especially KF (**6**), have become synthetic targets in several laboratories. Some papers about total syntheses of some kahalalides have been published.[27,95−98]

5.1. Total Synthesis of KB

Kahalalide B (**2**) is the first kahalalide peptide that was totally synthesized.[96] It is a cyclic depsipeptide composed of seven different amino acids (Gly, Thr, Pro, Leu, Phe, Ser, and Tyr) and the fatty acid 5-methylhexanoic acid (5-MeHex), which is also present in the structure of other members of the series. Two different strategies (A and B in Scheme 1) have been applied to accomplish the synthesis of kahalalide B (**2**). In strategy A, heptapeptide **175** was synthesized from the H-Gly-O-resin (**174**), which was produced from the commercially available chlorotrityl chloride resin (**166**) first by a sequential attachment of L-Thr, L-Pro, D-Leu, L-Phe, D-Ser, and L-Tyr derivative using Fmoc/t-Bu stragety and DIPCDI/HOBt as the coupling reagent. This was followed by capping with 5-methylhexanoc acid at the N-terminus. Cleavage of the resin from **175** with a TFA/DCM mixture (1:99) afforded the linear peptide **176**, which was subjected to macrocyclization using 1*H*-benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)-DIPEA. After removal of side chain protection with a TFA/H_2O mixture (95:5), kahalalide B (**2**) was produced in 16% yield. In strategy B, there were some different points in the synthesis of kahalalide B (**2**). First, the limited incorporation of the first amino acid of the sequence was performed with Fmoc-Thr(t-Bu)-OH in the presence of DIEA. In contrast, in strategy A, the first amino acid was incorporated by using Fmoc-Gly-OH. Second, in strategy A, cyclization (the step between **176** and **177**) was conducted in solution through the ester bond between the carboxyl group of Gly and the side chain hydroxyl of D-Ser, but in strategy B, the ester bond was formed on the solid phase and the cyclization (the step between **181** and **2**) occurred through an amide bond between the carboxyl group of Thr and the amine group of Gly (see Scheme 1). Finally, the cyclization step was performed at a concentration of 10^{-3} M with PyBOP-DIEA (3.6 equiv) in DMF for 23 h (strategy A) or 1 h (strategy B). The lower nucleophilicity of the hydroxyl compared to that of the amine probably led to the difference in time for the

Table 4. Clinical Trials of KF

effect	model	results	refs
efficacy	phase I study in patients with androgen-refractory prostate cancer in which KF (20−930 mg/m^2) was administered as a daily 1 h intravenous infusion for 5 days every 3 weeks	Thirty-three patients were treated; one patient showed a significant decrease in PSA level (>50%) associated with clinical improvement (pain relief), while three patients exhibited stable disease for 2 (n = 2) or 7 (n = 1) months. The MTD was 560 mg/m^2/day.	84
efficacy	phase I study in patients with various solid tumors in which KF was administered as a continuous weekly 1 h intravenous infusion at doses ranging from 266 to 1200 g/m^2	Twenty-five patients were treated, and three patients achieved a clinical benefit: one hepatocarcinoma patient who received 24 infusions consisting of 400 g/m^2/week, one squamous carcinoma cavum patient who received nine infusions at the same dose, and one NSCLC patient who received 16 infusions at a dose of 530 g/m^2/week. The MTD was 1200 g/m^2/week.	85
safety	two phase I trials in which 60 cancer patients were administered KF as a 1 h intravenous infusion	Grade 4 AI was consistently the DLT and tended to coincide with LDH elevation and an ALT:AP ratio of >5.0, indicating hepatocellular damage; these effects were reversible and dose-dependent.	89
safety and efficacy	phase I study in patients with advanced solid tumors in which KF was administered weekly as a 1 h intravenous infusion at a starting dose of 266 g/m^2/day	Thirty-eight patients were enrolled and received once-weekly KF 1 h infusions at doses between 266 and 1200 g/m^2. Dose-limiting toxicities included transient grade 3/4 increases in transaminase blood levels. The maximal tolerated dose for the KF schedule was 800 g/m^2, and the recommended dose for phase II studies was 650 g/m^2. No accumulated toxicity was found. This schedule provided a favorable safety profile and hints of antitumor activity.	86
efficacy and safety	phase I study in patients with androgen or metastatic-refractory prostate cancer in which KF was administered as a 1 h intravenous infusion for 5 consecutive days every 3 weeks with a starting dose of 20 g/m^2/day	Thirty-two patients were treated at nine dose levels (20−930 g/m^2/day). The maximal tolerated dose on this schedule was 930 g/m^2/day. The recommended dose for phase II studies is 560 g/m^2/day.	83
efficacy and safety	phase II study in patients with advanced malignant melanoma (AMM) in which KF was administered weekly as a 1 h intravenous infusion with a dose of 650 g/m^2	Twenty-four patients were recruited. No objective responses were observed, but the duration of stable disease suggested some degree of antitumor activity.	87
response, safety, and tolerability	phase II study in patients with advanced non-small-cell lung cancer (NSCLC) in which KF was administered weekly as a 1 h intravenous infusion with a dose of 650 g/m^2	Thirty-one patients were enrolled in this phase II trial. The primary results for efficacy showed no complete responses. One patient had a partial response; stability occurred in eight patients and disease progression in 11 patients. Six patients had stable disease lasting for more than 3 months. The duration of stable disease suggested some antitumor activity of KF in this indication, while its toxicity was clinically negligible.	90
efficacy, safety, and tolerability	phase II study in patients with hepatocarcinoma (HC) in which KF was administered as a 1 h intravenous infusion with a dose of 650 g/m^2 over 1 h per week until the disease failed to progress or unacceptable toxicity	Twenty-two patients were recruited. No objective response was observed. Stable disease occurred in nine patients with a median duration of 4.8 months. Median progression free survival was 2.4 months. KF was well tolerated in this patient population, and stable disease was the best response observed in previously untreated patients with hepatocarcinoma (HC).	91

Scheme 1

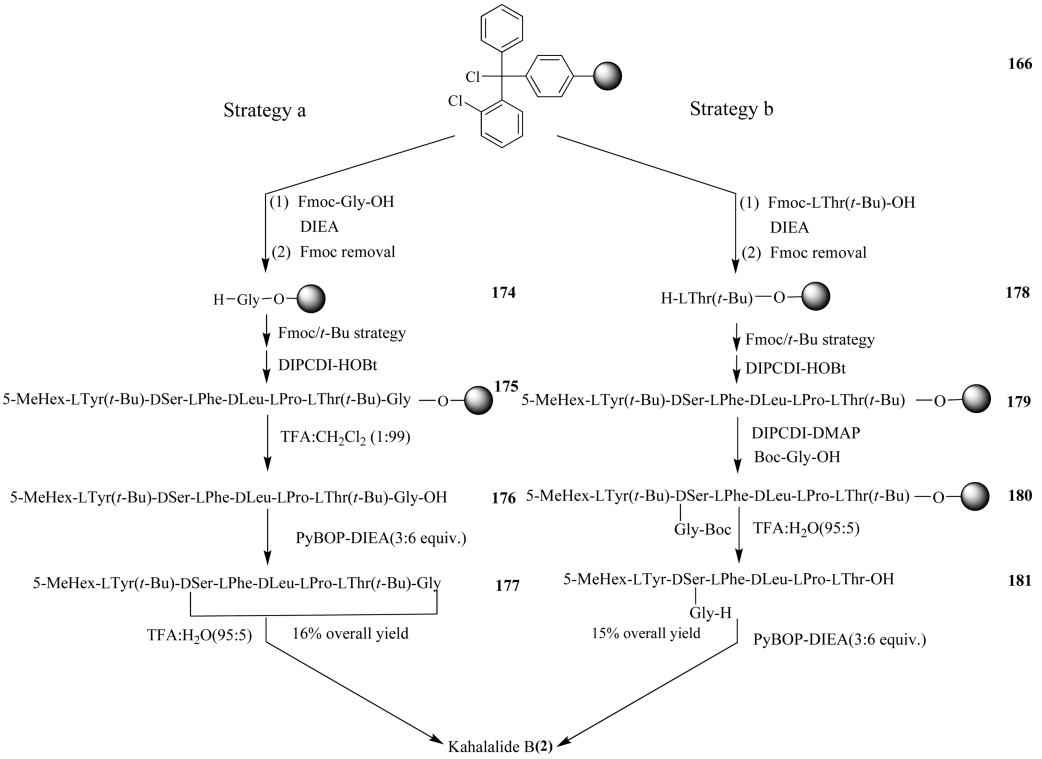


Table 5. Synthesis of KF Analogues following Scheme 2

	analogue	step	residue replaced	residue incorporated
25	[Etg^2]-KF	A5	Fmoc-Thr-OH 2	Fmoc-Etg-OH 2
26	[D-Etg^2]-KF	A5	Fmoc-Thr-OH 2	Fmoc-D-Etg-OH 2
27	[(*Z*)-Dhf^2]-KF	A5	Fmoc-Thr-OH 2	Fmoc-D/L-Phe-OH 2
28	[Dha^2]-KF	A5	Fmoc-Thr-OH 2	Fmoc-Ser-OH
29	[D-Thr^2]-KF	A5	Fmoc-Thr-OH 2	Fmoc-D-Thr-OH 2
30	[D-*allo*-Thr^2]-KF	A5	Fmoc-Thr-OH 2	Fmoc-D-*allo*-Thr-OH 2
31	[Gly^2]-KF	A5	Fmoc-Thr-OH 2	Fmoc-Gly-OH
32	[Aib^2]-KF	A5	Fmoc-Thr-OH 2	Fmoc-Aib-OH
33	[Trp^3]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Trp-OH
34	[hCh^3]-KF	A5	Fmoc-Phe-OH 3	Fmoc-hCh-OH
35	[Phe(3,4-Cl$_2$)^3,4(S)MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Phe(3,4-Cl$_2$)-OH
36	[Phe(F$_5$)^3,4(S)MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Phe(F$_5$)-OH
37	[Phe(4-I)^3,4(S)MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Phe(4-I)-OH
38	[Phe(4-NO$_2$)^3,4(S)MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Phe(4-NO$_2$)-OH
39	[Phe(4-F)^3,4(S)MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Phe(4-F)-OH
40	[Tyr(Me)^3,4(S)MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Tyr(Me)-OH
41	[Thi^3,4(S)-MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Thi-OH
42	[Tic^3,4(S)-MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Tic-OH
43	[Tyr^3,4(S)-MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Tyr(t-Bu)-OH
44	[Oic^3,4(S)-MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Oic-OH
45	[*N*MePhe^3,4(S)MeHex^{14}]-KF	A5	Fmoc-Phe-OH 3	Fmoc-*N*Me-Phe-OH
46	[Phe(2-Cl)^3]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Phe(2-Cl)-OH
47	[Phe(3-Cl)^3]-KF	A5	Fmoc-Phe-OH 3	Fmoc-Phe(3-Cl)-OH
48	[Phe(4-Cl)^3]-KF	A4	4(*S*)-MeHex 14	5-MeHex
		A5	Fmoc-Phe-OH 3	Fmoc-Phe(4-Cl)-OH

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Table 5. Continued

	analogue	step	residue replaced	residue incorporated
49	[Phe(3,4-F_2)^3]-KF	A4	4(*S*)-MeHex 14	5-MeHex
		A5	Fmoc-Phe-OH 3	Fmoc-Phe(3,4-F_2)-OH
50	[Nal^3]-KF	A4	4(*S*)-MeHex 14	5-MeHex
		A5	Fmoc-Phe-OH 3	Fmoc-Nal-OH
51	[Bip^3]-KF	A4	4(*S*)-MeHex 14	5-MeHex
		A5	Fmoc-Phe-OH 3	Fmoc-Bip-OH
52	[Phg^3]-KF	A4	4(*S*)-MeHex 14	5-MeHex
		A5	Fmoc-Phe-OH 3	Fmoc-Phg-OH
53	[Val^4]-KF	B1	Fmoc-DVal-OH 1	Fmoc-Val-OH
54	[D-Dapa^6]-KF	B2	Fmoc-D-*allo*-Thr-OH 6	Fmoc-D-Dapa
55	[D-Thr^6]-KF	B2	Fmoc-D-*allo*-Thr-OH 6	Fmoc-D-Thr-OH
56	[D-Ser^6]-KF	B2	Fmoc-D-*allo*-Thr-OH 6	Fmoc-D-Ser-OH
71	[N$^\varepsilon$(Me)3-Lys^8,4(S)-MeHex^{14}]-KF	B3	Fmoc-Orn(Boc)-OH 8	Fmoc-Lys(Boc)-OH
		B4	5-MeHex 14	4(*S*)-MeHex
		–	additional step	DIPEA/MeI
72	[Lys^8]-KF	B3	Fmoc-Orn(Boc)-OH 8	Fmoc-Lys(Boc)-OH
73	[Glu^8]-KF	B3	Fmoc-Orn(Boc)-OH 8	Fmoc-Glu(t-Bu)-OH
		B2	Fmoc-D-*allo*-Ile-OH 7	none
75	[Orn(N$^\delta$Tfa)^8,4(S)MeHex^{14}]-KF	B7	5-MeHex 14	4(*S*)-MeHex
		–	additional step	TFA/DIPEA
76	[Orn(N$^\delta$Tfa)^8,Thr(OTfa)^{12},4(S)MeHex^{14}]-KF	B7	5-MeHex 14	4(*S*)-MeHex
		–	additional step	TFA/DCM (1:1), 3 days
77	[Thr(OTfa)^{12},4(S)MeHex^{14}]-KF	B7	5-MeHex 14	4(*S*)-MeHex
		–	additional step	TFA/DCM (1:1), 3 days
78	[noD-*allo*-Ile^7,noOrn^8,noD-Pro^9,noD-Val^{10},noVal^{11}, noThr^{12},noD-Val^{13}]-KF	B2	Fmoc-D-*allo*-Ile-OH 7	none
		B3	Fmoc-Orn(Boc)-OH 8	none
		B3	Fmoc-D-Pro-OH 9	none
		B3	Fmoc-D-Val-OH 10	none
		B3	Fmoc-Val-OH 11	none
		B3	Fmoc-Thr(t-Bu)-OH 12	none
		B3	Fmoc-D-Val-OH 13	none
79	[noOrn^8]-KF	B3	Fmoc-Orn(Boc)-OH 8	none
80	[noOrn^8,noD-Pro^9]-KF	B3	Fmoc-Orn(Boc)-OH 8	none
		B3	Fmoc-D-Pro-OH 9	none
81	[noOrn^8,noD-Pro^9,noD-Val^{10}]-KF	B3	Fmoc-Orn(Boc)-OH 8	none
		B3	Fmoc-D-Pro-OH 9	none
		B3	Fmoc-D-Val-OH 10	none
82	[noOrn^8,noD-Pro^9,noD-Val^{10},noVal^{11}]-KF	B3	Fmoc-Orn(Boc)-OH 8	none
		B3	Fmoc-D-Pro-OH 9	none
		B3	Fmoc-D-Val-OH 10	none
		B3	Fmoc-Val-OH 11	none
83	[noOrn^8,noD-Pro^9,noD-Val^{10},noVal^{11},noThr^{12}]-KF	B3	Fmoc-Orn(Boc)-OH 8	none
		B3	Fmoc-D-Pro-OH 9	none
		B3	Fmoc-D-Val-OH 10	none
		B3	Fmoc-Val 11	none
		B3	Fmoc-Thr(t-Bu)-OH 12	none
84	[noOrn^8,noD-Pro^9,noD-Val^{10}, noVal^{11},noThr^{12},noD-Val^{13}]-KF	B3	Fmoc-Orn(Boc)-OH 8	none
		B3	Fmoc-D-Pro-OH 9	none
		B3	Fmoc-D-Val-OH 10	none
		B3	Fmoc-Val-OH 11	none
		B3	Fmoc-Thr(t-Bu)-OH 12	none
		B3	Fmoc-D-Val-OH 13	none

Table 5. Continued

	analogue	step	residue replaced	residue incorporated
85	[noVal^{11},noThr^{12},noD-Val^{13}]-KF	B3	Fmoc-Val-OH 11	none
		B3	Fmoc-Thr(t-Bu)-OH 12	none
		B3	Fmoc-D-Val-OH 13	none
86	[Pro^{9}, 4(S)MeHex^{14}]-KF	B3	Fmoc-D-Pro-OH 9	Fmoc-Pro-OH
87	[D-Pip^{9},4(S)MeHex^{14}]-KF	B3	Fmoc-D-Pro-OH 9	Fmoc-D-Pip-OH
88	[D-Tic^{9},4(S)MeHex^{14}]-KF	B3	Fmoc-D-Pro-OH 9	Fmoc-D-Tic-OH
89	[5(*R*)-Ph-Pro^{9},4(S)-MeHex^{14}]-KF	B3	Fmoc-D-Pro-OH 9	Fmoc-5(*R*)-Ph-Pro-OH
90	[Val^{10},D-Val^{11}]-KF	B3	Fmoc-D-Val-OH 10	Fmoc-Val-OH
		B3	Fmoc-Val-OH 11	Fmoc-D-Val-OH
91	[hCh^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-hCh-OH
92	[hCh^{11},D-Cha^{13}]-KF	B3	Fmoc-Val-OH 11	Fmoc-hCh-OH
		B3	Fmoc-D-Val-OH 13	Fmoc-D-Cha-OH
93	[D-Cha^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-D-Cha-OH
94	[Gly^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-Gly-OH
95	[Phe^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-Phe-OH
96	[Ala^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-Ala-OH
97	[Leu^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-Leu-OH
98	[D-Val^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-D-Val-OH
99	[Pro^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-Pro-OH
100	[Gln^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-Gln-OH
101	[Orn^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-Orn(Boc)-OH
102	[Thr^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-Thr(t-Bu)-OH
103	[Glu^{11}]-KF	B3	Fmoc-Val-OH 11	Fmoc-Glu(O-t-Bu)-OH
106	[Gly^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-Gly-OH
107	[Phe^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-Phe-OH
108	[Ala^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-Ala-OH
109	[Leu^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-Leu-OH
110	[D-Thr^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-D-Thr(t-Bu)-OH
111	[Pro^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-Pro-OH
112	[Gln^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-Gln-OH
113	[Orn^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-Orn(Boc)-OH
114	[Glu^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-Glu(O-t-Bu)-OH
115	[Val^{12}]-KF	B3	Fmoc-Thr(t-Bu)-OH 12	Fmoc-Val-OH
116	[Gly^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-Gly-OH
117	[D-Phe^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-D-Phe-OH
118	[D-Ala^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-D-Ala-OH
119	[D-Leu^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-D-Leu-OH
120	[Val^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-Val-OH
121	[D-Pro^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-D-Pro-OH
122	[D-Thr^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-D-Thr(t-Bu)-OH
123	[D-Glu^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-D-Glu(O-t-Bu)-OH
124	[D-Gln^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-D-Gln-OH
125	[Orn^{13}]-KF	B3	Fmoc-D-Val-OH 13	Fmoc-D-Orn(Boc)-OH
126	[no5-MeHex^{14}]-KF	B4	5-MeHex 14	none
127	[Ac^{14}]-KF	B4	5-MeHex 14	AcOH
128	[Tfa^{14}]-KF	B4	5-MeHex 14	TFA
129	[But^{14}]-KF	B4	5-MeHex 14	But-OH
130	[3-MeBut^{14}]-KF	B4	5-MeHex 14	3-MeBut-OH
131	[3,3-dMeBut^{14}]-KF	B4	5-MeHex 14	3,3-dMeBut-OH
132	[4-MePen^{14}]-KF	B4	5-MeHex 14	4-MePen-OH
133	[(c/t)MecHex^{14}]-KF	B4	5-MeHex 14	(c/t)-MecHex-OH
134	[4(*R*)-MeHex^{14}]-KF	B4	5-MeHex 14	4(*R*)-MeHex-OH
135	[Hep^{14}]-KF	B4	5-MeHex 14	Hep-OH
136	[6,6-dFHep^{14}]-KF	B4	5-MeHex 14	6,6-dFHep-OH

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Table 5. Continued

	analogue	step	residue replaced	residue incorporated
137	[Pfh^{14}]-KF	B4	5-MeHex 14	Pfh-OH
138	[Oct^{14}]-KF	B4	5-MeHex 14	Oct-OH
139	[Und^{14}]-KF	B4	5-MeHex 14	Und-OH
140	[Palm^{14}]-KF	B4	5-MeHex 14	Palm-OH
141	[Icos^{14}]-KF	B4	5-MeHex 14	Icos-OH
142	[2,4-hexadie^{14}]-KF	B4	5-MeHex 14	2,4-hexadie-OH
143	[Bza^{14}]-KF	B4	5-MeHex 14	Bza-OH
144	[*p*-MeBza^{14}]-KF	B4	5-MeHex 14	*p*-MeBza-OH
145	[*p*-TflBza^{14}]-KF	B4	5-MeHex 14	*p*-TflBza-OH
146	[Pipe^{14}]-KF	B4	5-MeHex 14	Pipe-OH
147	[3,5-dFPhAc^{14}]-KF	B4	5-MeHex 14	3,5-dFPhAc-OH
148	[*p*-TflPhAc^{14}]-KF	B4	5-MeHex 14	*p*-TflPhAc-OH
149	[*p*-TflCinn^{14}]-KF	B4	5-MeHex 14	*p*-TflCinn-OH
150	[4-dMeaBut^{14}]-KF	B4	5-MeHex 14	4-dMeaBut-OH
151	[4-GuBut^{14}]-KF	B4	5-MeHex 14	4-GuBut-OH
152	[6-Ohep^{14}]-KF	B4	5-MeHex 14	6-Ohep-OH
153	[4-Ac-OBut^{14}]-KF	B4	5-MeHex 14	4-Ac-OBut
154	[4-OHBut^{14}]-KF	B4	5-MeHex 14	4-OHBut-OH
155	[4-(4-AcOBut)OBut^{14}]-KF	B4	5-MeHex 14	4-(4-Ac-OBut)OBut-OH
156	[D-*allo*-IleiBut^{14}]-KF	B4	5-MeHex 14	Fmoc-D-*allo*-Ile-OH
			additional step	*i*But-OH
157	[Lit^{14}]-KF	B4	5-MeHex 14	Lit-OH
158	[Lit(OTfa)^{14}]-KF	B4	5-MeHex 14	Lit-OH
		B7	—	trifluoroacetylion
159	[D-Val^{1},D-Phe^{3},Val^{4},*allo*-Ile^{5},*allo*-Thr^{6},*allo*-Ile^{7}, D-Orn^{8},Pro^{9},Val^{10},D-Val^{11},D-Thr^{12},Val^{13}]-KF	A1	Fmoc-D-Val-OH 4	Fmoc-Val-OH
		A2	Fmoc-D-*allo*-Ile-OH 5	Fmoc-*allo*-Ile-OH
		A2	Fmoc-D-*allo*-Thr-OH 6	Fmoc-*allo*-Thr-OH
		A2	Fmoc-D-*allo*-Ile-OH 7	Fmoc-*allo*-Ile-OH
		A3	Fmoc-Orn(Boc)-OH 8	Fmoc-D-Orn(Boc)-OH
		A3	Fmoc-D-Pro-OH 9	Fmoc-Pro-OH
		A3	Fmoc-D-Val-OH 10	Fmoc-Val-OH
		A4	Fmoc-Val-OH 11	Fmoc-D-Val-OH
		A4	Fmoc-Thr(t-Bu)-OH 12	Fmoc-D-Thr(t-Bu)-OH
		A4	Fmoc-D-Val-OH 13	Fmoc-Val-OH
160	[D-Cha^{4},D-Cha^{5},D-Cha^{7}]-KF	B1	Fmoc-D-Val-OH 4	Fmoc-D-Cha-OH
		B2	Fmoc-D-*allo*-Ile-OH 5	Fmoc-D-Cha-OH
		B2	Fmoc-D-*allo*-Ile-OH 7	Fmoc-D-Cha-OH
161	[D-Val^{5},D-Val^{7}]-KF	B2	Fmoc-D-*allo*-Ile-OH 5	Fmoc-D-Val-OH
		B2	Fmoc-D-*allo*-Ile-OH 7	Fmoc-D-Val-OH
162	[Phe(3,4-Cl$_2$)^{3},*p*CF3 Cinn^{14}]-KF	4	5-MeHex 14	*p*-CF3 Cinn-OH
		5	Fmoc-hCh-OH 3	Fmoc-Phe(3,4-Cl2)-OH
163	[noVal^{11},noThr^{12},noD-Val^{13},Mst^{14}]-KF	B3	Fmoc-Val-OH 11	none
		B3	Fmoc-Thr(t-Bu)-OH 12	none
		B3	Fmoc-D-Val-OH 13	none
		B4	5-MeHex 14	Mst-OH
164	[Thr(OTfa)^{12},Lit(OTfa)^{14}]-KF	A4	5-MeHex 14	LiOH
		A7	additional step	TFA/DCM (1:1)
165	[no5-MeHex^{14},*N*-(Hep)2-D-Val^{13}]-KF	A4	additional step	heptanaldehyde/NaBH$_3$CN/DMF and AcOH (9:1)

cyclization step. Thus, strategy B is better than strategy A. The synthesis of kahalalide B (**2**) was achieved on a solid support, and this strategy should be useful for the synthesis of other cyclodepsipeptides.

5.2. Total Synthesis of KF

Several synthetic strategies have been successfully developed for the total synthesis of KF (**6**) (Table 5). The first successful synthesis of KF (**6**) (Scheme 2) is the linear solid phase synthesis.[27]

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

This methodology involves elongation of the synthetic chain on the solid phase. With the linear peptide in hand, cyclization in solution follows, and finally, deprotection allows preparation of the natural compound in a straightforward manner. Moreover, the solid phase methodology is easy to scale up and could be applied to generate a wide variety of new analogues. The Fmoc/t-Bu strategy and 2-chlorotrityl chloride resin allowed cleavage of the peptide under mild acidic conditions. Next, amino acid D-*allo*-Thr and the Thr precursor of (*Z*)-Dhb were both introduced without protection of the hydroxyl function. For the formation of all the amide bonds, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) was used. The alloc group was removed under standard conditions before the peptide was deprotected from the resin. The cyclization reaction was then performed with benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) using DMF as a solvent. Finally, the deprotection of the Boc group afforded the natural product KF (**6**).

The original solid phase synthesis was based on an orthogonal protecting group scheme using a chlorotrityl chloride resin together with Fmoc and allyloxycabonyl (Alloc) as temporary protecting groups, and t-Bu and Boc for the side chain protection of Thr and Orn, respectively. In 2005, *p*-nitrobenzyloxycarbonyl (*p*-NZ) was used for the permanent protection of ornithine in the synthesis of derivatives of KF (**6**), which contains acid labile residues.[95]

Furthermore, several convergent strategies (scheme 3) have been developed for the synthesis of KF (**6**).[98] Convergent strategies are defined as those in which peptide fragments are coupled to give the desired target molecule. The condensation of peptide fragments should lead to fewer problems in the isolation and purification of intermediates. The general convergent strategy for the synthesis of KF (**6**) included dividing the peptide into fragments, the N-terminal and the fragment containing the cycle. Four strategies were applied to prepare the three distinct N-components (Scheme 3). All four strategies were based on the solid phase synthesis of a branched peptide using a tri- and tetra-orthogonal protecting scheme and subsequent cyclization and deprotection of the N-terminal function in solution. In strategy I, pentapeptide **168** was formed from the Fmoc-D-Val-resin, prepared from the resin (**166**) by a sequential incorporation of D-*allo*-Ile, D-*allo*-Thr, and D-*allo*-Ile derivatives by using the Fmoc/t-Bu strategy and a DIPCDI/HOBt mixture as the coupling reagent and the esterification of the β-hydroxyl group of D-*allo*-Thr with Alloc-Val-OH using a DIPCDI/DMAP mixture. The macrocyclization of **184** by the HOBt/DIPEA/DIPCDI mixture was followed by the deprotection of Fmoc or *p*-NZ groups to afford the N-component **185**. The corresponding C-component 5-MeHex-D-Val-Thr(t-Bu)-Val-D-Val-D-Pro-OH was synthesized from the Fmoc-D-Pro-resin by a sequential attachment of D-Val, Thr, and D-Val derivatives using the Fmoc/t-Bu method and a DIPCDI/HOBt mixture as the coupling reagent and then being capped with 5-methylhexanoic acid at the N-terminus. Finally, the condensation of the N-component **185** and the corresponding C-component using a PyAOP/DIEA mixture yielded the product KF (**6**). In strategy 2, the synthesis of **185** started with a form of **186** by the incorporation of Alloc-Phe-*Z*-Dhb-OH onto the resin **166**. For chain elongation to the heptapeptide **187** from **186**, five amino acids were sequentially attached. Ester linkage between **187** and Fmoc-Val-OH was formed by a DIPCDI/DIPEA mixture. The removal of the Fmoc group from **188** by using a TFA/CH_2Cl_2 mixture (1:99) yielded **189**, which was followed by the cyclization and deprotection to afford **185**. Here HOBt and DIPCDI were used for macrocyclization; $Pd(PPh_3)_4$ and $PhSiH_3$ were applied to successfully remove the Alloc group, and $SnCl_2$ was effective for removal of the *p*-NZ group. For strategies I and II, epimerization was not observed. In strategy III, the removal of the Alloc group of **168** by using $Pd(PPh_3)_4$ and $PhSiH_3$ was followed by the attachment of Alloc-Phe-*Z*-Dhb-OH in the presence of HOBt and DIPCDI to afford **190**. The Boc group was introduced as an N^{α}-amino protecting group of D-*allo*-Ile in **190** after the Fmoc and Alloc groups had been removed. **191** was subjected to macrocyclization using a HOBt/DIPEA/DIPCDI mixture and removal of the Boc group using a TFA/H_2O mixture (19:1) to produce the second N-component **192**, which was then condensed with the corresponding C-component in solution using a PyAOP/DIEA mixture to afford the final KF (**6**). In strategy IV, pentapeptide **193** was synthesized from **186** by a sequential attachment of D-Val, D-*allo*-Ile, and D-*allo*-Thr derivatives using the Fmoc/t-Bu method. The Boc group was used as an N^{α}-amino protecting group of D-*allo*-Thr in **193** to form **194**, which was subjected to Fmoc-Val-OH coupling and the removal of the Fmoc group. The third N-component **196** was synthesized from **195** by macrocyclization using a HOBt/DIPEA/DIPCDI mixture and removal of the Boc group using a TFA/H_2O mixture (19:1). The condensation of **196** with the corresponding C-component produced KF (**6**). Epimerization of the C-terminal amino acids of the C-component in strategies III and IV was measured (4% for the case of Orn in strategy III and >10% for D-*allo*-Ile in strategy IV). Among these four strategies depicted in Scheme 3 showed, strategies I and II are better than strategies III and IV because the C-terminal amino acid of the C-component is D-Pro, which prevents epimerization during the coupling of the fragment in solution. The advantage of strategy II over strategy I was the fact that smaller amounts of the precious Alloc-Phe-*Z*-Dhb-OH were used.

5.3. Total Synthesis of KA

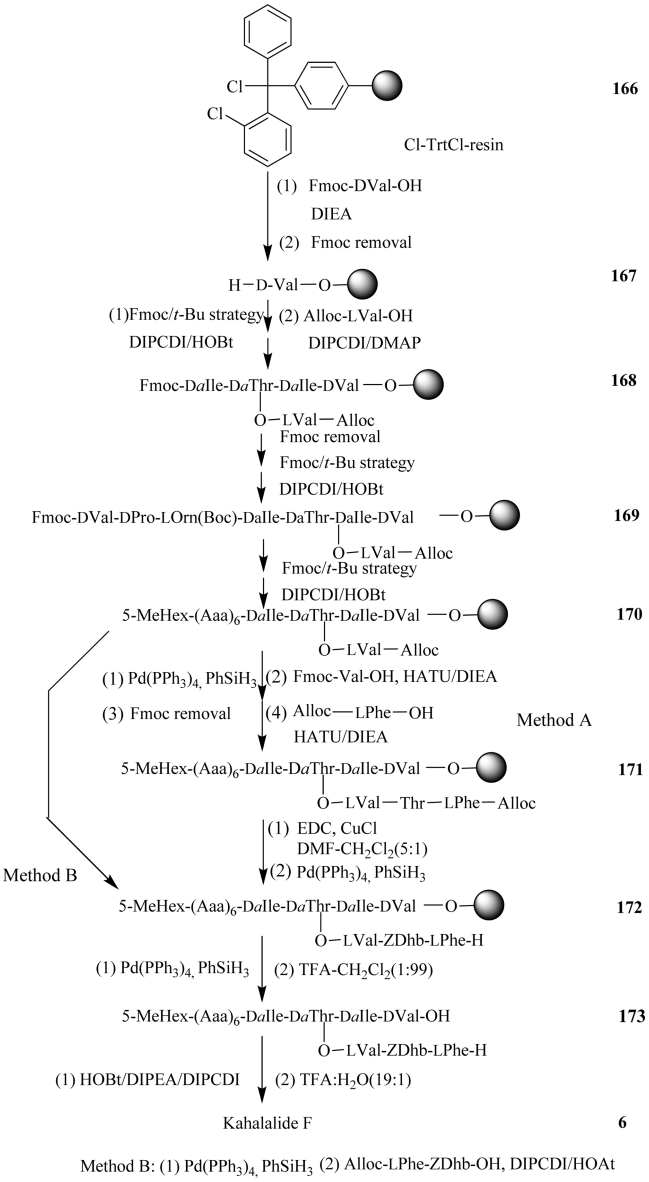
As Scheme 4 shows, the total synthesis of kahalalide A (**1**) began with the attachment of Fmoc-D-Phe onto the commercially available sulfonamide resin (**197**) giving **198**, which was incorporated with Fmoc-D-Leu, Fmoc-L-Thr(t-Bu), Fmoc-D-Phe, and (*S*)-2-methylbutyric acid to provide **199**. Deprotection of the Thr side chain in **199** was followed by ester bond formation with Fmoc-L-Ser(t-Bu). An attachment of Fmoc-L-Thr(t-Bu) and Fmoc-D-Leu afforded the key linear heptapeptides **200**. The safety-catch linker was then activated by sulfonamide alkylation with iodoacetonitrile, and macrocyclative cleavage of depsipeptide **201** into the solution resulted from trityl deprotection of the free amine. Acidic cleavage of the t-Bu ethers is the last step of the synthesis of kahalalide A (**1**) in 15−20% overall yield.

6. STRUCTURE−ACTIVITY RELATIONSHIP

6.1. Structure−Activity Relationship Study of KA

With the total synthesis successfully accomplished, Bourel-Bonnet et al. investigated the SAR of kahalalide A (**1**) by synthesizing kahalalide A analogues. The results highlighted the importance of the free Ser and Thr side chains and the constrained depsipeptide framework for biological activity. The methylbutyrate side chain is flexible and can be replaced with

Scheme 2



other hydrophobic groups, as evidenced by increased activity with hexanoate.[97]

6.2. Structure−Activity Relationship Study on KF and Its Analogues

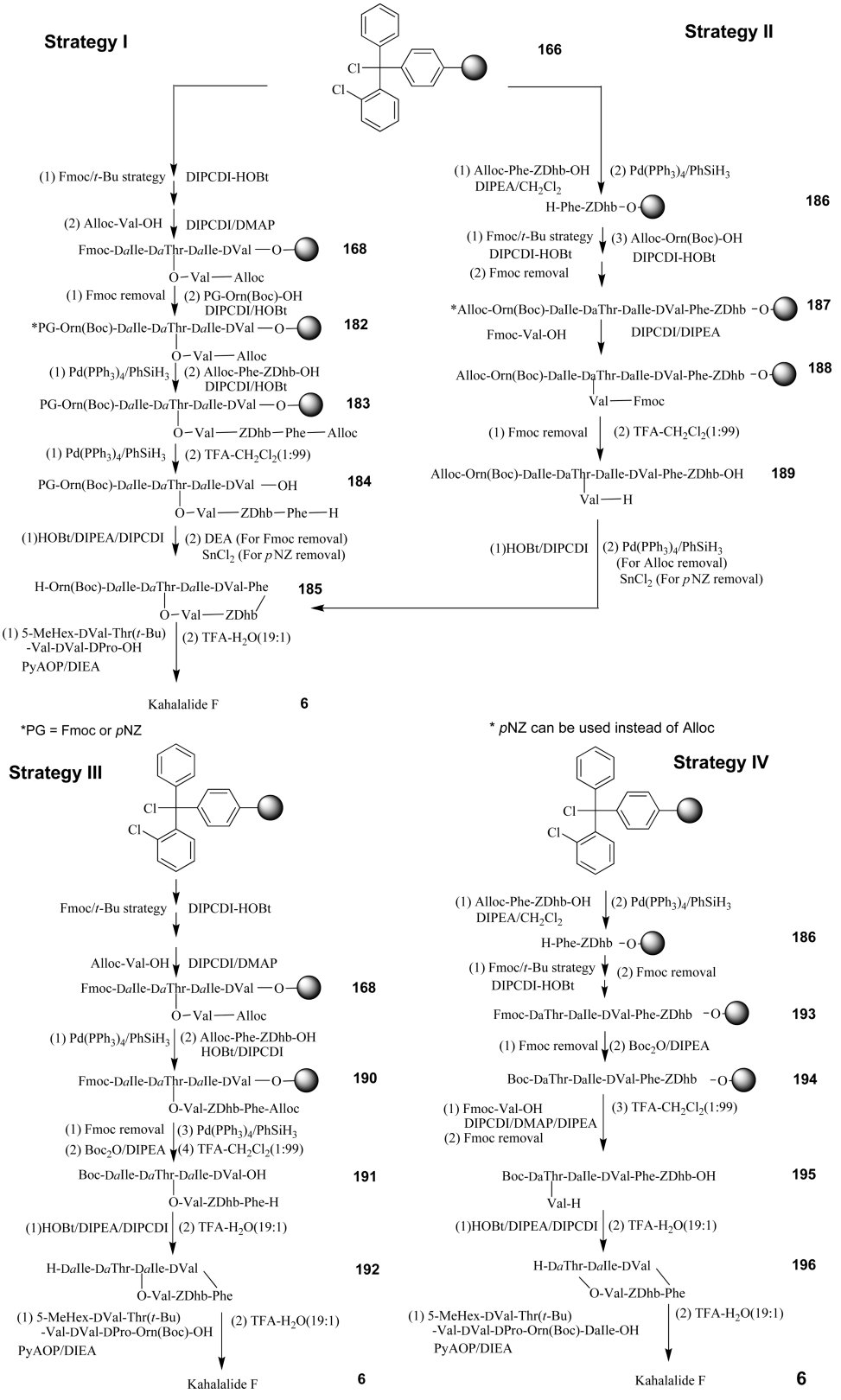
Approximately 143 new KF analogues (Table 6 and Figure 3) have been successfully synthesized or semisynthesized by two groups to improve pharmacological properties and examine the role of each residue in the biological activity of KF (**6**) to examine the structure−activity relationship of KF (**6**).[69,70] In 2007, our group obtained 10 new KF analogues (**57**−**64**, **104**, and **105**) by reaction of KF (**6**) as a starting point with diverse reactants through the amino group of Orn (acetylation with common coupling reagents) or the hydroxyl group of Thr.[69]

6.2.1. Semisynthetic Analogues of KF. In 2007, our group focused on the secondary alcohol of Thr and the primary amine of Orn as the key functional groups to modify from the bacterially produced natural products because they play a crucial role in the bioactivity of this class of compounds.[69] For spectroscopic and bioassay comparison, we first converted KF (**6**) to kahalalide G (**7**) under mild basic conditions. Thus, KF (**6**) was subjected to hydrolysis in a H_2O/MeOH mixture (2:1) in the presence of potassium carbonate at room temperature, causing catalytic cleavage of macrolactone to the target ring-opened product. Further studies continued with the acetylation of KF (**6**). Treatment of free base KF (**6**) with an excess 2:1 volumetric ratio of acetic anhydride to $BF_3 \cdot OEt_2$ at room temperature after 5 min led to major product (68%), monoacetate-KF (**104**). Addition of the Dess−Martin periodinane (DMP) to a solution of KF (**6**) in acetonitrile furnished the oxidation of the secondary alcohol to the corresponding oxo-KF (**105**) in good yield (75%). Some KF analogues were obtained by modification of the primary amine group on L-Orn to the related secondary amines as potential pharmacophores. KF (**6**) was reduced to the corresponding monoalkyl- or dialkyl-amino-KF by the stepwise reductive N-alkylation of the amino groups in the presence of a carboxyaldehyde and hydride reducing agent. This stepwise one-pot procedure includes the initial formation of the intermediate carbinol amine, which then dehydrates to form an imine. Then in situ reduction of this carbinol imine produces the alkylated amine. Sodium triacetoxyborohydride [$NaBH(OAc)_3$] was used as the hydride reducing agent that offers mild borohydride reduction and exhibits remarkable selectivity because the steric and electron withdrawing effects of the three acetoxy groups stabilize the boron−hydrogen bond and are responsible for its mild reducing properties. The reductive alkylation of KF was best performed under optimized conditions by the exposure of parent molecule to 5 equiv of the known aldehyde in methanol for 30 min at room temperature prior to portionwise addition of 2 equiv of triacetoxyborohydride under the same conditions. The reaction time was designed from a few hours to a couple of days and gave the desired products (**57**−**63**) in good to very good yields (Scheme 5). Furthermore, one KF analogue (**64**) was simply synthesized by treatment of DEAC-carboxylic acid and KF (**6**) in the presence of EDC and HBTU in DMF at room temperature for 1 h.

In 2008, the Albericio group reported seven new KF semisynthetic analogues (**65**−**70** and **74**) by modifying the primary amine of L-Orn in KF (**6**).[70] Compound **65** was easily obtained by adding DIPEA, tHex-OH, HOBt, and DIPCDI sequentially to a solution of KF (**6**) in a DMF/CH_2Cl_2 mixture (20:80) at 23 °C. The same procedure was applied to produce compounds **66**−**69** only by replacing tHex-OH with TFB-OH, cHP-OH, (+)-MTPA, or Fmoc-PEG-OH. Compound **69** was dissolved in a piperidine/DMF mixture (20:80) and stirred at 23 °C for 30 min to produce compound **70** in 75% yield. In addition, KF (**6**), D-Biotine, and HATU were dissolved in anhydrous DCM under an Ar atmosphere, and NMM was added, yielding compound **74**.

6.2.2. Synthetic Analogues of KF. Giralt, Albericio, and his co-workers synthesized ~125 novel KF analogues by solid phase synthesis (Scheme 2).[70] The route is very similar to the total synthesis route of KF (**6**) with minor modifications. The whole route consists of seven steps, including the elongation of the synthetic chain on the solid phase, subsequent cyclization, and final deprotection in solution. The tetrapeptide resin **168** was synthesized from the D-Val resin **167**, which was prepared from the commercially available chlorotrityl chloride resin (**166**), by a sequential attachment of D-*allo*-Ile, D-*allo*-Thr, and D-*allo*-Ile derivatives using the Fmoc/t-Bu strategy and a DIPCDI/HOBt

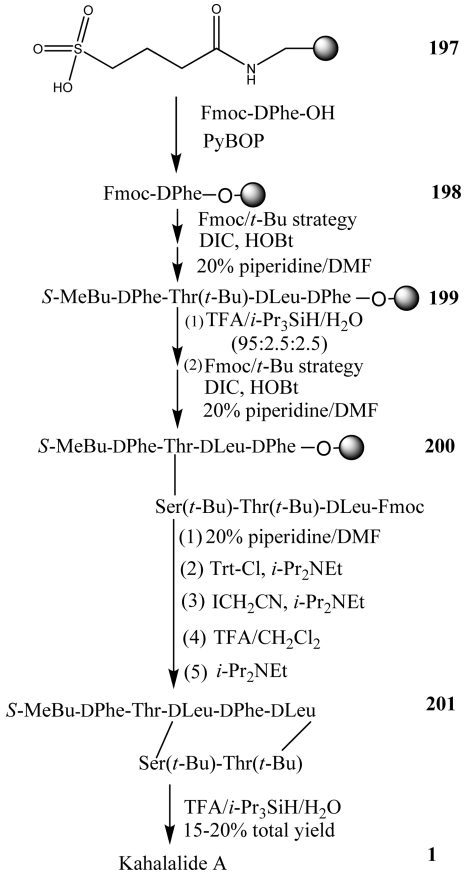
Scheme 3



mixture as the coupling reagent. An ester linkage between **166** and Allo-Val-OH was produced by using diisopropylcarbodiimide (DIPCDI) in the presence of DMAP. For elongation of the chain to the decapeptide **170** from **168**, six amino acids were sequentially attached and capped with 5-methylhexanoic acid at the N-terminus. Construction of (*Z*)-Dhb was conducted via two

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Scheme 4



methods: (A) after side chain elongation from **170** to the tridecapeptide **171**, stereoselective formation of the (*Z*)-Dhb residue on the resin by Fukase's method using EDCI and cuprous chloride and (B) direct introduction of the dipeptide, Alloc-Phe-(*Z*)-Dhb-OH, to **172** with a DIPCDI/HOAt mixture. The Alloc group in **172** was deprotected with $Pd(PPh_3)_4$ and phenylsilane, and cleavage from the resin with a TFA/CH_2Cl_2 mixture (1:99) afforded the linear depsipeptide **173**, which was subjected to macrocyclization using a DIPCDI/HOBt/DIEA mixture, after removal of side chain protection with a TFA/H_2O mixture (19:1). The yield of KF (**6**) was 10−14%. In Scheme 2, two alternative routes for incorporating the amino acid *Z*-didehydroaminobutyric acid are listed. Method B was chosen for most analogues. These 125 KF analogues (Figure 1 and Table 6) were synthesized following the procedure described for KF (**6**) described in Scheme 2, and antitumor activities of these compounds are listed in Table 7.

6.2.3. Structure−Activity Relationship of KF. KF (**6**) structure can be divided into three domains. Domain A includes the macrocycle formed by the six C-terminal amino acids closed by an ester bond between the carboxylic acid of Val and the hydroxyl function of D-*allo*-Thr. It is known that the ring is essential for biological activity because of the absence of activity of the acyclic natural analogue of KF, kahalalide G (**7**). The presence of the non-natural amino acid (*Z*)-Dhb and D-amino acids and the lack of a C-terminal amino acid as a result of the lactamization can contribute highly enzymatic stability to this domain and increase the rigidity of the domain, which are crucial for activity. The analogues at Phe suggested that a hydrophobic residue is required in that position. The introduction of any residues that can increase the hydrophobicity of that position can increase the activity. The L/D configuration of the carbon of the backbone is crucial. Therefore, the conformation of the ring is important for maintaining the activity. Domain B contains a peptide tail; the absolute configuration of any residue is crucial, and all analogues in which the chirality of any residue was reversed lost their activity. The domain not only can be a link between the other two domains but also can adopt or induce folding and interactions with the target protein. The amino acid Orn is important for conserving or enhancing the activity. In general, the compounds with aliphatic groups coupled to the amino group of Orn were the most active KF analogues. Several analogues exhibiting enhanced potency in several human cancer cell lines relative to KF (**6**) were prepared by direct reaction on the amino group of Orn in KF (**6**) with diverse reagents. Domain C is the N-terminal aliphatic acid that is also crucial for maintaining the activity. Any introduction of polar groups that can generate hydrogen bonds decreased the activity. Compounds with no terminal acid or a short aliphatic group had activity lower than that of KF (**6**). The SAR of KF (**6**) is summarized in Table 8.

KF (**6**) has a more defined structure than expected and is highly sensitive to stereotopical changes that affect the chirality of α-carbon of the residue. KF (**6**) is not sensitive to side chain substitutions in almost every residue, and for almost every side chain, it was possible to find a distinct side chain that could preserve or even improve the activity. A more hindered replacement in each side chain was able to improve the activity, and the activity can be further increased by enhancing the hydrophobicity at any point on the molecule, solubility in water being a limiting factor.

7. MECHANISM OF ACTION OF KF

7.1. KF Induces Oncosis Opposed to Apoptosis

The primary mechanism of action of KF (**6**) remains to be elucidated. The negative results of the NCI COMPARE analysis[99] suggested that the compound may exhibit its cytotoxicity by a unique mechanism of action. There are two forms of cell death that occur in cells: apoptosis and oncosis.[114] Most anticancer drugs are thought to induce apoptosis, which is defined as an active and programmed process characterized by a variety of morphological changes, including cell shrinkage, cytoplasmic condensation, ladder DNA degradation, and nuclear fragmentation resulting in cell death.[114] However, soon after exposure to KF (**6**), cells start a death process, including great swelling and a series of profound morphological alterations. KF (**6**) induces a rapid and profound alteration of cell architecture, including extensive vesiculation of cytoplasmic organelles, dilation of the endoplasmic reticulum elements, and cytoskeletal degradation. The integrity of crucial organelles such as lysosomes, mitochondria, and endoplasmic reticulum is largely damaged. Although chromatins clump irregularly into small, condensed masses, the nuclear structure is basically preserved and no DNA degradation is found.[100−104] These features are typical of the process named oncosis, which is a passive death process resulting from physical or chemical lethal injury.[114] Furthermore, several biochemical results also support the induction of KF (**6**) by oncosis as opposed to apoptosis. First, KF (**6**) has no effect on the cell cycle because flow cytometry analysis revealed that KF (**6**) induced neither cell cycle arrest nor apoptotic hypodiploid peak.[103] Second, neither protein and nucleic acid syntheses nor topoisomerase (I or II) can be inhibited by KF (**6**).[29] Third,

Table 6. KF Analogues[a]

analogue	Val[1]	(*Z*)-Dhb[2]	Phe[3]	D-Val[4]	D-*allo*-Ile[5]	D-*allo*-Thr[6]	D-*allo*-Ile[7]	Orn[8]	D-Pro[9]	D-Val[10]	Val[11]	Thr[12]	D-Val[13]	5-MexHex[14]
25	Etg													
26	D-Etg													
27	(*Z*)-Dhf													
28	Dha													
29	D-Thr													
30	D-*allo*-Thr													
31	Gly													
32	Aib													
33		Trp												
34		hCh												
35		Phe(3,4-Cl_2)												4
36		Phe(F_5)												4
37		Phe(4-I)												4
38		Phe(4-NO_2)												4
39		Phe(4-F)												4
40		Tyr(Me)												4
41		Thi												4
42		Tic												4
43		Tyr												4
44		Oic												4
45		*N*-MePhe												4
46		Phe(2-Cl)												
47		Phe(3-Cl)												
48		Phe(4-Cl)												
49		Phe(3,4-F_2)												
50		Nal												
51		Bip												
52		Phg												
53				Val										
54						D-Dapa								
55						D-Thr								
56						D-Ser								
57								Orn(4-FB)						
58								Orn(4-FB)$_2$						
59								Orn(4-PM)						
60								Orn(2-TM)						
61								Orn (2-TM)$_2$						
62								Orn(nHex)						
63								Orn(nHex)$_2$						
64								Orn(DEAC)						
65								Orn(tHex)						
66								Orn(TFB)						
67								Orn(cHP)						
68								Orn(Mosh)						
69								Orn(Fmoc-PEG)						
70								Orn(PEG)						
71								*N*-(Me)$_3$-Lys						4
72								Lys						
73								Glu						
74								Orn(Biot)						
75								Orn(N^{δ}Tfa)						4
76								Orn(N^{δ}Tfa)				Thr(OTfa)		4
77												Thr(OTfa)		4

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Table 6. Continued

analogue	Val[1]	(*Z*)-Dhb[2]	Phe[3]	D-Val[4]	D-*allo*-Ile[5]	D-*allo*-Thr[6]	D-*allo*-Ile[7]	Orn[8]	D-Pro[9]	D-Val[10]	Val[11]	Thr[12]	D-Val[13]	5-MexHex[14]
78							no	no	no	no	no	no	no	no
79								no						
80								no	no					
81								no	no	no				
82								no	no	no	no			
83								no	no	no	no	no		
84								no	no	no	no	no	no	
85											no	no	no	
86									Pro					4
87									D-Pip					4
88									D-Tic					4
89									5(*R*)-Ph-Pro					4
90										Val	D-Val			
91											hCh			
92											hCh		D-Cha	
93													D-Cha	
94											Gly			
95											Phe			
96											Ala			
97											Leu			
98											D-Val			
99											Pro			
100											Gln			
101											Orn			
102											Thr			
103											Glu			
104												Thr(Ac)		
105												Thr(Oxo)		
106												Gly		
107												Phe		
108												Ala		
109												Leu		
110												D-Thr		
111												Pro		
112												Gln		
113												Orn		
114												Glu		
115												Val		
116													Gly	
117													D-Phe	
118													D-Ala	
119													D-Leu	
120													Val	
121													D-Pro	
122													D-Thr	
123													D-Glu	
124													D-Gln	
125													D-Orn	
126														No
127														Ac
128														Tfa
129														But
130														3-MetBut

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Table 6. Continued

analogue	Val[1]	(*Z*)-Dhb[2]	Phe[3]	D-Val[4]	D-*allo*-Ile[5]	D-*allo*-Thr[6]	D-*allo*-Ile[7]	Orn[8]	D-Pro[9]	D-Val[10]	Val[11]	Thr[12]	D-Val[13]	5-MexHex[14]
131														3,3-dMeBut
132														4-MePen
133														(c/t)-MeHex
134														4(*R*)-MeHex
135														Hep
136														6,6-dFhep
137														Pfh
138														Oct
139														Und
140														Palm
141														Icos
142														2,4-Hexadie
143														Bza
144														*p*-MeBza
145														*p*-TflBza
146														Pipe
147														3,5-dFPhAc
148														p-TflPhAc
149														*p*-TflCinn
150														4-dMeaBut
151														4-GuBut
152														6-Ohep
153														4-Ac-Obut
154														4-OHBut
155														4-(4-Ac-Obut)Obut
156														D-*allo*-Ile-IBut
157														Lit
158														Lit(OTfa)
159	D-Val		D-Phe	Val	*allo*-Ile	*allo*-Thr	*allo*-Ile	D-Orn	Pro	Val	D-Val	D-Thr	Val	
160				D-Cha	D-Cha		D-Cha							
161					D-Val		D-Val							
162			Phe(3,4-Cl_2)											*p*-TflCinn
163											no	no	no	Mst
164												Thr(OTfa)		Lit(OTfa)
165													$N(Hep)_2$-D-Val	

[a] The chemical structure of each modification can be found in Figure 3. The numeral 4 in the 5-MeHex column indicates the presence of 4(*S*)-MeHex.

several markers of caspase-dependent apoptosis were negative after KF (**6**) exposure, including the externalization of phosphatidylserine, the release of cytochrome *c* from mitochondria, and cleavage of caspase-3 and PARP. Moreover, molecular or chemical inhibition of caspases by ectopic overexpression of Bcl-2 or a pan-caspase inhibitor (zVAD-fmk), respectively, failed to protect against KF (**6**) cytotoxicity. Specific inhibitors of cathepsin B (CA-074 Me and zFA-fmk) or D (pepstatin A) also failed to protect against cell death induced by KF (**6**).[29,103] Fourth, KF (**6**) exhibits strong cytotoxicity against both wild-type p53 and mutated p53 tumor cells in the NCI panel.[30−32] These results suggest that cytotoxicity induced by KF (**6**) is predominantly due to a process of necrotic cell death involving oncosis rather than apoptosis, which is verified by treatment of the yeast *Saccharomyces cerevisiae* with isoKF.[105,114,115]

7.2. Multiple Targets of KF Action

Much progress has been made with respect to targets involved in the action of KF (**6**), and multiple targets have been identified. First, human HeLa cervical cancer cells and monkey COS-1 fibroblasts treated with KF (**6**) were found to become dramatically swollen and produce many large vacuoles that appeared to be a consequence of changes in lysosomal membranes that were verified by the fact that lysosomes of KF-treated cells showed a dramatic enlargement and the lysosomal pH increased.[101] The analysis of lysosomes of human prostate cancer PC3 cells treated with KF (**6**) via fluorescent acidotropic probes LysoTracker Green and Acridine Orange showed that KF (**6**) exposure can alter the membrane permeability of lysosomes.[103] Thus, it appears that lysosomes are targets for kahalalide F action. Conjugation of KF (**6**) with gold nanoparticles (GNP) was found to enhance in vitro antitumor activity because there is a synergic effect between KF (**6**) and the GNP that can favor penetration and targeting to the lysosome of HeLa cells.[102]

Second, further studies of the putative effects of KF (**6**) on human prostate PC3 and breast cancer SKBR-3 cells by using electron microscopy showed that KF (**6**) can rupture the plasma membrane.[103] Recently, treatment of with isoKF (**22**) caused

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

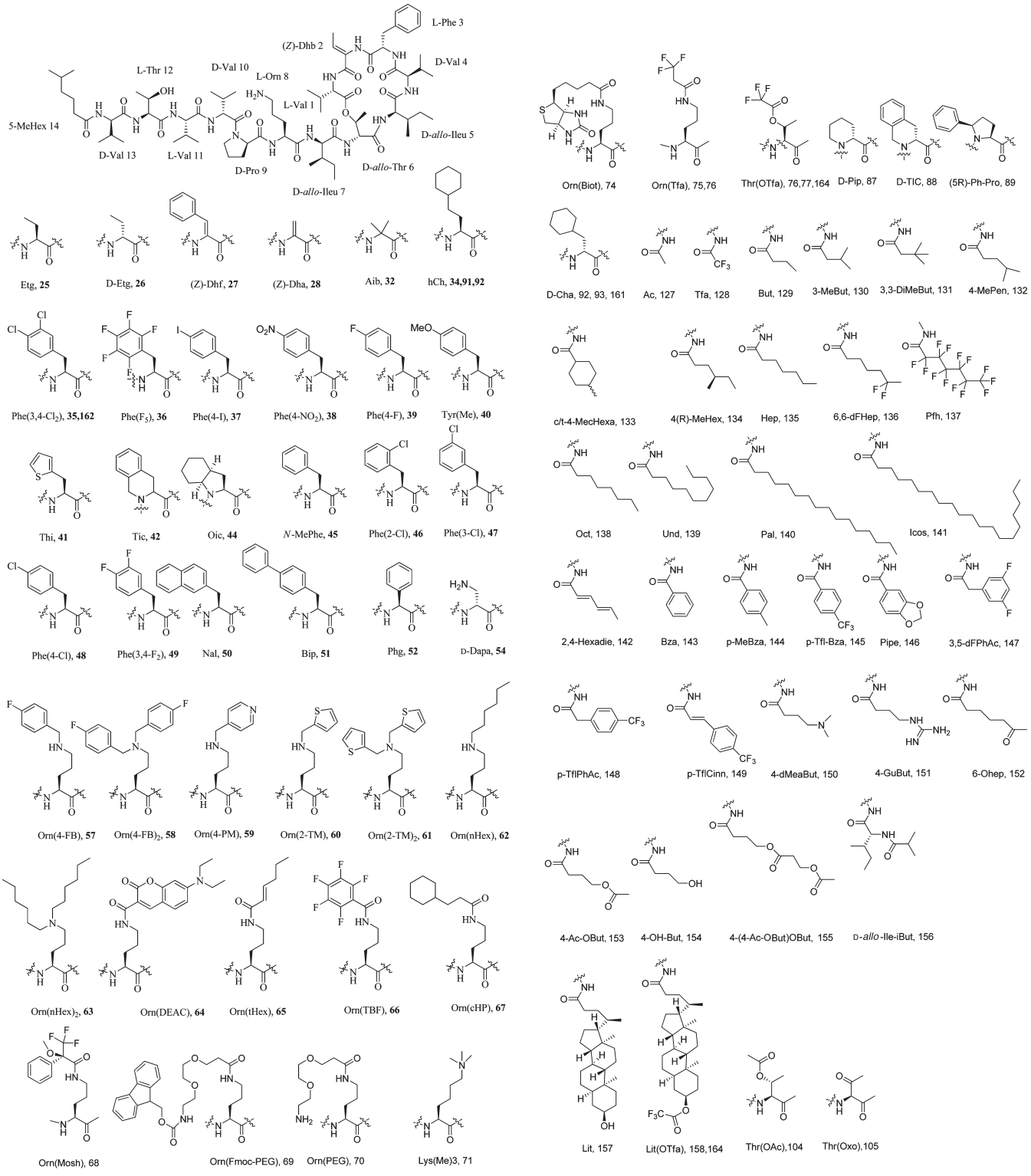
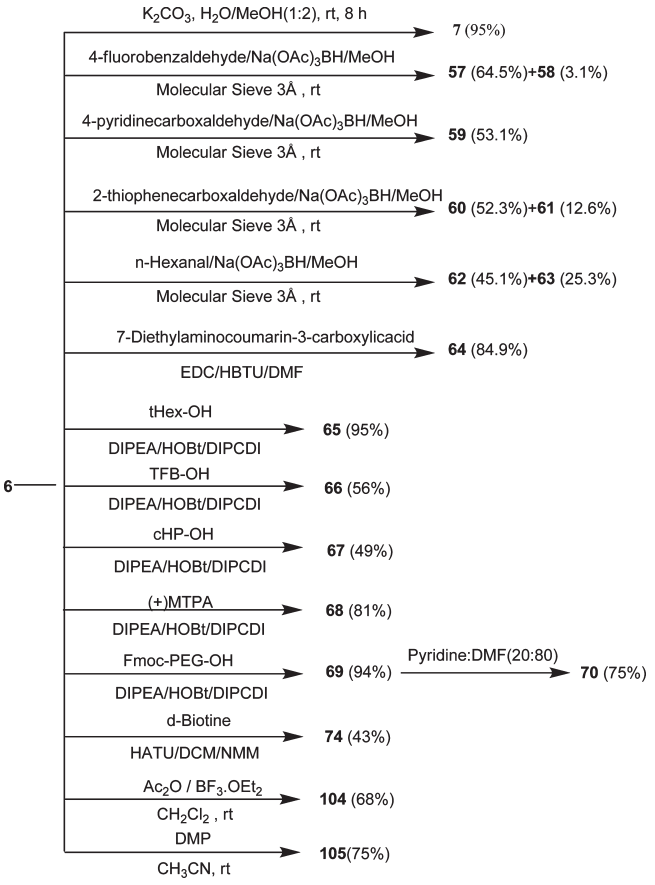


Figure 3. Building blocks used for the substitution of residues in KF (**6**).

invaginations of the plasma membrane, which make the cells permeable and metabolically inactive.[114] The plasma membrane is supposed to be the first target of KF (**6**) action, and the breakage of the plasma membrane causes changes in the osmotic balance of the cell, which induces alterations, including cytoplasmic swelling, vesiculation of cytoplasmic organelles, and vacuolization of mitochondria and endoplasmic reticulum. Therefore, the mechanism of action of KF (**6**) has been postulated to be similar to those of other cytotoxic compounds, which induce cell death through the formation of new ion channels in the membrane and/or by changing the activity of existing channels. For example, KF (**6**) was supposed to be similar to monensin, a Na^+/H^+ ionophore produced by *Streptomyces cinnamonensis*.[101] Although KF (**6**) could interact with the lipid bilayer, it is unlikely

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Scheme 5



to form pores by itself because the molecule is too small to directly span the lipid bilayer. A minimum of 20 amino acids is required to form the pore, and KF (**6**) is composed of 14 amino acids. One possibility is that KF (**6**) forms multimers. It is also possible that the drug interferes with the membrane, which can result in the formation or modification of ion channels. 2-Hydroxylated fatty acid-containing ceramides are found to be involved in the mechanism of action of isoKF (**22**) in *S. cerevisiae* and human colon cancer line HCT116. Furthermore, overexpression of fatty acid 2-hydroxylase (FA2H) and exogenous addition of 2-hydroxylated fatty acids increase the sensitivity of mammalian cells to isoKF (**22**). Accordingly, two possibilities are proposed to explain the mechanism of action of isoKF (**22**). One is that fatty acid 2-hydroxylation allows hydrogen bonding of isoKF directly to sphingolipids to facilitate ion channel formation or permeability to the drug. Another is that 2-hydroxylation affects the formation of some kind of membrane microdomain that is important for drug membrane association.[114] Third, the sensitivity to KF (**6**) in a panel of human tumor cell lines derived from breast (SKBR3, BT474, and MCF7), vulval (A431), NSCLC (H460, A549, SW1573, and H292), and hepatic (SKHep1, HepG2, and Hep3B) carcinoma significantly correlated with protein expression levels of ErbB3 (HER3) but not other ErbB receptors. Exposure to KF (**6**) for 4 h induced downregulation of ErbB3 expression in sensitive cell lines, as well as inhibition of the PI3K-Akt/PKB signaling pathway, which is directly linked to ErbB3.[105−107] Moreover, ectopic expression of a constitutively active Akt mutant had a protective effect against KF (**6**) cytotoxicity. This suggests ErbB3 and the Akt pathway are major determinants of KF (**6**) action on these cell lines. Recently, reverse chemical proteomics with T7 cDNA display has been used to identify human ribosomal protein S25 (RPS 25) as a

Table 7. Activity Data (GI_{50}) of KF Analogues (micromolar)

	DU-145	LN-caP	IGROV	IGROV ET	SK-BR 3	MEL-28	A-549	K-562	PANC-1	HT-29	LOVO	LOVO DOX	HELA	HELA APL
6	0.93	0.96	0.49	0.55	0.36	1.19	1.16	1.93	1.77	0.43	0.33	0.30	0.97	0.98
25	3.05	–	2.81	1.64	–	4.48	4.97	21.60	3.09	7.35	3.03	2.72	4.76	7.16
26	3.26	–	2.72	1.64	–	3.10	3.23	7.02	4.09	3.66	2.63	1.76	4.28	7.16
27	1.39	–	2.78	2.12	0.60	4.69	1.50	–	4.54	5.13	0.84	–	1.50	1.34
28	1.11	1.44	0.63	0.35	0.46	1.10	1.20	2.47	4.12	0.64	0.31	0.39	1.66	0.89
29	18.40	–	–	–	–	11.70	11.50	–	–	8.52	–	–	–	–
30	7.53	–	4.86	3.48	6.15	33.10	4.49	–	4.88	11.80	3.44	–	8.58	7.52
31	1.41	2.74	1.64	0.72	1.66	1.39	1.63	2.53	4.16	2.26	1.84	0.83	1.75	1.83
32	1.64	3.64	1.79	1.08	2.13	2.18	4.15	5.71	4.08	2.35	2.32	1.59	4.57	2.79
33	0.83	2.06	0.09	0.46	0.35	3.42	1.61	4.84	7.69	1.05	0.63	0.51	1.36	1.36
34	0.46	0.38	0.28	0.23	0.17	1.02	0.76	1.42	1.12	0.29	0.27	0.22	0.30	0.32
35	0.27	–	0.38	0.25	0.28	0.59	0.11	6.47	2.09	0.29	0.43	0.30	0.49	1.02
36	1.37	0.50	2.07	2.03	0.56	1.28	1.85	–	2.11	1.09	0.47	0.50	3.75	1.45
37	0.15	0.35	0.67	1.19	0.31	0.16	0.23	–	1.20	0.40	0.37	0.13	0.60	0.31
38	0.26	0.48	0.68	1.05	0.37	0.19	0.56	–	1.29	0.42	0.32	0.09	0.78	0.57
39	0.89	0.51	0.81	0.97	0.52	0.70	1.01	–	1.46	0.45	0.39	0.41	0.96	0.74
40	0.86	–	0.44	0.30	0.28	1.19	0.53	3.55	3.12	0.23	0.32	0.24	0.77	1.01
41	1.38	–	2.44	1.71	0.82	1.58	1.76	4.92	5.98	1.29	0.75	0.39	1.59	1.77
42	4.16	–	3.33	2.43	2.30	1.62	1.75	4.89	6.79	1.89	2.82	0.72	1.92	2.06
43	6.89	–	3.96	3.39	2.10	2.37	5.09	4.92	6.80	2.21	1.83	1.03	3.62	6.29
44	1.46	–	1.82	1.59	0.70	1.15	1.46	4.75	2.58	1.66	1.21	0.36	1.56	1.26

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Table 7. Continued

	DU-145	LN-caP	IGROV	IGROV ET	SK-BR 3	MEL-28	A-549	K-562	PANC-1	HT-29	LOVO	LOVO DOX	HELA	HELA APL
45	1.23	–	0.86	1.00	0.32	1.35	1.30	2.14	1.92	0.27	0.39	0.32	0.53	1.11
46	0.18	0.09	0.07	0.10	0.05	0.39	0.42	1.85	1.51	0.09	0.04	0.04	0.27	0.31
47	0.10	–	0.04	0.07	0.05	0.18	0.19	0.87	0.87	0.04	0.02	0.04	0.10	0.19
48	0.11	–	0.05	0.09	0.04	0.21	0.23	0.70	1.14	0.04	0.02	0.06	0.19	0.22
49	0.04	–	0.04	0.05	0.03	0.13	0.14	0.73	0.58	0.03	0.01	0.02	0.10	0.16
50	0.05	–	0.03	0.05	0.02	0.14	0.11	0.29	0.61	0.04	0.01	0.03	0.07	0.08
51	0.17	–	0.25	0.13	0.07	0.19	0.05	3.22	1.24	0.21	0.18	0.18	0.22	0.48
52	0.87	–	0.50	0.81	0.44	1.11	1.09	1.81	1.66	8.20	0.42	0.41	1.73	1.62
53	4.99	–	5.65	1.22	–	7.45	4.98	37.40	1.68	16.40	5.49	15.50	3.87	7.17
54	8.80	6.88	9.55	5.68	8.66	6.40	9.48	9.66	9.71	13.20	11.50	–	7.45	7.04
55	32.00	19.30	73.20	3.76	8.20	8.20	7.16	25.30	586.00	17.60	16.40	6.03	17.70	9.52
56	5.04	3.37	16.70	0.62	0.75	1.90	1.44	19.00	267.00	0.98	0.46	0.35	3.53	1.93
65	0.66	–	0.18	0.21	0.06	0.81	0.62	1.24	1.67	0.13	0.08	0.14	0.41	0.50
66	0.73	–	0.18	0.33	–	0.98	1.04	0.98	1.58	0.12	0.08	0.15	0.29	0.49
67	0.08	–	0.05	0.10	0.04	0.09	0.11	0.06	0.21	0.07	0.04	0.09	0.08	0.17
68	0.89	–	0.25	0.34	0.09	1.04	1.01	1.11	1.55	0.15	0.09	0.17	0.54	0.55
69	0.02	–	0.04	0.07	0.04	0.07	0.04	0.01	0.15	0.09	0.04	0.20	0.06	0.05
70	0.74	1.82	0.18	0.20	0.25	0.95	0.49	0.58	1.25	0.10	0.26	0.18	0.71	0.52
71	1.02	1.06	0.61	0.64	0.37	1.11	1.39	1.84	4.16	0.79	0.36	–	0.94	1.11
72	1.29	1.33	0.56	0.65	0.39	1.47	1.44	9.35	5.11	0.52	0.43	0.30	0.91	0.51
73	21.70	1.88	1.95	3.54	–	5.89	10.90	3.32	2.50	2.67	0.79	7.95	–	–
74	0.85	0.82	0.37	0.62	0.32	0.96	0.99	4.80	1.99	0.36	0.32	0.33	0.38	0.99
75	1.33	1.46	0.31	0.49	0.11	1.91	1.14	3.75	3.80	0.27	0.31	0.28	0.50	1.21
76	1.46	1.14	0.37	0.77	0.33	17.60	1.23	3.83	2.38	0.33	0.28	0.36	0.66	0.85
77	0.62	0.61	0.29	0.23	0.20	0.72	0.77	0.85	1.43	0.24	0.21	0.19	0.29	0.50
78	5.10	–	14.00	4.40	–	14.50	9.71	73.00	4.35	32.00	14.90	30.20	0.74	14.00
79	3.67	–	–	–	–	3.67	3.67	–	–	3.67	–	–	–	–
80	–	–	–	–	–	-	23.00	–	–	9.89	–	–	–	–
81	4.30	–	–	–	–	4.30	4.30	–	–	4.30	–	–	–	–
82	9.36	–	–	–	–	9.36	9.36	–	–	9.36	–	–	–	–
83	–	–	–	–	–	-	50.10	–	–	162.00	–	–	–	–
84	11.50	–	–	–	–	11.50	11.50	–	–	11.50	–	–	–	–
85	69.40	9.85	10.70	11.60	–	8.41	36.60	3.48	2.92	25.60	19.10	25.00	–	–
86	9.38	21.30	7.57	5.00	–	6.48	3.36	8.22	7.69	7.56	11.50	13.20	6.60	4.99
87	2.28	5.79	1.39	0.89	–	1.59	0.80	6.45	7.62	1.16	2.25	1.22	3.08	1.51
88	2.39	4.33	1.52	1.06	–	3.63	1.43	7.90	7.38	1.32	2.62	2.29	3.40	3.64
89	8.93	10.30	7.20	3.05	–	6.16	3.19	7.82	5.17	7.19	7.33	12.60	6.28	4.75
90	3.39	–	–	–	–	3.39	3.39	–	–	3.39	–	–	–	–
91	0.35	0.64	0.28	0.38	0.23	0.33	0.19	0.49	1.04	0.24	0.24	0.11	0.39	0.46
92	3.30	3.38	2.39	3.03	2.06	3.18	3.60	1.79	2.90	2.90	2.81	1.21	2.95	4.34
93	1.41	2.98	1.27	1.09	0.79	1.73	1.97	2.10	7.15	2.01	2.09	0.83	1.30	1.45
94	8.95	3.05	6.44	5.78	19.00	6.51	9.65	2.57	7.21	13.50	10.70	–	7.58	7.16
95	0.61	0.86	0.34	0.19	0.33	0.73	0.72	1.74	2.54	0.27	0.29	–	0.43	0.82
96	3.90	2.80	2.00	1.25	3.25	3.45	3.73	4.32	9.79	2.59	2.68	–	3.10	3.27
97	0.94	1.03	0.46	0.35	0.45	0.96	1.28	2.10	3.29	0.61	0.31	–	1.02	0.94
98	3.13	2.44	2.09	1.39	3.69	6.34	5.09	4.29	4.43	4.84	3.31	–	5.02	3.40
99	8.72	3.47	9.47	5.63	18.50	6.34	9.40	9.57	7.99	13.10	16.10	–	7.39	6.98
100	8.54	3.12	9.27	5.51	18.10	6.21	8.07	9.38	7.24	12.90	14.30	–	7.23	6.83
101	1.23	1.76	1.75	1.35	4.46	1.52	2.92	0.22	1.73	4.61	3.61	7.59	1.28	1.11
102	7.56	5.41	5.18	1.91	2.07	7.72	7.56	9.83	5.51	11.00	2.97	2.99	6.47	7.00
103	7.48	5.30	9.13	4.69	15.20	7.58	7.41	9.64	8.78	19.30	16.30	15.60	7.69	6.87
106	0.57	0.23	0.08	0.20	0.63	0.21	0.52	0.13	0.52	0.89	0.60	2.39	0.25	0.23
107	0.19	0.06	0.18	0.25	0.30	0.15	0.07	0.02	0.14	0.23	0.33	0.38	0.13	0.15
108	1.42	1.38	2.01	1.23	5.82	1.24	0.64	0.82	1.16	1.67	2.05	1.08	1.27	1.28

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Table 7. Continued

	DU-145	LN-caP	IGROV	IGROV ET	SK-BR 3	MEL-28	A-549	K-562	PANC-1	HT-29	LOVO	LOVO DOX	HELA	HELA APL
109	0.96	1.05	0.64	0.75	0.43	1.27	0.64	0.54	1.11	1.00	0.42	0.28	1.06	1.17
110	5.21	4.17	8.12	9.19	10.70	6.94	6.84	1.93	3.14	18.50	17.00	11.60	7.48	8.00
111	4.84	6.62	4.52	7.41	3.08	2.44	1.61	1.48	5.14	4.63	4.66	2.86	2.10	2.95
112	0.91	–	0.36	0.48	0.15	1.17	1.14	1.90	1.74	0.45	0.26	0.30	1.81	1.70
113	6.67	6.75	4.70	7.62	2.88	5.27	3.27	1.62	8.05	5.90	4.94	2.88	3.42	6.67
114	2.48	1.07	0.86	1.13	1.92	4.83	1.63	8.13	4.12	1.31	1.71	9.65	2.55	2.06
115	0.18	0.33	0.18	0.26	0.17	0.21	0.26	0.72	1.12	0.30	0.28	0.13	0.13	0.32
116	5.69	–	10.50	5.36	–	6.95	0.72	38.50	0.50	10.90	8.76	11.10	0.67	7.37
117	3.90	2.28	2.01	2.24	2.34	2.64	2.65	3.40	5.44	2.34	2.62	–	2.21	1.79
118	1.81	–	2.13	1.61	–	1.99	1.46	7.41	1.66	3.03	1.98	0.80	1.03	1.68
119	1.34	1.79	1.05	1.31	–	1.09	1.92	2.72	2.91	0.66	0.61	0.33	2.09	1.00
120	0.55	0.85	1.53	2.89	–	1.00	1.33	0.31	1.51	0.64	1.30	1.76	2.31	1.04
121	2.06	1.16	2.49	4.70	–	1.05	2.83	1.31	2.75	5.16	4.57	3.51	2.89	1.18
122	1.47	1.95	1.17	1.00	–	1.22	1.55	8.51	3.91	1.06	0.37	0.33	2.03	1.04
123	2.84	–	2.01	1.31	–	3.73	3.25	7.02	5.31	2.91	1.95	2.03	2.94	2.91
124	1.46	–	1.57	1.33	–	1.34	0.23	6.80	0.17	2.30	1.46	0.61	0.45	2.42
125	1.16	0.85	1.13	1.03	1.72	1.14	0.75	0.88	1.42	0.77	2.30	2.04	1.37	1.10
126	5.98	–	11.10	4.01	–	8.05	5.39	40.50	5.49	17.80	9.46	16.70	5.16	7.75
127	7.11	–	–	–	–	7.11	7.11	–	–	7.11	–	–	–	–
128	3.42	–	–	–	–	6.84	6.84	–	–	3.42	–	–	–	–
129	1.74	–	–	–	–	3.48	3.48	–	–	1.74	–	–	–	–
130	2.07	–	2.25	1.36	–	1.98	1.68	7.79	8.47	2.98	1.63	0.88	2.09	2.33
131	0.34	–	–	–	–	3.42	1.71	–	–	0.34	–	–	–	–
132	0.34	–	–	–	–	3.42	0.34	–	–	0.34	–	–	–	–
133	0.69	1.07	0.53	0.60	0.35	1.01	1.04	2.94	2.66	0.55	0.24	0.34	0.90	0.63
134	1.69	2.98	0.83	0.55	0.54	2.01	1.69	2.07	8.01	0.73	0.40	0.25	2.33	1.28
135	0.85	1.09	0.35	0.29	0.23	0.81	1.18	2.74	2.36	0.50	0.32	0.26	0.97	0.78
136	4.52	1.84	1.33	0.99	–	2.94	2.03	2.96	2.04	1.96	0.44	1.81	–	–
137	0.53	0.76	0.85	0.70	0.29	0.73	0.64	0.83	2.32	0.51	1.16	0.29	0.96	0.60
138	0.58	0.78	0.68	0.53	0.34	0.93	1.05	1.00	2.83	0.58	0.32	0.30	0.95	0.53
139	0.18	0.42	0.22	0.17	0.06	0.19	0.16	1.67	1.63	0.37	0.29	0.10	0.14	0.24
140	0.15	0.13	0.19	0.35	0.04	0.14	0.14	0.12	0.20	0.25	0.30	0.26	0.28	0.33
141	15.70	1.65	5.17	4.69	3.40	1.89	1.88	9.14	1.79	3.95	3.81	–	5.44	1.52
142*	6.85	–	–	–	–	6.85	3.43	–	–	0.69	–	–	–	–
143	1.21	1.19	1.00	0.63	0.87	1.12	1.32	0.43	5.38	1.34	0.35	–	1.21	1.04
144	1.00	1.03	0.48	0.37	0.46	1.09	1.07	1.08	2.92	0.61	0.33	–	0.93	0.95
145	0.31	0.34	0.25	0.14	0.30	0.84	0.20	9.18	1.84	0.22	0.25	–	0.35	0.52
146	–	–	–	–	–	–	0.64	–	–	0.41	–	–	–	–
147	1.84	1.65	1.37	0.89	1.27	2.34	1.62	9.29	7.73	1.72	0.79	–	1.41	2.02
148	5.28	1.63	1.00	0.87	–	2.80	1.84	2.23	2.17	1.68	0.46	1.85	–	–
149	1.00	0.50	0.40	0.14	–	0.38	0.74	1.39	1.82	0.42	0.25	0.60	–	–
150*	6.77	–	–	–	–	6.77	6.77	–	–	6.77	–	–	–	–
151	8.62	3.93	9.36	5.56	18.30	6.27	9.28	9.46	9.51	13.00	15.00	–	7.30	6.90
152	7.56	5.36	9.22	4.74	7.27	7.66	7.49	9.75	8.87	19.50	8.59	5.05	7.77	6.94
153*	6.70	–	–	–	–	6.70	6.70	–	–	6.70	–	–	–	–
154*	6.89	–	–	–	–	6.89	6.89	–	–	6.89	–	–	–	–
155*	0.63	–	–	–	–	0.63	0.63	–	–	0.63	–	–	–	–
156*	6.46	–	–	–	–	6.46	6.46	–	–	6.46	–	–	–	–
157	5.10	–	14.00	4.40	–	14.50	9.71	73.00	4.35	32.00	14.90	30.20	0.74	14.00
158	0.39	0.85	0.38	0.35	0.12	0.21	1.06	0.97	1.64	0.39	0.34	0.23	1.07	1.04
159	40.90	21.30	161.00	15.90	12.30	8.20	7.16	25.30	586.00	17.60	16.40	13.30	17.70	9.52
160	0.15	0.30	0.21	0.26	0.25	0.17	0.16	0.36	1.32	0.36	0.28	0.14	0.26	0.23
161	1.36	2.34	1.83	2.17	2.12	1.64	2.40	22.00	4.71	2.50	2.46	1.43	1.40	2.73
162	0.11	–	0.17	0.12	0.21	0.13	0.05	4.19	1.36	0.19	0.21	0.23	0.18	0.31

dx.doi.org/10.1021/cr100187n |*Chem. Rev.* 2011, 111, 3208–3235

Table 7. Continued

	DU-145	LN-caP	IGROV	IGROV ET	SK-BR 3	MEL-28	A-549	K-562	PANC-1	HT-29	LOVO	LOVO DOX	HELA	HELA APL
163	44.60	7.21	9.10	8.92	–	4.15	32.10	3.00	2.45	23.10	17.70	22.60	–	–
164	0.70	0.73	0.41	0.64	0.08	0.20	0.95	1.07	1.66	0.46	0.29	0.34	1.66	1.33
165	24.90	3.72	8.39	19.50	14.10	7.86	6.64	6.16	6.84	14.20	14.00	11.00	2.37	1.81

Table 8. Structure and Activity of KF

	amino acids	SAR	domain	SAR
1	L-Val	L configuration is important	A	ring is essential
2	(*Z*)-Dhb	double bond is crucial		L/D configuration of the α-carbon of the backbone that maintains the conformation of the ring is crucial
3	L-Phe	A hydrophobic residue can increase the activity		
4	D-Val	D configuration is important		
5	D-*allo*-Ile	D configuration is important		
6	D-*allo*-Thr	configurations of two chiral centers are very important		
7	D-*allo*-Ile	D configuration is important	B	configuration of the α-carbons of the amino acids is crucial; domain adopts or induces some sort of folding and/or interactions with other molecules
8	L-Orn	aliphatic groups coupled to the amino group of Orn can increase the activity		
9	D-Pro	D configuration and the ring are important		
10	D-Val	D configuration is important		
11	L-Val	L configuration is important		
12	L-Thr	L configuration is important, and aliphatic substitutions can increase the activity		
13	D-Val	D configuration is important		
14	5-MeHex	long aliphatic group is essential	C	domain does not interact selectively with any other molecule, and its main function is as an aliphatic buoy

binding partner for KF (**6**). KF (**6**) binds to phage-displayed RPS25 in a dose-dependent manner with a conservative dissociation constant of ~50 μM.[108]

8. CONCLUSION

In the 17 years following the discovery of the kahalalides, a large body of research (isolation, structure elucidation, synthesis, biological activities, clinical trials, and mechanisms of action) has been conducted. The mechanism of action of KF (**6**) is not completely understood, although significant advances have been made in recent years. It is likely that the PI3K/Akt signaling pathway coupled to ErbB3 receptors could be the target of KF (**6**) action. There is no report regarding ErbB3 inhibiting drugs in the clinic, so KF (**6**) would be a promising candidate for inhibiton of ErbB3 receptors in tumor cells.

KF (**6**) is currently under phase II clinical trials and isoKF (Irvalex) under phase I clinical trials. The preliminary results of the phase II clinical study of KF (**6**) in patients with advanced NSCLC, hepatocarcinoma (HC), and advanced malignant melanoma (AMM) revealed an excellent tolerability profile with no serious adverse events, a positive response, and stable disease occurring in a number of patients.

KF (**6**) is a splendid example of the important role marine natural product chemistry can play in the discovery of fundamental scientific and medical knowledge. The past 17 years of kahalalide research have challenged us and our colleagues in the field with an intricately complex puzzle.

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ACKNOWLEDGMENT

We thank Dr. Megan Huggett (Hawaii Institute of Marine Biology, Kane'ohe, HI) for the photograph used in Figure 2.

ABBREVIATIONS

(c/t)-MecHex-OH	(*cis*/*trans*)-4-methylcyclohexane-carboxylic acid
3,5-dFPhAc-OH	(3,5-difluorophenyl)acetic acid
4-GuBut-OH	4-guanidinobutyric acid
6,6-dFHep-OH	6,6-difluoroheptanoic acid
6-Ohep-OH	6-oxoheptanoic acid
Ala	alanine
Alloc	allyloxycarbonyl
AMM	advanced malignant melanoma
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Bip	2-amino-3-biphenyl-4-ylpropionic acid
Boc	*tert*-butyloxycarbonyl
Bza-OH	benzoic acid
cDNA	cDNA
CE	cremophor EL/ethanol
CEW	cremophor EL/ethanol/water
Cha	cyclohexylalanine or 2-amino-3-cyclohexyl-propionic acid
cHP	3-cyclohexylpropionic acid
CID	collision-induced dissociation
Cl-TrtCl-resin	2-chlorotrityl chloride resin
DEAC	(7-diethylamino)coumarin-3-carboxylic acid
DEHP	diethylhexyl phthalate
Dha	2-aminoacrylic acid
Dhb	α,*β*-didehydro-α-aminobutyric acid
DHB	2,5-dihydroxybenzoic acid
DIPCDI	*N*,*N*′-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMP	Dess−Martin periodinane
DMF	*N*,*N*-dimethylformamide
DSC	differential scanning calorimetry
EDC	1-ethyl-3-[3′-(dimethylamino)-propyl]carbodiimide
EDCI	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
Fmoc	9-fluorenylmethoxycarbonyl
GI_{50}	growth inhibition at 50%
Glu	glutamic acid
Gly	glycine
HATU	*N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methyl-methanaminium hexafluorophosphate *N*-oxide
HBTU	*N*-[(1*H*-benzotriazol-1-yl)-(dimethylamino)methylene]-*N*-methyl-methanaminium hexafluorophosphate *N*-oxide
hCh	homocyclohexylalanine or 2-amino-4-cyclohexy-l-butyric acid
Hep-OH	heptanoic acid
HOAc	acetic acid
HOAt	1-hydroxy-7-azabenzotriazole(3-hydroxy-3*H*-1,2,3-triazolo[4,5-*b*]pyridine)
HOBt	1-hydroxybenzotriazole
Ile	isoleucine
isoKF	4(*S*)-methylhexanoic kahalalide F
FDAA	1-fluoro-2,4-dinitrophenyl-5-L-alanine amide
KF	kahalalide F
HC	hepatocellular carcinoma
HSV II	herpes simplex II virus
Hyp	4-hydroxyproline
LC_{50}	lethal concentration at 50%
LcV	lymphocyte
Leu	leucine
Lit-OH	litocholic acid

Lys	lysine
MCF-7	Michigan Cancer Foundation-7 (a human breast adenocarcinoma cell line)
MeHex-OH	methylhexanoic acid
MeOH	methanol
MLR	mixed lymphocyte reaction
Mst-OH	myristic acid or tetradecanoic acid
MTD	maximal tolerated dose
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
NaI	2-amino-3-naphthalen-2-ylpropionic acid
NMM	*N*-methylmorpholine
NSCLC	non-small-cell lung cancer
Oct-OH	octanoic acid
Oic	octahydroisoindole-1-carboxylic acid
Orn	ornithine
p-CF3Bza-OH	4-trifluoromethylbenzoic acid
PARP	poly(ADP-ribose) polymerase
PEG	polyethylene glycol
Phe	phenylalanine
Pfh-OH	perfluoroheptanoic acid
Phg	aminophenylacetic acid
PI3K	phosphoinositide 3-kinases
Pip	pipecolic acid
Pipe-OH	benzo[1,3]dioxole-5-carboxylic acid
p-NZ	*p*-nitrobenzyloxycarbonyl
p-MeBza-OH	4-methylbenzoic acid
p-TflCinn-OH	3-(4-trifluoromethylbenzyl)acrylic acid
p-TflPhAc-OH	3-(4-trifluoromethylbenzyl)acetic acid
Pro	proline
PyAOP	7-azabenzotriazol-1-yl-*N*-oxy-tris-(pyrrolidino)phosphonium hexafluoro-phosphate
PyBOP	benzotriazol-1-yl-*N*-oxy-tris-(pyrrolidino)phosphonium hexafluoro-phosphate
RH	relative humidity
RPS	ribosomal proteins
SAR	structure−activity relationships
Ser	serine
t-Bu	*tert*-butyl
TCA	trichloroacetic acid
TCFU	tumor colony-forming unit
Tfa	trifluoroacetyl
TFA	trifluoroacetic acid
TFB	2,3,4,5-tetrafluorobenzoic acid
TFAA	trifluoroacetic anhydride
TGI	total growth inhibition
Thi	2-amino-3-thiophen-2-ylpropionic acid
Thr	threonine
Tic	1,2,3,4-tetraisoquinoline-3-carboxylic acid
Trp	tryptophan
Tyr	tyrosine
Und-OH	undecanoic acid
Val	valine

REFERENCES

(1) Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Discov.* **2009**, *8*, 69.
(2) Marris, E. *Nature* **2006**, *443*, 904.
(3) Harvey, A. L. *Drug Discov. Today* **2008**, *13*, 894.
(4) Newman, D. J. *J. Med. Chem.* **2008**, *51*, 2589.
(5) Lam, K. S. *Trends Microbiol.* **2007**, *15*, 279.
(6) Rishton, G. M. *Am. J. Cardiol.* **2008**, *101*, 43D.
(7) Baker, D. D.; Chu, M.; Oza, U.; Rajgarhia, V. *Nat. Prod. Rep.* **2007**, *24*, 1225.
(8) Ojima, I. *J. Med. Chem.* **2008**, *51*, 2587.
(9) Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Discov.* **2005**, *4*, 206.
(10) Harvey, A. L. *Curr. Opin. Chem. Biol.* **2007**, *11*, 480.
(11) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216.
(12) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2007**, *70*, 461.
(13) Haefner, B. *Drug Discovery Today* **2003**, *8*, 536.
(14) Hamann, M. T.; Scheuer, P. J. *J. Am. Chem. Soc.* **1993**, *115*, 5825.
(15) Hamann, M. T.; Otto, C. S.; Scheuer, P. J.; Dunbar, D. C. *J. Org. Chem.* **1996**, *61*, 6594.
(16) Goetz, G.; Nakao, Y.; Scheuer, P. J. *J. Nat. Prod.* **1997**, *60*, 562.
(17) Kan, Y.; Fujita, T.; Sakamoto, B.; Hokama, Y.; Nagai, H. *J. Nat. Prod.* **1999**, *62*, 1169.
(18) Horgen, F. D.; delos Santos, D. B.; Goetz, G.; Sakamoto, B.; Kan, Y.; Nagai, H.; Scheuer, P. J. *J. Nat. Prod.* **2000**, *63*, 152.
(19) Dmitrenok, A.; Iwashita, T.; Nakajima, T.; Sakamoto, B.; Namikoshi, M.; Nagai, H. *Tetrahedron* **2006**, *62*, 1301.
(20) Ashour, M.; Edrada, R.; Ebel, R.; Wray, V.; Wätjen, W.; Padmakumar, K.; Muller, W. E. G.; Lin, W. H.; Proksch, P. *J. Nat. Prod.* **2006**, *69*, 1547.
(21) Tilvi, S.; Naik, C. G. *J. Mass Spectrom.* **2007**, *42*, 70.
(22) Rao, K. V.; Na, M.; Cook, J. C.; Peng, J.; Matsumoto, R.; Hamann, M. T. *J. Nat. Prod.* **2008**, *71*, 772.
(23) Gao, J.; Caballero-George, C.; Wang, B.; Rao, K. V.; Shilabin, A. G.; Hamann, M. T. *J. Nat. Prod.* **2009**, *72*, 2172.
(24) Goetz, G.; Yoshida, W. Y.; Scheuer, P. J. *Tetrahedron* **1999**, *55*, 7739.
(25) Goetz, G.; Yoshida, W. Y.; Scheuer, P. J. *Tetrahedron* **1999**, *55*, 11957.
(26) Bonnard, I.; Manzanares, I.; Rinehart, K. L. *J. Nat. Prod.* **2003**, *66*, 1466.
(27) López-Macià, A.; Jimenez, J. C.; Royo, M.; Giralt, E.; Albericio, F. *J. Am. Chem. Soc.* **2001**, *123*, 11398.
(28) Hamann, M. T. *Curr. Opin. Mol. Ther.* **2004**, *6*, 657.
(29) Faircloth, G.; Cuevas, C. *Prog. Mol. Subcell. Biol.* **2006**, *43*, 363.
(30) Scheuer, P. J.; Hamann, M. T.; Gravalos, D. G. E.P. Patent 19940300780, 1994; *Chem. Abstr.* **1995**, *122*, 17167.
(31) Scheuer, P. J.; Hamann, M. T.; Gravalos, D. G. U.S. Patent 6011010, 2000; *Chem. Abstr.* **2000**, *132*, 59154.
(32) Scheuer, P. J.; Hamann, M. T.; Gravalos, D. G. U.S. Patent 6274551, 2001; *Chem. Abstr.* **2001**, *135*, 170762.
(33) Ruffles, G. K.; Faircloth, G. T.; Nuyen, B.; Weitman, S. WO Patent 2002036145, 2002; *Chem. Abstr.* **2002**, *136*, 359641.
(34) Jimeno, J.; López-Lázaro, L.; Ruiz-Casado, A.; Izquierdo, M. A.; Paz-Ares, L.; Trigo, J. M.; Schellens, J. . WO Patent 2003033012, 2003; *Chem. Abstr.* **2003**, *138*, 331673.
(35) Faircloth, G. T.; Elices, M.; Sasak, H.; Avilés Marin, P. M.; Cuevas Marchante, M. D. C. WO Patent 2004035613, 2004; *Chem. Abstr.* **2004**, *140*, 357671.
(36) Ruffles, G. K.; Faircloth, G. T.; Nuyen, B.; Weitman, S. U.S. Patent 20040067895, 2004; *Chem. Abstr.* **2002**, *136*, 359641.
(37) Izquierdo Delso, M. A. WO Patent 2004075910, 2004; *Chem. Abstr.* **2004**, *141*, 236712.
(38) Ruffles, G. K.; Faircloth, G. T.; Nuyen, B.; Weitman, S. EP Patent 1330258, 2005; *Chem. Abstr.* **2002**, *136*, 359641.
(39) Hill, R. T.; Hamann, M. T.; Enticknap, J.; Rao, K. V. WO Patent 2005042720, 2005; *Chem. Abstr.* **2005**, *142*, 444570.

(40) Jimeno, J.; Lopez-Lázaro, L.; Ruiz-Casado, A.; Izquierdo, M. A.; Paz-Ares, L.; Trigo, J. M.; Schellens, J.; Paz-Ares, L. U.S. Patent 20050054555, 2005; *Chem. Abstr.* **2003**, *138*, 331673.
(41) Tubby, D. G.; Francesch Solloso, A.; López Macia, Á.; Cuevas Marchante, C.; Albericio Palomera, F.; Acosta Crespo, G.; Cruz Ricondo, L. J.; Giralt, Iledo, E.; Gracia Cantador, C.; Isidro Lobet, A., Eds.; Pharma Mar, S. A. WO Patent 2005103072, 2005; *Chem. Abstr.* **2005**, *143*, 440762.
(42) Faircloth, G. T.; Elices, T.; Sasak, H.; Aviles Marin, P. M.; Cuevas Marchante, M. D. C. U.S. Patent 20060234920, 2006; *Chem. Abstr.* **2004**, *140*, 357671.
(43) Hill, R. T.; Enticknap, J.; Hamann, M. T.; Rao, K. V. U.S. Patent 20070196901, 2007; *Chem. Abstr.* **2005**, *142*, 444570.
(44) Izquierdo Delso, M. A. U.S. Patent 20070032412, 2007; *Chem. Abstr.* **2004**, *141*, 236712.
(45) Scheuer, P. J.; Hamann, M. T.; Gravalos, D. G. U.S. Patent RE39496, 2007; *Chem. Abstr.* **2001**, *135*, 170762.
(46) Tubby, D. G.; Francesch Solloso, A.; López Macia, Á.; Cuevas Marchante, C.; Albericio Palomera, F.; Acosta Crespo, G.; Cruz Ricondo, L. J.; Giralt, Iledo, E.; Gracia Cantador, C.; Isidro Lobet, A. U.S. Patent 20070207948, 2007; *Chem. Abstr.* **2005**, *143*, 440762.
(47) Faircloth, G. T.; del Carmen Cuevas Marchante, M. U.S. Patent 20080119397, 2008; *Chem. Abstr.* **2004**, *140*, 357671.
(48) Faircloth, G. T.; del Carmen Cuevas Marchante, M. U.S. Patent 20080318848, 2008; *Chem. Abstr.* **2004**, *140*, 357671.
(49) Delso, M. A. I. U.S. Patent 20080090757, 2008; *Chem. Abstr.* **2004**, *141*, 236712.
(50) Delso, M. A. I. U.S. Patent 7323444, 2008; *Chem. Abstr.* **2004**, *141*, 236712.
(51) Faircloth, G. T.; del Carmen Cuevas Marchante, M. U.S. Patent 7507708, 2009; *Chem. Abstr.* **2004**, *140*, 357671.
(52) Faircloth, G. T.; Nuijen, B.; Weitman, S. U.S. Patent 7473681, 2009; *Chem. Abstr.* **2002**, *136*, 359641.
(53) Hosta, L.; Pla, M.; Cruz, L. J.; Kogan, M.; Albericio, F. WO Patent 2009095480, 2009; *Chem. Abstr.* **2009**, *151*, 212475.
(54) Albericio, F.; Fernández, A.; Giralt, E.; Gracia, C.; López, P.; Varon, S.; Cuevas, C.; López, Á.; Francesch, A.; Jiménez, J. C.; Royo, M. U.S. Patent 20070117743, 2005; *Chem. Abstr.* **2005**, *142*, 298335.
(55) Albericio, F.; Fernández, A.; Giralt, E.; Gracia, C.; López, P.; Varon, S.; Cuevas, C.; López, Á.; Francesch, A.; Jiménez, J. C.; Royo, M. WO Patent 2005023846, 2005; *Chem. Abstr.* **2005**, *142*, 298335.
(56) Albericio, F.; Giralt, E.; Jimenez, J. C.; López, Á.; Manzanares, I.; Rodrigues, I.; Royo, M. WO Patent 2001058934, 2001; *Chem. Abstr.* **2001**, *135*, 167039.
(57) Albericio, F.; Giralt, E.; Jimenez, J. C.; López, Á.; Manzanares, I.; Rodrigues, I.; Royo, M. U.S. Patent 20040214755, 2004; *Chem. Abstr.* **2001**, *135*, 167039.
(58) Albericio, F.; Giralt, E.; Jimenez, J. C.; López, Á.; Manzanares, I.; Rodrigues, I.; Royo, M. EP Patent 1254162, 2007; *Chem. Abstr.* **2001**, *135*, 167039.
(59) Albericio, F.; Giralt, E.; Jimenez, J. C.; López, Á.; Manzanares, I.; Rodrigues, I.; Royo, M. U.S. Patent 20080318849, 2008; *Chem. Abstr.* **2001**, *135*, 167039.
(60) Albericio, F.; Giralt, E.; Jimenez, J. C.; López, Á.; Manzanares, I.; Rodrigues, I.; Royo, M. U.S. Patent 7482429 2009: *Chem. Abstr.* **2001**, *135*, 167039.
(61) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.
(62) Becerro, M. A.; Goetz, G.; Paul, V. J.; Scheuer, P. J. *J. Chem. Ecol.* **2001**, *27*, 2287.
(63) Rumpho, M. E.; Worful, J. M.; Lee, J.; Kannan, K.; Tyler, M. S.; Bhattacharya, D.; Moustafa, A.; Manhart, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17867.
(64) Rumpho, M. E.; Summer, E. J.; Manhart, J. R. *Plant Physiol.* **2000**, *123*, 29.
(65) Rumpho, M. E.; Summer, E. J.; Green, B. J.; Fox, T. C.; Manhart, J. R. *Zoology* **2001**, *104*, 303.
(66) Green, B. J.; Li, W. Y.; Manhart, J. R.; Fox, T. C.; Summer, E. J.; Kennedy, R. A.; Pierce, S. K.; Rumpho, M. E. *Plant Physiol.* **2000**, *124*, 331.
(67) Green, B. J.; Fox, T. C.; Manhart, J. R.; Rumpho, M. E. *Symbiosis* **2005**, *40*, 31.
(68) Cruz, L. J.; Luque-Ortega, J. R.; Rivas, L.; Albericio, F. *Mol. Pharmaceutics* **2009**, *6*, 813.
(69) Shilabin, A. G.; Kasanah, N.; Wedge, D. E.; Hamann, M. T. *J. Med. Chem.* **2007**, *50*, 4340.
(70) Jimenez, J. C.; López-Macià, A.; Gracia, C.; Varon, S.; Carrascal, M.; Caba, J. M.; Royo, M.; Francesc, A. M.; Cuevas, C.; Giralt, E.; Albericio, F. *J. Med. Chem.* **2008**, *51*, 4920.
(71) Córdoba, S.; Zapata, I.; Romero, J.; de la Torre, A.; López-Martin, J. A.; Vargas, J. A.; Sánchez-Prieto, R.; Corbacho, C.; Regueiro, C. A.; Magallón, R. *Eur. J. Cancer Suppl.* **2003**, *1*, S173.
(72) Stokvis, E.; Rosing, H.; López-Lázaro, L.; Rodriguez, I.; Jimeno, J. M.; Supko, J. G.; Schellens, J. H. M.; Beijnen, J. H. *J. Mass Spectrom.* **2002**, *37*, 992.
(73) Stokvis, E.; Rosing, H.; López-Lázaro, L.; Jimeno, J. M.; Supko, J. G.; Schellens, J. H. M.; Beijnen, J. H. *Br. J. Clin. Pharmacol.* **2002**, *53*, 543.
(74) Nuijen, B.; Bouma, M.; Floriano, P.; Manada, C.; Rosing, H.; Stokvis, E.; Kettenes-van den Bosch, J. J.; Bult, A.; Beijnen, J. H. *J. Liq. Chromatogr. Relat. Technol.* **2001**, *24*, 3141.
(75) Sparidans, R. W.; Stokvis, E.; Jimeno, J. M.; López-Lázaro, L.; Schellens, J. H. M.; Beijnen, J. H. *Anticancer Drugs* **2001**, *12*, 575.
(76) Nuijen, B.; Bouma, M.; Talsma, H.; Manada, C.; Jimeno, J. M.; López-Lázaro, L.; Bult, A.; Beijnen, J. H. *Drug Dev. Ind. Phar.* **2001**, *27*, 767.
(77) Nuijen, B.; Bouma, M.; Manada, C.; Jimeno, J. M.; Lazaro, L. L.; Bult, A.; Beijnen, J. H. *Invest. New Drugs* **2001**, *19*, 273.
(78) Nuijen, B.; Bouma, M.; Manada, C.; Jimeno, J. M.; López-Lázaro, L.; Bult, A.; Schellens, J. H. M.; Beijnen, J. H. *Clin. Cancer Res.* **2000**, *6*, 4556s.
(79) Nuijen, B.; Bouma, M.; Manada, C.; Jimeno, J. M.; Bult, A.; Beijnen, J. H. *PDA J. Pharm. Sci. Technol.* **2001**, *55*, 223.
(80) Luber-Narod, J.; Smith, B.; Grant, W.; Jimeno, J. M.; López-Lázaro, L.; Scotto, K.; Shtil, A.; Faircloth, G. T. *Clin. Cancer Res.* **2000**, *6*, 4510s.
(81) Faircloth, G. T.; Grant, W.; Smith, B.; Supko, J.; Brown, A.; Geldof, A.; Jimeno, J. *Proc. Am. Assoc. Cancer Res.* **2000**, *41*, 3823.
(82) Supko, J. G.; Lu, H.; Jimeno, J. M.; Grant, W.; Faircloth, G. T. *Clin. Cancer Res.* **1999**, *5*, 3792s.
(83) Rademaker-Lakhai, J. M.; Horenblas, S.; Meinhardt, W.; Stokvis, E.; de Reijke, T. M.; Jimeno, J. M.; López-Lázaro, L.; Martin, J. A. L.; Beijnen, J. H.; Schellens, J. H. M. *Clin. Cancer Res.* **2005**, *11*, 1854.
(84) Beijnen, J. H.; Rademaker-Lakhai, J. M.; Horenblas, S.; Meinhardt, W.; Stokvis, E.; de Reijke, T. M.; Jimeno, J. M.; Lazaro, L. L.; Martin, J. A. L.; Schellens, J. *J. Clin. Oncol. Suppl.* **2004**, *22*, 2076.
(85) Ciruelos, E.; Trigo, J. M.; Pardo, B.; Paz-Ares, L.; Estaun, N.; Cuadra, C.; Domínguez, M. J.; Marin, A.; Ruiz, A.; Jimeno, J.; Izquierdo, M. A. *Eur. J. Cancer Suppl.* **2002**, *38*, 33.
(86) Pardo, B.; Paz-Ares, L.; Tabernero, J.; Ciruelos, E.; Garcia, M.; Salazar, R.; López, Á.; Blanco, M.; Nieto, A.; Jimeno, J.; Izquierdo, M. A.; Trigo, J. M. *Clin. Cancer Res.* **2008**, *14*, 1116.
(87) Paz-Ares, L.; Rubio, J.; Espinosa, E.; López-López, J. J.; Alonso, L.; Manzano, J. L.; Plazaola, A.; Nieto, A.; de las Heras, B.; Algarra, S. M. *Ann. Oncol.* **2006**, *17* (Suppl. 9), ix165.
(88) Martin-Algarra, S.; Espinosa, E.; Rubio, J.; López-López, J. J.; Manzano, J. L.; Carrion, L. A.; Plazaola, A.; Tanovic, A.; Paz-Ares, L. *Eur. J. Cancer* **2009**, *45*, 732.
(89) Jimeno, J.; Faricloth, G.; Fernandez Sousa-Faro, J. M.; Scheuer, P.; Rinehart, K. *Mar. Drugs* **2004**, *2*, 14.
(90) Provencio, M.; Izquierdo, A.; Viñolas, N.; Paz-Ares, L.; Feliu, J.; Constenla, M.; Heras, B. a. d. l.; Erustes, M.; Izquierdo, M. A.; Rosell, R. *Ann. Oncol. Suppl.* **2006**, *17*, 225.
(91) Galan, C.; Casado, E.; Ramos, F. J.; Vega-Villegas, E.; Rivera, F.; López, Á.; de las Heras, B.; Erustes, M.; Soto-Matos, A.; Giron, C. G. *Ann. Oncol. Suppl.* **2006**, *17*, 318.
(92) Provencio, M.; Sanchez, A.; Gasent, J.; Gomez, P.; Rosell, R. *Clin. Lung Cancer* **2009**, *10*, 295.

(93) Ling, Y. H.; Aracil, M.; Jimeno, J.; Perez-Soler, R.; Zou, Y. Y. *Eur. J. Cancer* **2009**, *45*, 1855.
(94) Yin, J. M.; Aviles, P.; Lee, W.; Ly, C.; Guillen, M. J.; Munt, S.; Cuevas, C.; Faircloth, G. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2735.
(95) López, P. E.; Isidro-Llobet, A.; Gracia, C.; Cruz, L. J.; Garcia-Granados, A.; Parra, A.; Alvarez, M.; Albericio, F. *Tetrahedron Lett.* **2005**, *46*, 7737.
(96) López-Macià, A.; Jimenez, J. C.; Royo, M.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **2000**, *41*, 9765.
(97) Bourel-Bonnet, L.; Rao, K. V.; Hamann, M. T.; Ganesan, A. *J. Med. Chem.* **2005**, *48*, 1330.
(98) Gracia, C.; Isidro-Llobet, A.; Cruz, L. J.; Acosta, G. A.; Alvarez, M.; Cuevas, C.; Giralt, E.; Albericio, F. *J. Org. Chem.* **2006**, *71*, 7196.
(99) Garcia-Fernandez, L. F.; Reyes, F.; Sanchez-Puelles, J. M. *Pharm. News* **2002**, *9*, 495.
(100) Córdoba, S.; Zapata, I.; Romero, J.; Torre, A. d. l.; López-Martin, J. A.; Vargas, J. A.; Sánchez-Prieto, R.; Corbacho, C.; Regueiro, C. A.; Magallón, R. *Eur. J. Cancer Suppl.* **2003**, *1*, S173.
(101) Garcia Rocha, M.; Bonay, P.; Avila, J. *Cancer Lett.* **1996**, *99*, 43.
(102) Hosta, L.; Pla-Roca, M.; Arbiol, J.; López-Iglesias, C.; Samitier, J.; Cruz, L. J.; Kogan, M. J.; Albericio, F. *Bioconjugate Chem.* **2009**, *20*, 138.
(103) Suarez, Y.; Gonzalez, L.; Cuadrado, A.; Berciano, M.; Lafarga, M.; Munoz, A. *Mol. Cancer Ther.* **2003**, *2*, 863.
(104) Faircloth, G. T.; Smith, B.; Grant, W.; Jimeno, J. M.; Garcia-Gravalos, L.; Scotto, K.; Shtil, A. *Proc. Am. Assoc. Cancer Res.* **2001**, *42*, 213.
(105) Sewell, J. M.; Mayer, I.; Langdon, S. P.; Smyth, J. F.; Jodness, D. I.; Guichard, S. M. *Eur. J. Cancer* **2005**, *41*, 1637.
(106) Janmaat, M. L.; Kruyt, F. A.; Jimeno, J. M.; Rodriguez, J. A.; Giaccone, G. *Proc. Am. Assoc. Cancer Res.* **2004**, *45*, 5328.
(107) Janmaat, M. L.; Rodriguez, J. A.; Jimeno, J.; Kruyt, F. A. E.; Giaccone, G. *Mol. Pharmacol.* **2005**, *68*, 502.
(108) Piggott, A. M.; Karuso, P. *ChemBioChem* **2008**, *9*, 524.
(109) Salazar, R.; Casado, E.; Martin, A. L.; Pardo, B.; Roca, J.; Rodriguez, R.; Garcia, M.; Tabernero, J.; de las Heras, B.; Paz-Ares, L. *J. Clin. Oncol. Suppl.* **2005**, *23*, 2059.
(110) Gomez, S. G.; Bueren, J. A.; Faircloth, G. T.; Jimeno, J.; Albella, B. *Exp. Hematol.* **2003**, *31*, 1104.
(111) Stokvis, E.; Rosing, H.; López-Lázaro, L.; Schellens, J. H. M.; Beijnen, J. H. *Biomed. Chromatogr.* **2004**, *18*, 400.
(112) Rademaker-Lakhai, J. M.; Horenblas, S.; Meinhardt, W.; Stokvis, E.; de Reijke, T. M.; Jimeno, J. M.; López-Lázaro, J. J.; Martin, J. A. L.; Beijnen, J. H.; Schellens, J. H. M. *Br. J. Clin. Pharmacol.* **2003**, *56*, 469.
(113) Brown, A. P.; Morrissey, R. L.; Faircloth, G. T.; Levine, B. S. *Cancer Chemother. Pharmacol.* **2002**, *50*, 333.
(114) Herrero, A. B.; Astudillo, A. M.; Balboa, M. A.; Cuevas, C.; Balsinde, J.; Moreno, S. *Cancer Res.* **2008**, *68*, 9779.
(115) Sewell, J. M.; Mayer, I.; Langdon, S. P.; Smyth, J. F.; Jodness, D. I.; Guichard, S. M. *Eur. J. Cancer* **2005**, *41*, 1637.