Kahalalide F and ES285: Potent Anticancer Agents from Marine Molluscs

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Abstract. The marine environment is proving to be a very rich source of unique compounds with significant activities against cancer of several types. Finding the sources of these new chemical entities has made it necessary for marine and medical scientists to find enterprising ways to collaborate in order to sample the great variety of intertidal, shallow and deep-water sea life. Recently these efforts resulted in a first generation of drugs from the sea undergoing clinical trials. These include PharmaMar compounds: Yondelis, Aplidin, kahalalide F, ES285 and Zalypsis. Two of these compounds, kahalalide F and ES285, have been isolated from the Indopacific mollusc *Elysia rufescens* and the North Atlantic mollusc *Spisula polynyma*, respectively.

16.1 Introduction

Potent cytotoxic agents have a well-established role in the treatment of cancer. Many of the anticancer agents currently in use are of natural origin (i.e. vinca alkaloids, taxanes, anthracyclines), derived from terrestrial plants and microorganisms, or are natural product derivatives. The relevance of the sea as a source to discover novel anticancer compounds was validated by the discovery, development and marketing approval of 1-beta-D-arabinofuranosylcytosine (ARA-C). The available results clearly anticipated the potential of the marine ecosystem in cancer therapy. This chapter describes the progress made and the perspectives in clinical development of two innovative marine anticancer compounds: kahalalide F and ES285. The mechanistic data generated in parallel with the clinical program confirms the potential of the marine ecosystem in the discovery of new agents acting against new relevant cellular targets in cancer cell biology.

16.2 Kahalalide F

Kahalalide F is a partially cyclic depsipeptide with the unusual feature of having a short-chain fatty acid amide at its amino terminal residue

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(Fig. 16.1; Hamann and Scheuer 1993; López-Maciá et al. 2001). A number of other natural kahalalides (A, B, G) have also been described (Hamann et al. 1996); and many of them show activity against cancer and AIDS-related opportunistic infections. These compounds were discovered in the sea mollusc *Elysia rufescens* (a marine gastropod of the subclass Opisthobranchia) collected at Kahala Bay in Honolulu (Hawaii). Several species of animals in this class are known to acquire, process and accumulate chemicals produced by the algae on which they feed. Surprisingly, some species are able to retain chloroplasts from these algae that remain photosynthetically active within the animal for prolonged periods of time (Green et al. 2000). In the case of kahalalide F, the compound is found in the algae (*Bryopsis pennata*) on which the *Elysia* molluscs feed, albeit in a much reduced concentration (Becerro et al. 2001). In this natural setting, a biological role has been proposed for kahalalides as a deterrent to the feeding behaviour of predators of *Elysia* nudibranchs.

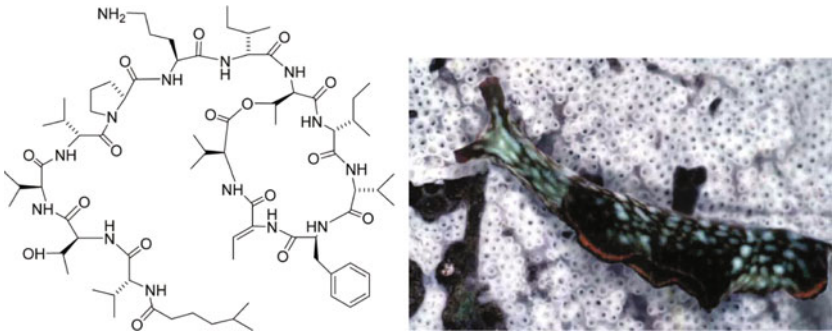


Fig. 16.1. Structure and source organism of kahalalide F

16.2.1 Mechanism of Action

The primary mechanism of kahalalide F action has not been identified; however, early experimental results have provided some insight into the physiological events that correlate with tumour cell killing by this compound. In the NCI COMPARE analysis, kahalalide F did not exhibit significant correlations to any other standard chemotherapeutic agent, suggesting that the compound may possess a unique way of achieving its biological effectiveness. In these in vitro studies, selectivity was noted to colon, central nervous system (CNS), melanoma, prostate and breast tumour cell lines, where PC-3 and DU-145 prostate tumour cell lines

happened to be most sensitive with LC_{50} concentrations below 1 μM (NCI data).

Neither protein nor nucleic acid syntheses have been found to be inhibited in cultured cells by sublethal concentrations of kahalalide F. Topoisomerase enzymatic activities (I or II) are also not affected; and no damage to DNA has been specifically correlated with exposure to kahalalide F. A cell cycle block in G0–G1 has been identified in a variety of tumour cell lines that include prostate (DU-145), cervical (HeLa), colon (HT-29), head and neck (HN30) and non-small cell lung carcinoma (NSCLC; HOP62) all with IC_{50} values in the 1 μM range (Córdoba et al. 2003).

Kahalalide F is strongly cytotoxic to both wild-type p53 and mutated p53 tumour cells in the NCI panel. A number of cell lines overexpressing multi-drug resistance proteins (e.g. PC-3 prostate, CACO-2 colon, UO-31 renal, MCF7 breast) as well as cell lines resistant to topoisomerase II inhibitors are sensitive to kahalalide F. This suggests that the compound may act independently of the respective resistance mechanisms.

Cultured cells exposed to biologically relevant concentrations of kahalalide F detach from their substrata and become markedly swollen. This is associated with the formation of large intracellular vacuoles (García-Rocha et al. 1996). Within minutes, these engorged vesicles move from the periphery of the cell to a perinuclear location, as observed by confocal laser scanning microscopy. Confocal fluorescence microscopy studies using a fluorescent acidophilic probe (LysoTracker Green), specific to the inner organelle membrane and confirmed by antibodies to the lysosomal-located enzyme cathepsin D (Fig. 16.2), have identified major and immediate effects on lysosomes. Moreover, there is an increase in lysosomal pH. However, cytoskeletal structures and in particular the microtubule network appear intact; and the morphologies of the endoplasmic reticulum and Golgi apparatus appear to be unaffected by the action of kahalalide F. Thus, it appears that its actions are mostly of a lysosomal nature.

It has been suggested that the subcellular effect might be explained if kahalalide F is inserted as an ionophore in membranes favouring an increase in cation permeability, thus causing a passive water influx and resulting in cisternal dilation. The hydrophobic nature of the compound would not be incompatible with a model in which membrane-associated events trigger cell death. These effects would be similar to those of compounds like the carboxylic ionophore, monensin (Tartakoff 1983).

It has also been suggested that the compound (NCI655128) blocks the EGF receptor and inhibits TGF-β gene expression, receptors that initiate an important signal transduction pathway for proliferation (Wosikowski et al. 1997). These growth factors control the tyrosine kinase class I subfamily that normally mediates signal transduction in the signalling pathway mediated by the '"ras" oncogene. This is overexpressed in many

cancer types, especially some breast and ovarian tumour cells. In vitro studies have shown that kahalalide F is selectively cytotoxic to neu^{+} cells overexpressing Her2, suggesting it may interfere with ErbB2 transmembrane tyrosine kinase activity, a key effector mediating this intracellular pathway. However, it does not inhibit autophosphorylation of the receptors or MEK kinase activity (unpublished data). More recently, kahalalide F has been shown to cause rapid and potent cytotoxic effects in the ErbB2 (HER2/neu) overexpressing breast cancer cell lines (i.e. SKBR3, BT474). This was associated with induction of a hypodiploid cell population, dramatic cell swelling and permeabilization of the plasma and lysosomal membranes (Suárez et al. 2003).

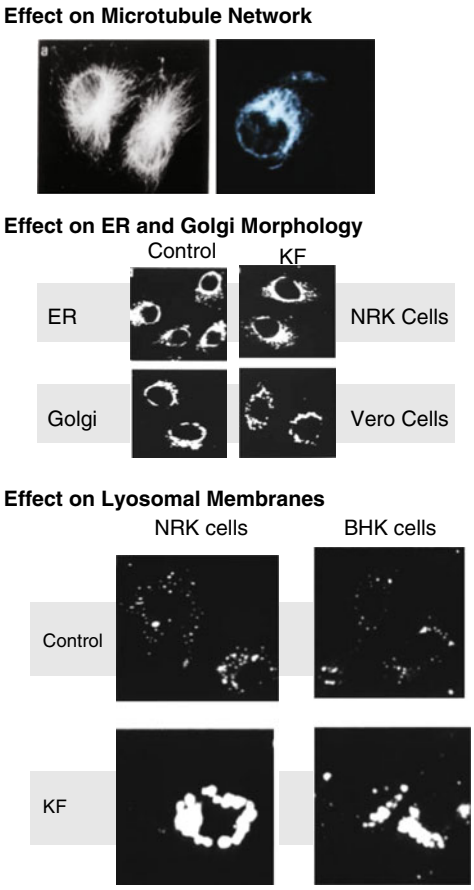


Fig 16.2. Effect on the micotuble network and membrane-bound organelles. (**a**) Effect on Microtubule Network. Typsinized COS-1 cells incubated in the absence or presence of 2μM KF. A reduction of cytoskeletal space is seen as well as the appearance of relatively intact microtubule network. (**b**) Effect on ER and Golgi Morphology Control and treated cells with 2μM Kf. Stained fo ER with anti-PDI (protein disulfide isomerase) and for Golgi apparatus with anti-Golgi alpha mannosidase and showing no difference in organelle morphologies. (**c**) Effect of Lyosomal Membranes Contol and treated cells with 2μM KF. Labeled with the fluorescent acidophilic probe LysoTracker Green and observed unde the laser scanning microscope showing an increase in lysosomal volume as a consequence of kahalalide F treated cells.

Several markers of caspase-dependent apoptosis were negative after kahalalide F exposure, including the externalization of phosphatidyl serine, 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 kahalalide F cytotoxicity. Specific inhibitors of cathepsin B (CA-074 Me, zFA-fmk) or D (pepstatin A) also failed to protect against cell death induced by kahalalide F. Taken together, these results suggest that kahalalide F induced cytotoxicity is predominantly due to a process of necrotic cell death involving oncosis rather than apoptosis. This effect has also been reported in hepatoma cell lines (Sewell et al. 2004).

The sensitivity to kahalalide F in a panel of human tumour cell lines derived from breast (SKBR3, BT474, MCF7), vulval (A431), NSCLC (H460, A549, SW1573, H292) and hepatic carcinoma (SKHep1, HepG2, Hep3B) significantly correlated with protein expression levels of ErbB3 (HER3) but not other ErbB receptors. Exposure to kahalalide F for 4 h induced down-regulation of ErbB3 expression in sensitive cell lines, as well as inhibition of the PI3K-Akt/PKB signalling pathway, which is directly linked to ErbB3 (Janmaat et al. 2004). Moreover, ectopic expression of a constitutively active Akt mutant had a protective effect against kahalalide F cytotoxicity. This suggests ErbB3 and the Akt pathway are major determinants of kahalalide F action on these cell lines.

16.2.2 Preclinical Pharmacology

Early preclinical data identified kahalalide F as a potent new chemical entity showing significant cytotoxic activity below 10 μM (IC_{50}) against solid tumour cell lines. Further evaluation demonstrated that this activity was selective for, but not restricted to, prostate tumour cells. Subsequent studies have identified tumour cells that overexpress the Her2/neu and Her3/neu oncogenes as potentially sensitive targets for kahalalide F. Moreover, kahalalide F cytotoxicity was not schedule-dependent (unpublished data) and it was not a strong multi-drug resistance substrate, as it was effective against many multi-drug resistant tumour cell lines (NCI data).

Preliminary in vitro screening studies identified micromolar activity of kahalalide F against mouse leukaemia (P388) and two human solid tumours: non-small cell lung (A549) and colon (HT-29). Gastric (HS746T, 0.01 μM) and prostate (PC-3, 0.08 μM) tumours were shown to be very sensitive as well. In vitro studies in cell lines of human origin evaluated by the NCI confirmed these results and identified selective activity against colon, NSCLC, melanoma, prostate and breast cancer cells, with potencies

ranging from 200 nM (prostate) to 10 μM (leukaemia). Extended in vitro selectivity studies reveals that kahalalide F was active against neu^{+} (Her2-overexpressing) human breast tumour cells (Suárez et al. 2003), some primary sarcoma lines, but not hormone-sensitive LNCAP tumour cells (Table 16.1).

Table 16.1. In vitro cytotoxic activity of kahalalide F against solid tumours

tumour	line	IC_{50} (Molar)
chondrosarcoma	CHSA	1.58 μM
osteosarcoma	OSA-FH	1.65 μM
prostate	PC-3	1.02 μM
prostate	DU-145	1.78 μM
prostate	LNCAP	not active
breast	SK-BR-3	2.50 μM
breast	BT-474	2.00 μM

Kahalalide F has also been evaluated in a human tumour colony-forming unit (TCFU) assay from surgically derived tumours (Table 16.2; Córdoba et al. 2003). In particular, complete inhibition against breast, colon, kidney, NSCLC, ovary, prostate, stomach and uterine tumour specimens. To date, prostate and stomach tumour specimens are the most sensitive, with preliminary IC_{50} activities of less than 10 nM in a limited number of specimens that have been tested.

Table 16.2. In vitro activity of kahalalide F in the TCFU assay: long-term exposure

primary tumour	10 nM	100 nM	1.0 μM
breast	0/10	1/10	9/10
colon	3/6	2/6	5/6
head and neck	0/1	0/1	0/1
kidney	0/4	1/4	2/4
NSCL	1/8	1/8	7/8
melanoma	1/2	0/2	1/2
neuroblastoma	0/1	0/1	1/1
ovary	2/10	2/10	9/10
peritoneum	0/1	0/1	1/1
prostate	1/1	1/1	1/1
stomach	1/1	1/1	1/1
unknown primary	0/1	0/1	1/1
uterus	0/1	0/1	1/1
	9/47(19%)	9/47 (19%)	39/47(83%)

[a] Less than 50% survival of TCFU's

In vivo antitumour activity was observed against human breast, colon, prostate and lung tumour cells xenografted into athymic mice. Interestingly, chemotherapy-resistant DU-145 (hormone refractory prostate) tumours, initially responded to kahalalide F at the maximum tolerated dose (MTD) and half MTD levels in the first cycle. One week after the first cycle was completed, a second cycle was started and tumour growth was inhibited further (Table 16.3). In similar fashion, studies with PC-3 xenografted tumours confirmed this tumour growth inhibition (Table 16.4).

Table 16.3. In vivo antitumour activity of kahalalide F to DU-145 human prostate tumours

group	injection dose ($\mu g\ kg^{-1}$)	regimen	cycle 1 volume (mm^3)	day 8 %T/C	cycle 2 volume (mm^3)	day 26 %T/C
vehicle	–	Q2D × 5, iv	598	100	1,398	100
MTD	490	Q2D × 5, iv	240	40	704	50
1/2 MTD	245	Q2D × 5, iv	347	58	697	50
1/4 MTD	123	Q2D × 5, iv	633	106	1,510	108

Table 16.4. In vivo antitumour activity of kahalalide F to PC-3 human prostate tumours

group	injection dose ($\mu g\ kg^{-1}$)	regimen	cycle 1 volume (mm^3)	day 10 %T/C	cycle 2 volume (mm^3)	day 35 %T/C
Vehicle	–	Q2D × 5, iv	734	–	3,512	–
MTD 4	490	Q2D × 5, iv	229	32	2,210	63
1/2 MTD	245	Q2D × 5, iv	489	67	2,466	70
1/4 MTD	123	Q2D × 5, iv	372	51	2,294	65

16.2.3 Preclinical Toxicology

The pharmacokinetic (PK) behaviour of kahalalide F was characterized in mice and confirmed in rats. In conjunction with in vitro and in vivo antitumour activity studies, a pattern of systemic exposure to the drug

associated with efficacious dosing regiments was developed (Nuijen et al. 2001; Brown et al. 2002; Gómez et al. 2003).

– A dose of 278 μg kg $^{-1}$ given as a rapid intravenous (i.v.) bolus injection afforded an initial plasma concentration of 1.55 μM. The plasma concentration time profile was distinctly bi-exponential with half-lives of 15.8 min and 4.4 h for the initial and terminal disposition phases, respectively.

– The apparent volumes of distribution of the drug were very large, more than 100 times body weight, suggesting that the compound distributed extensively into peripheral tissues. The total body clearance, 14.5 ml min $^{-1}$ kg^{-1}, was only 23% of hepatic blood flow.

– The C_{max} of the MTD i.v. dose was comparable to the in vitro IC_{50} values for the most sensitive human tumour cell lines.

No drug was found in mouse plasma 24 h after i.v. injection. There was no accumulation upon repeated i.v. injection at an interval of 24 h. Surprisingly, a slightly greater than MTD was too toxic when given as an i.v. single bolus. Daily serial i.v. injections of the MTD dose were not toxic. This preliminary data suggested that kahalalide F was rapidly eliminated from plasma with limited binding to extra vascular tissues. In addition, increased tolerance to repeat dosing without acute cumulative adverse effects may be favourable to clinical development of the drug, if confirmed in the human setting.

In rodents, a single 250 μg kg^{-1} dose bolus injection of kahalalide F was established as the MTD, although there were gender differences. The dose-limiting toxicity was predominantly renal, albeit reversibility was reached by the final necropsy day (day 29). Furthermore, minimal histologic evidence of nephrotoxicity was apparent at the half MTD. Signs of liver function alteration were seen on day 4 at the MTD dose, with recovery by day 29 necropsy. If the MTD was exceeded, there were lethalities with signs of neurotoxicity.

In fractionated dose studies in beagle dogs, slight non-regenerative anaemia was seen in males and females at all dose levels. The effect was typically most severe on day 8, with general recovery by days 15 and 22. No other haematological changes were observed. No correlative histologic changes, i.e. in the bone marrow, spleen or liver, were seen. No other indices of toxicity were seen and drug-related clinical signs or histologic lesions were not apparent.

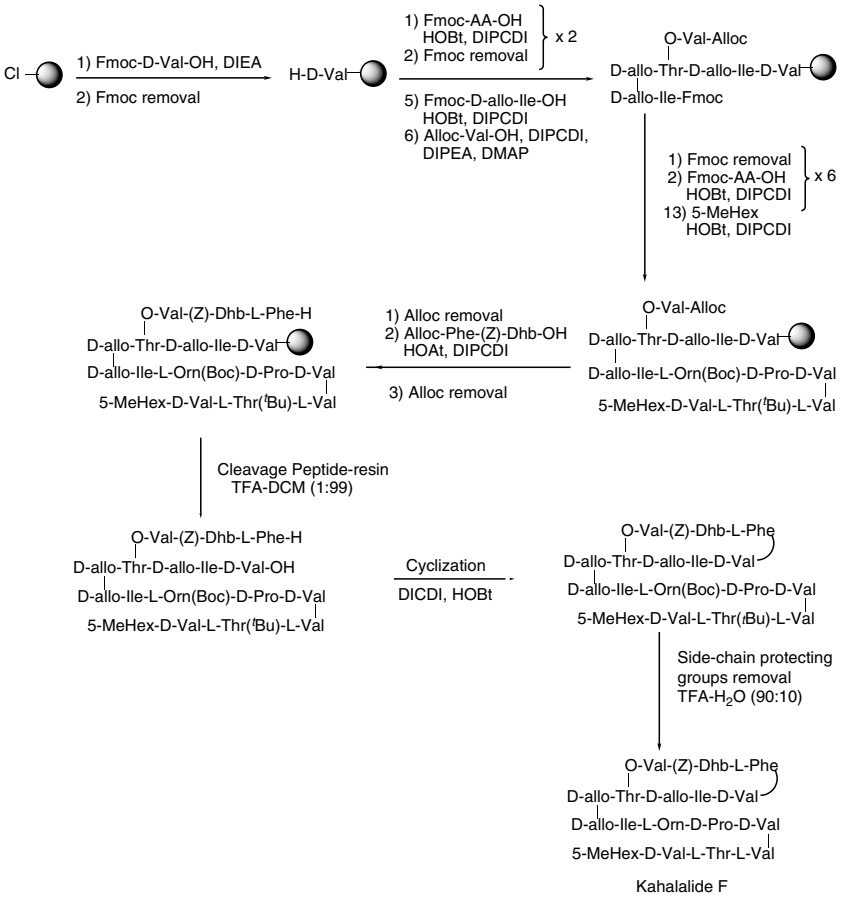
16.2.4 Synthesis of Kahalalide F

Thus far, only one synthesis of kahalalide F has been published, as described by López-Maciá et al. (2001). They used a solid-phase strategy to obtain kahalalide F in a multi-gram scale. The methodology involves

elongation of the synthetic chain in 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 used is easy to scale up and could be applied to generate a wide variety of new analogues.

After synthesis of the linear peptide, the Fmoc/tBu strategy and 2-chlorotrityl chloride resin allowed cleavage of the peptide under mild acid conditions. Next, aminoacids *d-allo*-Thr and the Thr precursor of the *Z*-Dhb were both introduced without protection of the hydroxyl function. For the formation of all the amide bonds, HATU/DIEA was used.

Before deprotecting the peptide from the resin, the alloc group was removed under standard conditions. The cyclization reaction was then performed with PyBOP/DIEA using DMF as a solvent. Finally, the deprotection of the Boc group afforded the natural compound (Scheme 16.1).



Scheme 16.1. Synthesis of kahalalide F

16.2.5 Clinical Trials

Results from a dose-escalating Phase I study in patients with advanced androgen-resistant prostate cancer were presented at the 2002 Annual Meeting of the American Society for Clinical Oncology (Schellens et al. 2002). As predicted, kahalalide F was found to have rapid plasma clearance in this study. Moreover, the dose of kahalalide F could be safely escalated up to 930 μg m^{-2} day^{-1}. Kahalalide F also demonstrated a favourable safety profile and treatment-related side-effects were non-cumulative and rapidly reversible.

Data from a Phase I study in patients with advanced solid tumours that had failed to respond to previous chemotherapy was presented at the 2002 EORTC-NCI-AACR annual meeting (Ciruelos et al. 2002). In this study, kahalalide F was administered as a weekly 1-h i.v. infusion and the dose could be escalated up to 1,200 μg m^{-2} $week^{-1}$. Signs of activity in a variety of cancers were observed at 400–1,200 μg m^{-2} $week^{-1}$. Overall, this data suggested a favourable safety profile for kahalalide F, with no reports from bone marrow or renal toxicities, mucositis, alopecia or general cumulative toxicity.

16.3 ES285

ES285 (Fig. 16.3) is a marine compound found in the mollusc *Mactromeris* (formerly *Spisula polynyma*) by Rinehart et al. (1998). ES285·HCl consists of a linear 18-carbon chain bearing amine and alcohol groups at positions 2 and 3, respectively. Each chiral centre is a single configuration (2*S*,3*R*). Drug substance is synthesized as the hydrochloride salt from commercially available raw materials. The molecular formula of the synthesized material is $C_{18}H_{39}NO{\cdot}HCl$, with the molecular weight 321.97. ES285 shows antitumour selectivity for certain slow-growing solid tumours, such as those of the liver, prostate and kidney, and it is currently in Phase I clinical trials in Europe.

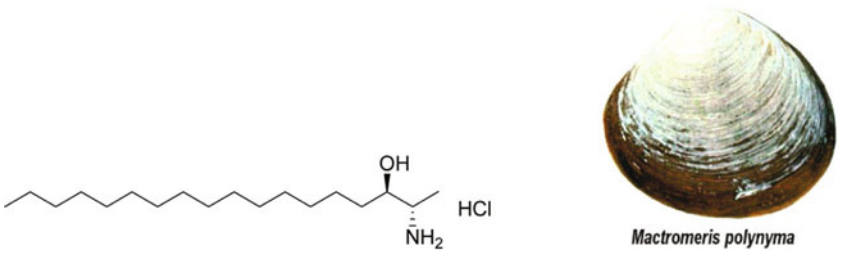


Fig. 16.3. Structure and source organism of ES285

16.3.1 Mechanism of Action

The mechanism of the action of ES285 is under investigation. Available data suggests that the antitumour activity of ES285 may be associated with disruption of the cytoskeleton in cancer cells. The in vitro cytotoxicity of ES285 is schedule-dependent from 1 to 24 h at 100 nM, 1 μM and 10 μM. Cell cycle analysis shows a delayed G2/M transition and an accumulation of cells in G1 after variable drug-washout experiments, regardless of pre-treatment duration of exposure (1 or 24 h; Salcedo et al. 2003).

Cultured tumour cells change their morphology in the presence of ES285, acquiring first a fusiform shape and later becoming rounded without focal adhesions (Fig. 16.4; Cuadros et al. 2000). The transition to bipolar, spindle-shaped cells is also associated with apoptosis that eventually leads to immediate cell death in most, but not all, cells. The selective induction of apoptosis in tumour cells is an area of active study for this compound.

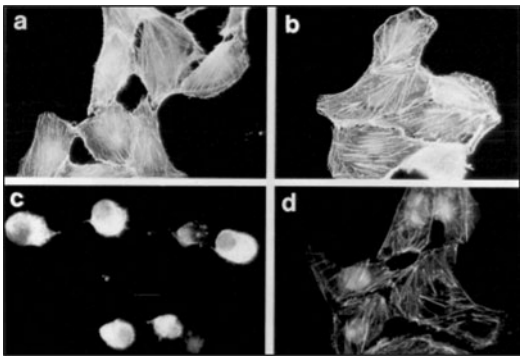


Fig 16.4. Change in cell morphology in the presence of ES285. a, appearance of normal stress fibers; **b**, increase in stress fibers from LPA; **c**, rounding of cells by ES-285; **d**, reduction of LPA-included stress fibers by ES-285.

Microscopic analysis of the cytoskeleton of treated cells indicates that there is an absence of actin stress fibres that are typically regulated by Rho, a small GTP-binding protein. Moreover, stimulation of Rho by lysophosphatidic acid (LPA) is blocked by ES285 (Fig. 16.4). These indirect findings led us to speculate that Rho may be a tentative target for ES285. In contrast, studies from other investigators using both overexpression and siRNA-mediated knockout of RhoA do not support a direct RhoA function or that of signalling pathways under the control of RhoA (Lacal et al. 2004).

ES285 may act through G protein-coupled endothelium differentiation gene (EDG) receptors, considering its structural homology with bioactive lipids such as sphingosine-1-phosphate. In particular, ES285 could utilize EDG receptors coupled to several G proteins and thus activate RhoA

through a signalling pathway originated from one of the former proteins. Recently, Salcedo (2005) showed that ES285 treatment effects were due to interaction with EDG receptors. However, this data also showed that these receptors were not essential for ES285-induced cell death.

ES285 produces cell vacuolation that precedes apoptosis. The resulting multi-nucleated, dividing cells are unable to separate (note G2/M arrest preceding apoptosis). Some vacuolated cells undergo blebbing and die quickly, while other cells take longer for this to occur. Further details are beginning to emerge of the molecular targets involved in the early and late events induced by ES285. Using either HeLa or Jurkat cell lines, ES285 has a specific effect on cell cycle distribution (Fig. 16.5). Following prolonged exposure, however, cells in early G1 become progressively more sensitive to the drug and, within 24 h, sub-G1 cells represent 70–80% of all apoptotic cells when exposed to 10 μM ES285.

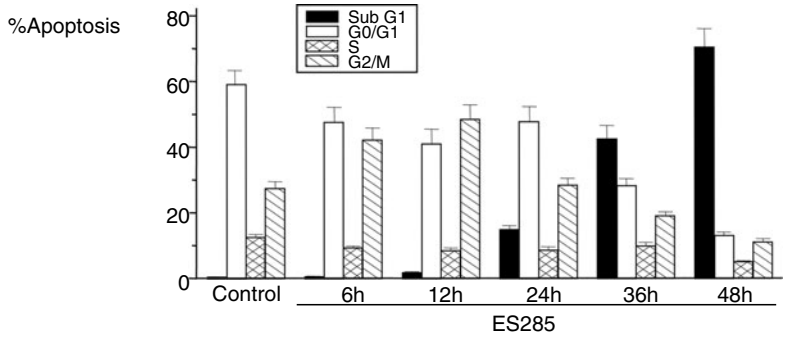


Fig. 16.5. Time-dependent induction of apoptosis in sub-G1 HeLa cells

After 24 h exposure, 10 μM ES285 produces internucleosomal DNA breakdown or apoptotic cell death. The Fas/FasL systems are not involved in this process. However, caspase 3 and its substrate, PARP, are activated by ES285 and subsequent markers of apoptosis evolve within 24 h. Potent and persistent activation of JNK is not affected by ES285. There is only a transient activation in 12 h. ERK activation, a survival signal, is clearly involved within 24 h in a strong time-dependent induction. Blocking ERK leads to an increased apoptotic response to ES285; and the use of cells transfected with antiapoptotic genes renders them unresponsive to ES285. Finally, ES285 induces mitochondrial release of cytochrome *c*.

Other tumour cell types exhibit a consistent pattern of delayed apoptosis as described earlier; and overall ES285 has shown that it can profoundly influence several targets in the induction pathway of apoptosis. For instance, this compound activates caspase 3 and 12 and modifies the phosphorylation level of p53, thus suggesting that ES285 triggers an atypical cell death program (study UIC/TRL 391: single i.v. dose in rats).

16.3.2 Non-Clinical Studies

Preliminary in vitro studies indicate that ES285 has potent activity against cell line subpanels containing solid tumours, lymphomas and leukaemias, with selectivity for certain solid tumours (i.e. colon, gastric, pancreas, pharynx, renal) at IC_{50} potencies in the nanomolar range. SK-HEP-1 hepatoma tumour cells deserve special mention because they showed an IC_{50} of 0.562 pM. The activities against solid tumour cell lines were generally tenfold more potent than those for leukaemias and lymphomas and, more specifically, the slow-growing adherent tumour cells seemed to be more sensitive to ES285. In vitro studies by the NCI have confirmed that the antitumour activity of ES285 ranges from 0.1 to 10 µM.

The ES285 effects appear to be long-lasting. In particular, human HCT-116 N7 colon tumour cells were pre-treated with various concentrations of ES285 for either 1 or 24 h (Fig. 16.6). After 1 h of pre-treatment and subsequent drug removal, the cytotoxic effects of 10 µM ES285 continued for up to 72 h. After 24 h of pre-treatment, in turn, cytotoxic activity continued to be present for both 1 and 10 µM concentrations of the drug. Moreover, following treatment with 0.1 µM ES285, cytotoxic activity remained for at least 48 h after 24 h exposure.

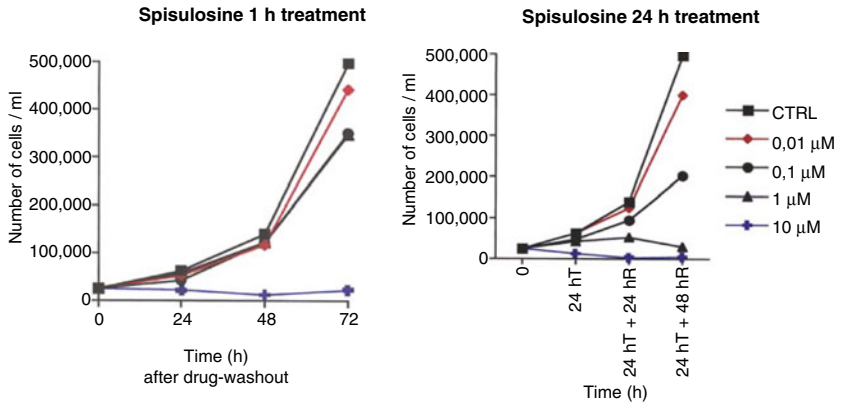


Fig. 16.6. Exposure-dependent in vitro cytotoxicity

ES285 is active in vivo against certain slow-growing solid human tumours (hepatoma, renal, prostate; Table 16.5).

The efficacy of ES285 has been demonstrated when administered as a continuous infusion in male athymic rats bearing a human liver adenocarcinoma tumour. Analysis of net tumour growth of the corresponding treated (T) groups relative to the vehicle control (C) group indicated that the optimal value of %T/C occurred on day 6 after group randomization, i.e. –80 and –73% for the high-dose and low-dose ES285 infusion groups, respectively.

Table 16.5. In vivo activity in mice of ES285 against human tumours

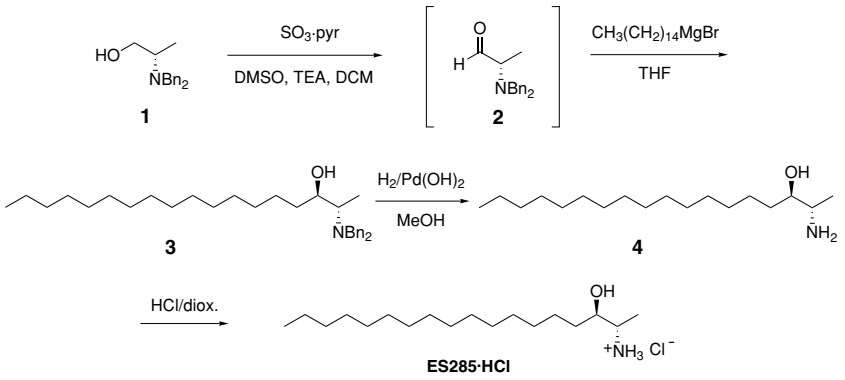
tumour type/line	MTD (mg kg^{-1})	regimen	%T/C	score	model
leukaemia					
ip P388	10	QD × 5, *ip*	104	–	survival
melanoma					
iv B16	10	QD × 9, *ip*	111	–	survival
Hepatoma					
iv SK-HEP-1	10	Q2D × 5, *iv*	122	+	survival
	10	QD × 9, *ip*	14	+++	hollow fibre
sc SK-HEP-1	5		19	+++	
	2.5		22	++	
colon cancer					
sc HT-29	10	QD × 9, *ip*	184	–	hollow fibre
pancreatic cancer					
sc PANC-1	10	QD × 9, *ip*	105	–	hollow fibre
Melanoma					
sc MRI-H-187	10	Q2D × 5, *iv*	48	+/–	xenograft
renal cancer					
sc MRI-H-121	25	Q4D × 3, *ip*	28	++	xenograft
prostate cancer					
sc PC-3	25	Q4D × 3–5, *ip*	<1	++++	xenograft
sc DU-145	25	Q4D × 3–5, *ip*	34	+	xenograft

Non-clinical toxicity has been tested in mice, rats, dogs and monkeys via the i.v. route. Across all species at high doses, the heart was the common target organ of toxicity and the histopathologic change noted was myocardial degeneration. However, the severity decreased inversely with the higher order of animal. Serum troponin I levels were predictive of toxicity in the monkey. Elevations in liver enzyme function tests were frequently observed in the dog, monkey and rat, with cellular changes noted in the rat liver (degenerative). The kidney indicative of nephropathy was also observed to be a target organ of toxicity in the mouse, rat and monkey. The tests and epididimides were affected in both rat and dog, in that degenerative cellular changes were noted. Although injection site changes were noted, predominantly in the rodents (mouse, rat), no such inflammatory, edematous or degenerative changes were noted in the monkey.

16.3.3 Synthesis of ES285

A synthetic scheme that allows preparation of sufficient quantities of ES285·HCl for non-clinical and clinical evaluation has been developed at PharmaMar (Scheme 16.2). ES285 is prepared in four steps from the

commercially available chiral starting material (*S*)-2 (*N*,*N*-dibenzylamino)-1-propanol, **1**.



Scheme 16.2. Synthesis of ES285

The chiral alcohol **1** is oxidized to the corresponding aldehyde, which is then coupled with the Grignard reagent derived from 1-bromopentadecane. The coupling reaction proceeds with high *anti*:*syn* selectivity with the desired isomer representing about 90% of the crude reaction mixture. Compound **3** is purified by preparative HPLC. Next, this intermediate is deprotected by hydrogenation to produce the free amine. Last, the hydrochloride salt of ES285 is generated under anhydrous conditions to produce the final drug substance. Using this approach, ES285 has been prepared on the multi-gram scale.

16.3.4 Clinical Trials

ES285 is currently under investigation in patients with advanced malignant solid tumours as a single agent. Four Phase I clinical trials are ongoing. All these trials are still at the dose-escalation stage and no reliable data is available at present.

16.4 Conclusions

The continuing PharmaMar exploration program has shown the potential use of marine ecosystems as a source of new anticancer compounds. As result of this program, we are developing five new chemical entities as anticancer agents. Further research and development of these marine-derived compounds requires the collaboration of a diverse range of disciplines,

including marine biology, biochemistry, toxicology, pharmacology and oncology.

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