Progress on Lamellarins[Φ]

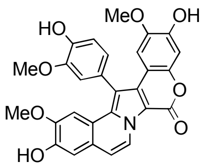
Daniel Pla,*[a,†] Fernando Albericio[a,b,c] and Mercedes Álvarez*[a,b,d]

Received (in XXX, XXX) Xth XXXXXXXXX 2011, Accepted Xth XXXXXXXXX 20XX
First published on the web Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

This review covers recent literature on the lamellarins, a family of marine natural products, and related analogs, encompassing synthetic strategies for total synthesis, structure-activity relationships (SAR), and studies on mechanisms of biological action, namely in the context of anti-tumor activity. It reviews work published from January 2008 to December 2010.

Introduction

Lamellarin D (Lam-D, Fig. 1), which comes from a large family of marine pyrrole alkaloids, is currently in preclinical development as an anti-cancer drug. Comprehensive reviews of this compound were published in 2007 and 2008.[1, 2]



Lam-D

Fig. 1 Structure of Lam-D

Recent advances in the pharmacological development of Lam-D include the identification of new cellular targets of this compound and new insights into its mechanism of action. The malate–aspartate shuttle was recently identified as a new target for Lam-D by proton NMR-based metabolomics,[3] and certain protein kinases relevant to cancer have recently been reported as targets of Lam-D, Lam-N and other lamellarins.[4] Lam-D exerts its anti-tumor activity through complementary pathways: a nuclear route via topoisomerase I (Topo I) inhibition,[5,6] and mitochondrial targeting by induction of mitochondrial permeability transition (MPT).[7]

Robust synthesis of Lam-D has enabled SAR and mechanism of action studies. Thus, the vast chemical space of the Lam structure has been explored through modified analogs, including 1-dearyl,[8] 2-dearyl,[9] lactone-free derivatives,[10] lactam,[11] and polymeric and peptidic nuclear location signal conjugates.[12, 13]

Herein, in addition to the recent synthetic strategies developed for total synthesis of Lam-D and its numerous naturally occurring and synthetic analogs, recent work on SAR studies and on using multi-target mechanisms to induce apoptosis are also discussed.

1. Synthesis of Lamellarins

1.a. Construction of the pyrrole core

Numerous synthetic strategies based on the formation of the five-membered pyrrole heterocycle have been developed for the pentacyclic core of the Lamellarins (Fig. 2).

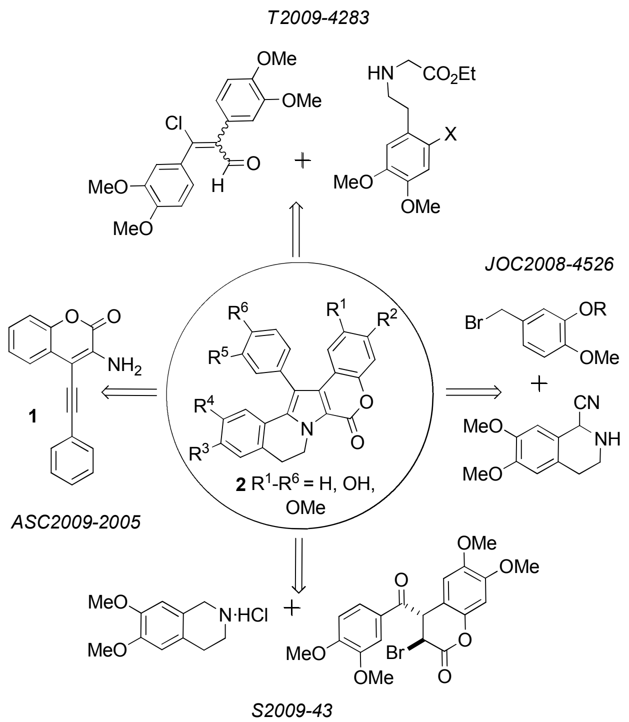
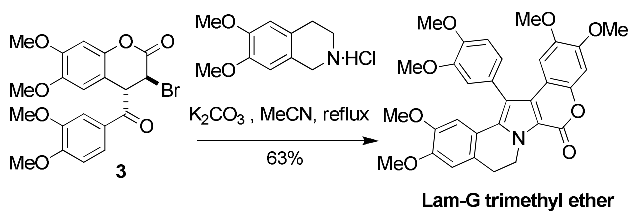


Fig. 2 Synthesis of lamellarins based on construction of the pyrrole core

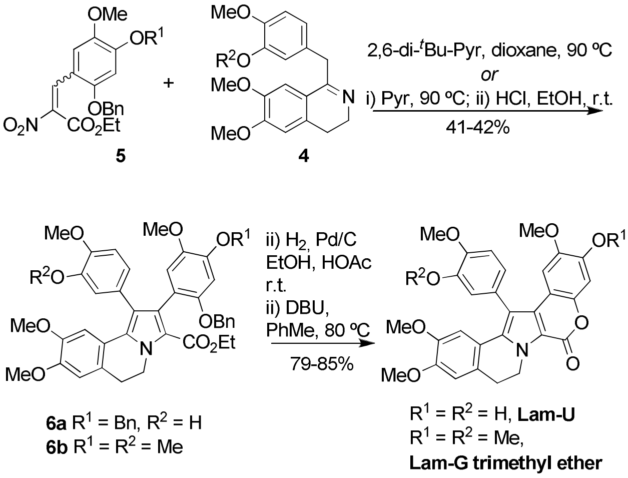
A new protocol for the construction of the Lamellarin scaffold **2** (Fig. 2 R[1]-R[6] = H) relies on Pd-catalyzed intramolecular hydroamination of the acetylenic aminocoumarin derivative **1**.[14] This procedure has not yet been exploited in natural product synthesis.

The key step in a recent synthesis of Lam-G trimethyl ether[15] is the final step: haloarylation of 3-bromo-4-(3,4-dimethoxybenzoyl)-6,7-dimethoxy-chroman-2-one **3** with 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (Scheme 1).



Scheme 1 Key step in the synthesis of Lam-G trimethyl ether[15]

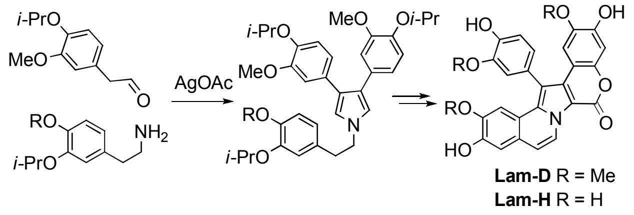
1-Benzyl-3,4-dihydroisoquinolines have been transformed to Lam-G trimethyl ether and Lam-U using short reaction sequences. Grob reaction of dihydroisoquinoline **4** with the nitrocinnamate **5** afforded pyrroles **6a** and **6b**.[16] Subsequent *O*-debenzylation and lactonization provided the aforementioned natural product, plus Lam-U (Scheme 2).



Scheme 2 Synthesis of Lam-G trimethyl ether and Lam-U[16]

Gupton *et al*.[17] reported formal syntheses of Lam-G trimethyl ether and ningalin B via formation of the polysubstituted pyrrole derivatives **7a** and **7b**, respectively, from a vinylogous iminium salt derivative. Subsequent transformations of these highly substituted pyrroles enabled efficient regio-controlled formal syntheses of the targets (Scheme 3).

In late 2010 Li *et al*. reported a new, concise synthesis of Lam-D and Lam-H by condensation of 3-isopropoxy-4-methoxyphenethylamine with two units of the corresponding phenethylaldehyde derivative, followed by AgOAc-mediated oxidative coupling to construct the pyrrole core. Their synthesis featured two additional induced oxidative cyclizations to form the lactone and the pyrrole−arene C-C bond. Thus, Lam-D and Lam-H were prepared in seven steps and in overall yields of 17% and 16%, respectively (Scheme 4).[18]

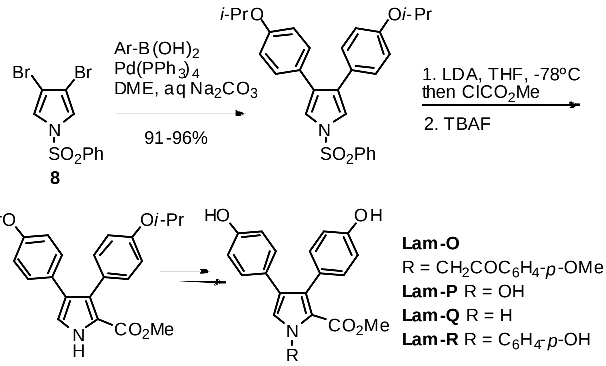


Scheme 4 Synthesis of Lam-D and Lam-H[18]

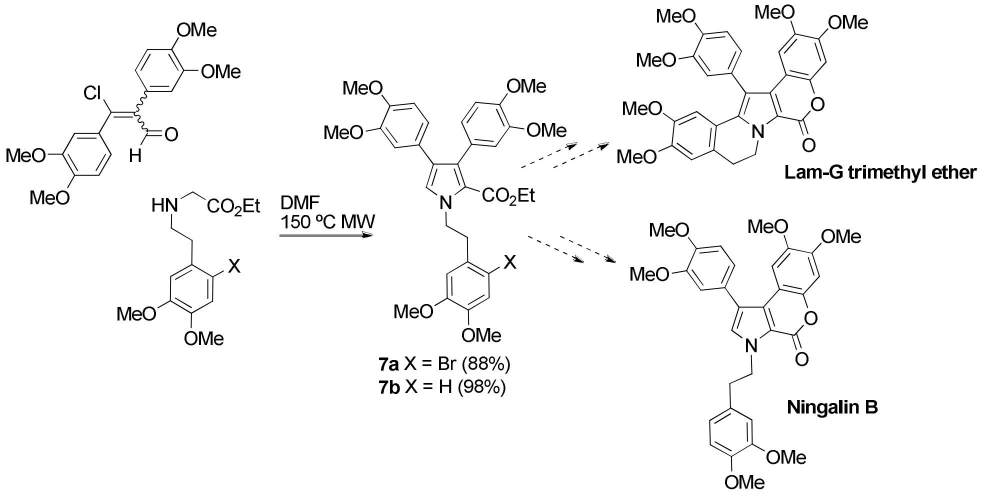
1.b. Cross-coupling reactions

Efforts towards Lamellarin synthesis have vastly benefited from Pd(0) catalyzed cross-coupling chemistry—namely, the Heck, Negishi and Suzuki reactions. Recently reported methodologies for these compounds using this chemistry are described below.

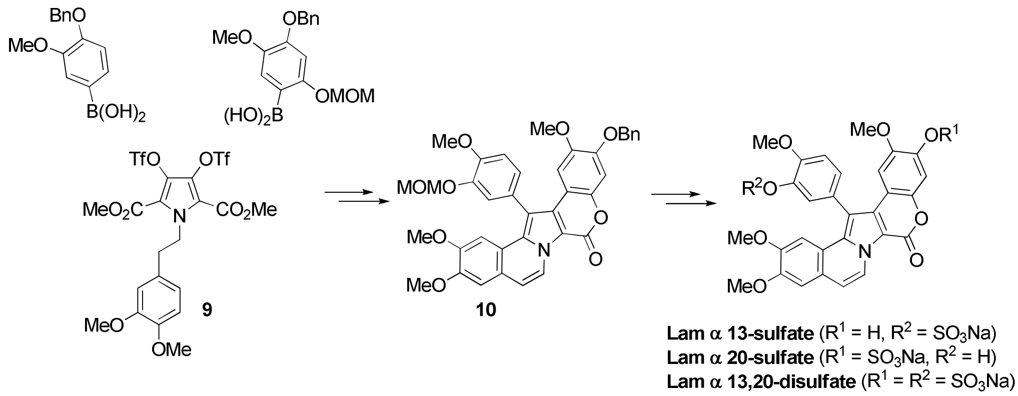
Total syntheses of Lam-O, P, Q, and R have been achieved by using as key synthetic steps the Pd-catalyzed Suzuki–Miyaura cross-coupling reaction of *N*-benzenesulfonyl-3,4-dibromopyrrole (**8**) with an arylboronic acid followed by directed α-lithiation.[19] *N*-Deprotection and substitution gave the natural products (Scheme 5).



Scheme 5 Synthesis of lamellarins O, P, Q, and R[19]



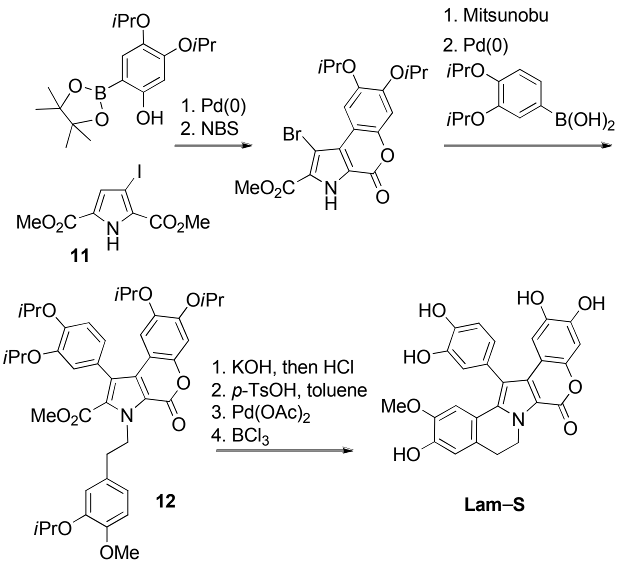
Scheme 3 Formal syntheses of Lam-G trimethyl ether and ningalin B[17]



Scheme 6 Synthesis of Lam-α 13-, 20-sulfate, and 13,20-disulfate[20,21]

Sequential Suzuki-Miyaura coupling of 3,4-dihydroxypyrrole bistriflate (**9**) as a key reaction[20, 21] was harnessed to prepare three Lam-α sulfate derivatives: Lam-α 13-sulfate, 20-sulfate, and 13,20-disulfate. The common intermediate **10**, in which 13-OH and 20-OH are protected with orthogonal protecting groups, was the shared precursor (Scheme 6).

A general method for the synthesis of *N*-unsubstituted 3,4-diarylpyrrole-2,5-dicarboxylates also has been developed.[22] The principal reactions comprised a Hinsberg-type synthesis of dimethyl *N*-benzyl-3,4-dihydroxypyrrole-2,5-dicarboxylate followed by palladium-catalyzed Suzuki-Miyaura coupling of its bis-triflate derivative. The *N*-benzyl protecting group of the resulting 3,4-diarylpyrrole-2,5-dicarboxylates was cleanly removed under hydrogenolysis or solvolysis. The authors observed that the regioselectivity of the Suzuki cross-coupling of dihalopyrrole esters was influenced by the reaction solvent.[23]

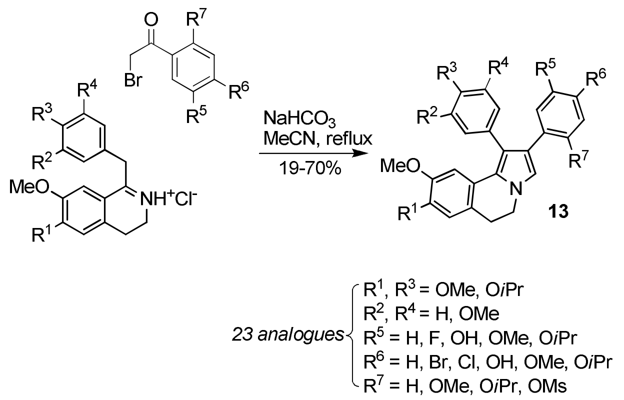


Scheme 7 Synthesis of Lam-S[24]

Banwell *et al.*[24] recently published the first total synthesis of Lam-S and a new synthesis for Lam-G trimethyl ether, based on the same strategy. Their route included two sequential Suzuki–Miyaura cross-coupling reactions to insert the two polyalcoxyaryl groups at positions 3 and 4 of the pyrrole derivative **11**. The resulting compound was then subjected to decarboxylative Heck cyclization and, in the case of Lam-S, subsequent *O*-*i*Pr ether deprotection. (Scheme 7).

1.c. Lactone-free Lam-D analogs, and azalamellarins

Hu *et al.* reported their work on developing 1,2-diphenyl-5,6-dihydropyrrolo[2,1-*a*]isoquinolines **13** as hybrids of combretastatin A4 and Lam-D with the aim of retaining the cytotoxic and anti-mitotic activities of the parent compounds (Scheme 8).[10] They exploited a previously described strategy for *N*-ylide-mediate pyrrole formation which involves dihydroisoquinoline hydrochlorides and phenacyl bromides (see Scheme 1). Their work complements, and is closely related with, the preparation of a library of open-chain analogs that had been reported a few years earlier.[9]

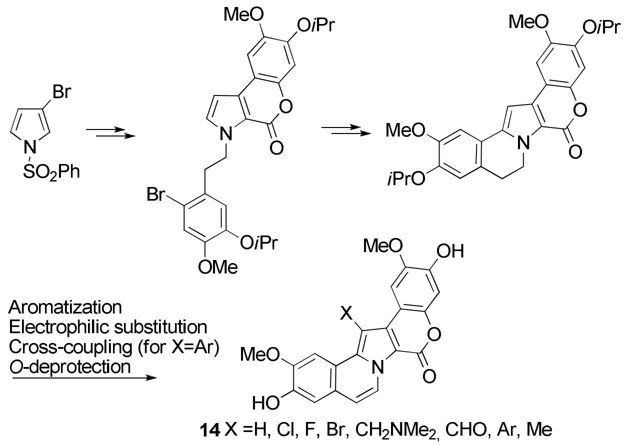


Scheme 8 Synthesis of combretastatin A4 and Lam-D hybrid-library [10]

Thasana *et al.* obtained a library of seventeen azalamellarins using microwave-assisted Cu^{I}-mediated C-N bond formation.[11]

1.d. 1- and 2-Dearyl Lam-D analogs

Iwao *et. al.* synthesized numerous Lam-D analogs (the 1-dearyl-Lam compounds **14**; Scheme 9)[8] using a methodology that they had previously developed.[19]



Scheme 9 Synthesis of 1-dearyl-Lam-D analogs[8]

Given the results of SAR studies on similar 2-dearyl analogs,[9] the aforementioned compounds created by the Iwao group should prove interesting for biological evaluation.

1.e Polyethylene glycol (PEG), dendrimeric, and nuclear location signal (NLS) bioconjugates of Lam-D

Mono- and di-PEG bioconjugates of Lam-D (**15a** & **15b**, and **16**, respectively) with improved solubility have been prepared from the corresponding partially protected (*O*-*i*Pr or *O*-Bn) phenolic derivatives **17a-c**; alternatively, tri-PEG conjugates (**18**) have been obtained directly from the parent Lam-D compound (Scheme 10).[12] A strategy similar to that described for the total synthesis of Lam-D[25] was exploited to prepare **19a-c** precursors of **15a,b** and **16** (Scheme 10).

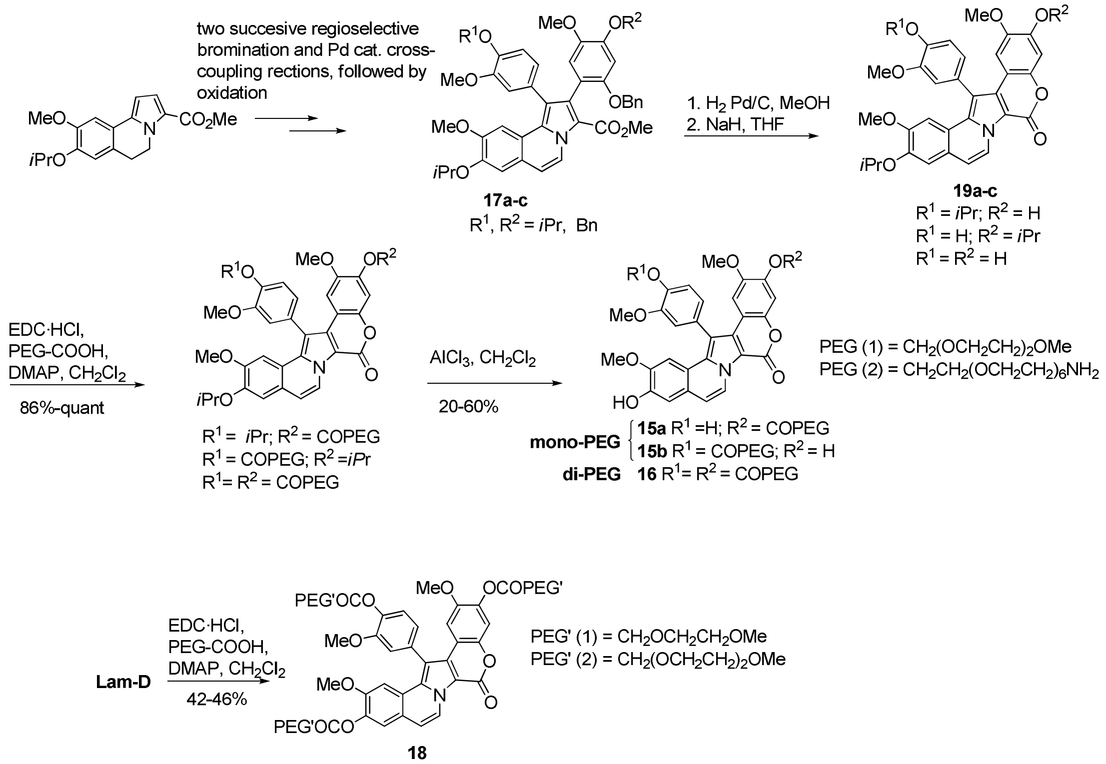
In the synthesis of the dendrimeric- and NLS-Lam-D bioconjugates **20** and **21**, the amino and guanidino groups of the oligomeric building blocks were protected with *N*-Boc.[13] The PEG-based dendrimer **22** was condensed with protected Lam-D **23** using EDC·HCl and DMAP as coupling agents. Simultaneous removal of the Boc and TBDPS-protecting groups with HF at low temperature afforded **20** (Scheme 11).

Pre-activation of the Boc-protected NLS-peptide **24** with *N,N,N',N'*- tetramethylchloroformamidinium hexafluorophosphate (TCFH) and NEt_3, followed by the addition of a solution the Boc-protected Lam-D **25** and DMAP, were required to form the bioconjugates, which was obtained in 45% yield. Elimination of the nine Boc protecting groups with 40% TFA in CH_2Cl_2 gave compound **21** in 17% yield (Scheme 11).

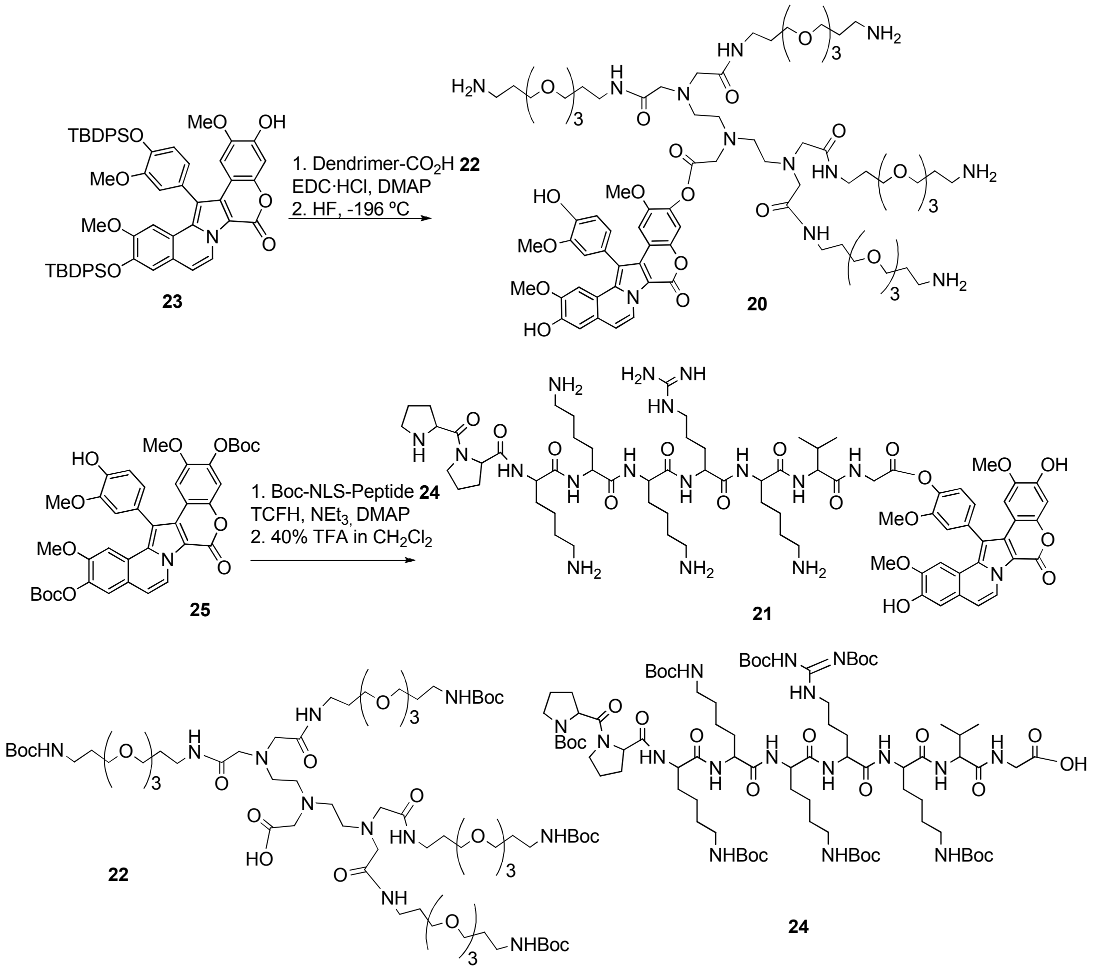
2. Molecular Structure-Activity Determinants, and SAR Studies

Natural products remain an underutilized source of leads for the pharmaceutical industry.[26] Preparing analogs of these products through medicinal chemistry approaches to explore maximum chemical space is a valuable method for improving and/or modulating the parent biological activity. Given their diverse anti-tumor activities, lamellarins have been the subject of several SAR studies in recent years.

Hu *et al.* prepared a library of structurally simplified lactone-free lamellarin (LF-Lam) analogs having a 1,2-diphenyl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline core structure. This library was partially inspired by the close structural resemblance



Scheme 10 Synthesis of mono-, di- and tri-PEG-bioconjugates of Lam-D[12]



Scheme 11 Preparation of dendrimeric- and NLS-bioconjugates of Lam-D[13]

between Lam-D and combretastatin A4, which contains a conserved *cis*-stilbene motif. They evaluated twenty-two novel, diversely-substituted analogs, which they then screened for anti-proliferative activities against five cancer cell lines. The compounds generally showed poor activity (sub-millimolar IC_{50} values); the most active of them are shown in Fig. 3 and listed in Table 1.

Table 1 Anti-proliferative activities of the three best LF-Lam analogs prepared by Hu *et al.* [10]

Compound	IC_{50} (μM)				
	A549	K-562	SMM-7721	SGC-7901	HCT-116
LF-Lam 1	> 94.1	4	> 94.1	> 94.1	1.4
LF-Lam 2	6.7	2.7	10.9	11.4	0.7
LF-Lam 3	23.3	5.3	15	22.7	13.8

Cell lines: A549, human non-small-cell lung carcinoma; HCT-116, human colon carcinoma; K-562, human erythromyeloblastoid leukemia; SGC-7901, human gastric adenocarcinoma; SMM-7721, human hepatocarcinoma.

Nevertheless, we believe these results reflect a partial but promising structural simplification. Selective removal of the *O*-*i*Pr groups would easily afford the corresponding free phenolic groups at positions C8 and C4', which are crucial for the activity of open-chain methyl ester lamellarin analogs.[9] The Hu group's results are in agreement with previous findings related to the presence of the OH at C4" of the aryl at position 2 of the pyrrolo[2,1-*a*]isoquinoline, which are also crucial role for activity.

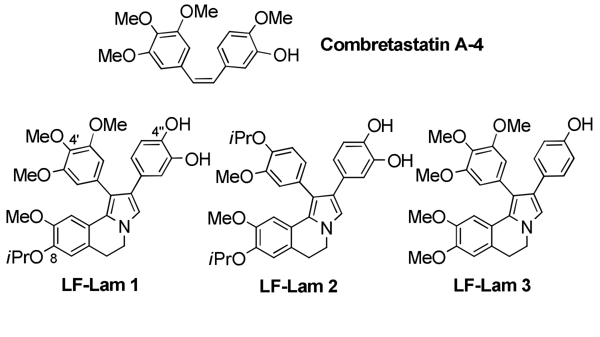


Fig. 3 Structures of combretastatin A4 and LF-Lam analogs[10]

Ruchirawat *et al.* extensively assessed the cytotoxic activities of 25 lamellarins against multiple cancer cell lines derived from six different cancer types (oral, lung, breast, liver, cervix, and blood cell).[27] The compounds comprised 22 naturally occurring lamellarins and three synthetic derivatives containing either a saturated or an unsaturated C8-C9 bond, and bearing different OMe, OH and H substitution patterns on the aryl rings. Their SAR studies were the first to reveal the importance of having an OH group at C10 for biological activity and also confirmed prior findings[1,2] that the C8=C9 double bond increases cytotoxicity; that the OH groups at C3 and C11 are crucial to activity; and that methylation of the OH groups at C12, C3' and C4' induces only subtle changes in the GI_{50} values.[27]

Lamellarins-D, -X, -ε, -M, -N, and dehydro-Lam-J were the most potent anti-tumor compounds of the family (Fig. 4): they had IC_{50} values ranging from sub-nanomolar (0.08 nM) to micromolar (3.2 μM).[27] Furthermore, they showed selectivity against cancer cells: these lamellarins were roughly 3 to 1,000,000 times more toxic to cancer cells from all eleven cell lines tested than to the control (normal) cells, MRC-5 human embryonic lung fibroblasts (Table 2).[27] In related work, Lam-D and its bioconjugates were similarly selective for cancer cells over BJ skin fibroblasts.[12, 13]

Hannongbua *et al.* have studied lamellarins using receptor-independent (RI) 4D quantitative SAR (QSAR) models.[28, 29] They obtained valuable 3D pharmacophore information from a set of 25 structurally complex lamellarins screened *in silico* against human hormone dependent T47D breast cancer cells. Overall, they identified formation of an intermolecular hydrogen bond, and the hydrophobic interactions of substituents at C10, C11 and C12, as the most important features for cytotoxicity against the cancer cells.

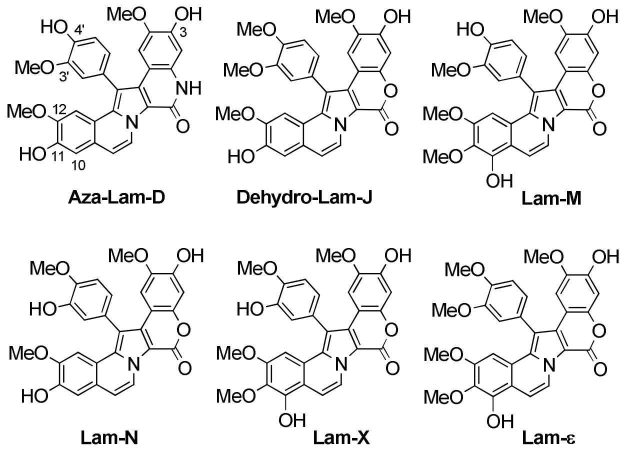


Fig. 4 Structures of aza-Lam-D; dehydro-Lam-J; and Lam-X, -ε, -M and -N[27, 11]

They also suggested that hydrophobic substitutions at C3' and C4' could enhance cytotoxicity.

Ruchirawat *et al.* prepared Lam-D analogs containing a lactam rather than a lactone. Their work was the first report of a SAR studies done for a change at this position.[11] The resulting azalamellarins were screened against four cancer cell lines (HuCCA-1, A-549, HepG2, and MOLT-3). Aza-Lam-D showed good activity (IC_{50} values in the micromolar range), comparable to that of its parent, Lam-D (Table 2). The amide *N*-H confers the aza-analogs with greater solubility in biological media compared to that of the parent compound. Interestingly, *N*-alkylated aza-analogs were not as potent as the parent compound.

For pharmacological enhancement through optimized use of chemical space, the Ruchirawat group suggested introducing

Table 2 Cytotoxicity of Lam-D; aza-Lam-D; dehydro-Lam-J; and Lam-X, -ε, -M and -N[27, 11]

Comp.	IC50 (μM)												
	Oral	Lung		Breast		Liver			C rvi x	Blood cell		Leuke mia	Fibroblast
	KB	A549	H69 AR	T47D	MDA -MB-231	HuCC A-1	HepG 2	S10 2	HeLa	P38 8	HL-60	MOL T-3	MRC-5
Lam-D	0.04	0.06	0.4	0.00008	0.4	0.08	0.02	3.2	0.06	0.1	0.04	0.049	9.2
Aza-Lam-D	NR	0.74	NR	NR	NR	0.12	0.13	NR	NR	NR	NR	0.03	NR
Dehydro -Lam-J	0.08	0.04	0.3	0.0001	0.4	0.006	0.01	2.1	0.08	0.08	0.04	NR	> 97.4
Lam-M	0.2	0.04	0.3	0.009	0.1	0.06	0.02	1.9	0.3	0.1	0.06	NR	13.4
Lam-N	0.06	0.04	0.06	0.0006	0.6	0.008	0.02	2.3	0.04	0.08	0.04	NR	> 100.1
Lam-X	0.08	0.3	0.3	0.006	0.08	0.04	0.2	1.6	0.09	0.3	0.2	NR	10.1
Lam-ε	0.3	0.3	2.3	0.006	0.3	0.07	0.1	2.1	0.3	0.3	0.1	NR	25.8

NR: not reported; Cell lines: A549, human non-small-cell lung carcinoma; H69AR, human multi-drug-resistant small-cell lung carcinoma; HeLa, human cervical adenocarcinoma; HepG2, human hepatocellular carcinoma; HL-60, human promyelocytic leukemia; HuCCA-1, human cholangiocarcinoma; KB, human oral epidermoid carcinoma; MDA-MB-231, human hormone-independent breast cancer 231; MOLT-3, T-lymphoblast (acute lymphoblastic leukemia); MRC-5, human fetal/embryonic lung fibroblast; P388, mouse lymphoid neoplasm; S102, human hepatocellular carcinoma; T47D, human hormone-dependent breast cancer.

additional polar functionalities (*e.g.* alternative H-bond donors, acceptors, and ionizable groups) into this family of compounds.[30]

Biopolymeric mono-, di- and tri-ester conjugates of Lam-D (at its phenolic sites) are more soluble than the parent compound and can be readily hydrolyzed in physiologic conditions.[12] For example, the monoPEG **15a** is up to 80-times more soluble in water than Lam-D. Moreover, a release of up to 97% of Lam-D has been achieved by incubation of **15a** for 6 h at 37 °C in Dubelcco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and 100 units/mL penicillin and streptomycin.[13] The cytotoxicity of the bioconjugates **15, 16, 18**, **20** and **21** was assessed against three human tumor cell lines (MDA-MB-231 breast, A-549 lung and HT-29 colon). Several compounds exhibited greater cellular internalization than did Lam-D, and more than 85% of the bioconjugates showed a lower GI_{50} than did Lam-D.[12,13] Cellular internalization was analyzed, and a nuclear distribution pattern was observed for bioconjugate **21**, which contains an NLS.[13] The mechanisms of action for Lam-D and these derivatives have been indentified as cell cycle arrest at G2 phase and apoptotic cell-death pathways.[12]

In summary, the various SAR studies and virtual screening models for lamellarins and related analogs discussed above have demonstrated the efficacy of targeted medicinal chemistry. The findings from this work may contribute to future drug development of synthetic analogs and to the selection of synthetic scaffolds.

3. Activities and Mechanism of Action of Lam-D

Topo I is a nuclear target of Lam-D

Inhibition of the nuclear DNA relaxation enzyme Topoisomerase I (Topo I) by Lam-D has recently been characterized in a model system of highly nicked double-stranded DNA (dsDNA) by using optical tweezers force-stretching experiments (Fig. 5a). In these experiments, normal Topo I activity—which ultimately leads to total repair of the DNA nicks—is shown by a higher force plateau in DNA stretching-relaxation plots and complete disappearance of the overstretching hysteresis (Figs. 5b and 5c, green curves). When Lam-D is added to the system, it prevents the religation step and blocks enzyme turnover, as seen by the absence of a higher force plateau and by a large, non-vanishing hysteresis between the stretching and relaxing paths of the force cycle (Figure 5d, green curve).[6]

"insert Figure 5 here"

Fig. 5 Optical tweezers (OT) force assay of Topoisomerase I (Topo I) activity. (a) Schematic of the experiments, in which each 3' biotinylated end of the dsDNA is attached to one streptavidin-coated bead. (b-d) OT Force-DNA extension measurements. b) Force-stretching experiment starting from a dsλDNA molecule with a few nicks, as indicated by the small hysteresis area (black curve). Stretching-relaxing cycles are indicated by half-bold half-thin plots. The cleavage activity of Topo I after 9 min of reaction generated an ssDNA-like domain, a heavily nicked dsDNA which exhibited the mechanical properties of ssDNA (red curve). After a further 11 min the ligating activity of Topo I removed the ssDNA-like domain (green curve). c) Force-stretching experiment starting with highly-nicked dsDNA, as indicated by the large hysteresis area (black curve). Topo I activity is reflected by a higher-level plateau, which indicates total religation of the initial DNA nicks, and by complete disappearance of the overstretching hysteresis (green curve, 18 min reaction time). The arrow indicates a drop in the stretching curve resulting from Topo I cleavage during the cycle. d) Force assay of Topo I activity in the presence of Lam-D. Starting λDNA exhibited a small hysteresis area (black curve) corresponding to a dsDNA molecule with only a few nicks. The cleavage activity of Topo I induced a large increase in hysteresis in the presence of Lam-D after the first two minutes of reaction (red curve). Stabilization by Lam-D of this intermediate complex resulted in inhibition of the religating activity of Topo I (green curve, acquired at 21 min reaction).[6, 31]

Inhibition of Topo I by Lam-D has also been demonstrated by atomic force microscopy (AFM) imaging of plectonemic DNA molecules with Topo I enzyme attachments that were treated with a combination of Lam-D and a reaction buffer used to relax supercoiled DNA (see Fig. 6).[6]

"insert Figure 6 here"

Fig. 6 AFM imaging of Topo I-treated plasmid DNA. a) Supercoiled circular DNA treated by Topo I in the presence of Lam-D. Horizontal scale is 0.75 µm, vertical scale is 2 nm for all images. b) Interpretive illustration. The green dots represent Topo molecules.[6, 31]

Nuclear penetration remains a challenge in the pharmacological development of Lam-D and related compounds. Albeit the aforementioned work elegantly demonstrates how Lam-D blocks the nuclear enzyme Topo I, if the drug cannot reach the nucleus, then this activity is useless. Confocal microscopy experiments on internalization of Lam-D and its PEG conjugates **15, 16** and **18** in three different cell lines show that these compounds chiefly end up in the cytoplasm[12], although Lam-D and the PEG-NH_2 compound **15a** were able to weakly reach the nucleus in one of the tested cell cultures. Chemical bioconjugation of Lam-D to a nuclear localization signal (NLS) peptide to give the Lam-D NLS bioconjugate **21** was explored as a strategy to enable active transport of the drug through the nuclear membrane. Experiments on the co-localization and cellular distribution of Lam-D NLS bioconjugate in three green fluorescent protein-Topo I (GFP-Topo I) transfected cell cultures (HT-29, A-549, and MDA-MD-231 cell lines), indicated that it localized to the nucleus in HT-29 and A-549 cells, while GFP-Topo I was located in the nucleus for all three cell types. Thus, the NLS peptidic sequence is at least partly responsible for nuclear import of **21** in those cell lines. The authors concluded that greater cytotoxicity correlated with greater nuclear localization.[13]

Induction of mitochondrial apoptosis independent of nuclear signaling

In early 2008 Marchetti *et al* made a huge breakthrough in understanding the cytotoxicity of Lam-D while studying non-small cell lung carcinoma (NSCLC) cells, which are typically resistant to apoptosis induced by standard chemotherapy. They observed that Lam-D did not trigger signs of nuclear apoptosis coupled to the nuclear translocation of apoptosis-inducing factor (AIF) in the cells, yet it induced activation of Bax, mitochondrial release of cytochrome c and AIF, and activation of caspase-3.[32]

In 2009 Bailly, Marchetti *et al.* reported further and more extensive studies using various tumor cell lines, including several leukemia and carcinoma lines.[5] Their results suggest that for all cell types tested, Lam-D exerts its cytotoxicity primarily by inducing mitochondrial apoptosis *independently* of nuclear signaling.[5] This work not only contributes to deciphering the apoptotic pathways activated by Lam-D but also provides evidence that Lam-D is still active even if the classical apoptotic pathways are blocked in cancer cells (*i.e.* when p53 is null/mutated, Topo I is mutated, and/or Bcl-2 is overexpressed).

In 2010 the same research group ultimately identified direct induction of mitochondrial permeability transition (MPT), which leads to swelling and cytochrome *c* release, as the mechanism behind the pro-apoptotic function of Lam-D.[7] These results indicate that functional mitochondria are required for Lam D-induced apoptosis. Furthermore, inhibition of mitochondrial respiration is responsible for MPT-dependent apoptosis of cancer cells induced by Lam-D.[7]

The aforementioned findings open the path to new cancer therapy strategies that target mitochondria, thereby reinforcing the potential value of mitochondriophilic drugs for this indication. Given the problems posed by drug resistant cancer cells, targeting mitochondria may prove an effective approach, as long as Lam-D or a related drug is sufficiently selective in affecting exclusively the mitochondria of cancer cells.[5,7,32]

A new target, the malate–aspartate shuttle

The ability to isolate and study mitochondria has enabled several interesting metabolic studies. Nevertheless, conclusions based only on these kinds of studies should be evaluated with prudence. Furthermore, state-of-the-art techniques such as proton NMR-based metabolomics are demonstrating that whole cells can be studied directly. For example, Morvan *et al.*[3] have applied proton NMR-based metabolomics to screen the effects of Lam-D and two other drugs (also natural marine products) in whole human MCF7 breast cancer cells. They observed that Lam-D provoked a severe decrease in DNA content in the MCF7 cells after 24 h treatment, followed by apoptosis. Metabolite profiling of the cells revealed major biochemical disorders following treatment with Lam-D: namely, accumulation of aspartate, glutamate, and lactate, which suggests that Lam-D targets the malate–aspartate shuttle.

Inhibition of protein kinases

Maijer *et al.* have also identified a new molecular target of lamellarins.[4] They reported that lamellarins inhibit several protein kinases relevant to cancer, including cyclin-dependent kinases, dual specificity tyrosine phosphorylation activated kinase 1A, casein kinase 1, glycogen synthase kinase-3 and PIM-1. They found a good correlation between inhibition of protein kinases by lamellarins and cell death. This suggests that specific kinases may contribute to these drugs' cytotoxicity, and could be harnessed together with the drugs' other bioactivities to achieve anti-tumor effects. Further studies in this area are needed.

Conclusions and Future Trends

The unique structures and biological activities of the lamellarins and related pyrrole-derived alkaloids have attracted significant synthetic efforts in recent years. Thus, elegant synthetic routes to these compounds or their key intermediates have been developed. Since 2008, Lam-G trimethyl ether has been synthesized using four novel strategies, in total yields ranging from 5% to 45%[15-17, 24]

These highly modular approaches can provide access to any member of the family; one need only choose the appropriately substituted and protected building blocks. Additionally, these chemistries have proven valuable for preparation of structurally simplified lamellarin analogs, affording sufficient quantities for SAR and other biological studies.

The initial SAR studies on lamellarins have provided the first evidence that an OH group at C10 position is important for biological activity.[27] Latter work demonstrated the importance of the C8=C9 double bond, and confirmed the importance of the OH groups at C3 and C11. To date, Lam-D, X, ε, M and N, and dehydro-Lam-J are the most potent anti-tumor compounds of this family.

Further SAR studies of the 1-dearyl Lam-D analogs synthesized by Iwao *et al.*[8] could provide useful data and a more thorough overview on structural simplification of the parent compound.

Incorporation of polar groups onto the core to facilitate further H-bonding interactions, as well as ionizable groups to enhance the solubility, can yield improved lamellarin analogs. For example, aza-Lam-D[11], which contains one more H-bond donor than the parent compound; several PEG analogs of Lam-D (**15**, **16**, **18**, and **20**) analogs;[12] and a peptide bioconjugate of Lam-D (**21**)[13] have shown enhanced bioactivity and physicochemical properties relative to the parent compound.

Future medicinal chemistry efforts should be addressed to provide derivatives and bioconjugates with greater specificity for biological targets. Given that Lam-D has been shown to inhibit the nuclear enzyme Topo I, medicinal chemists should prioritize achieving nuclear transport and release of this drug, and creating analogs able to cross the nuclear membrane on their own. Researchers should also explore if and how chemical modifications in the form of new lamellarin analogs could lead to improved or distinct mitochondrial/nuclear pathways of action.

Lastly, direct targeting of mitochondria by Lam-D can circumvent the drug resistance often encountered in tumor cells. Thus, Lam-D constitutes a new pro-apoptotic agent that may bypass certain forms of apoptosis resistance.[5]

Further studies can contribute to a better understanding of the mechanism of lamellarins and their analogs, and will be beneficial for the ongoing pharmacological optimization of this class of compounds.

Acknowledgments

This review was partially supported by CICYT (Grant CTQ 2009-07758), Generalitat de Catalunya (2009SGR 1024), and the Barcelona Science Park. DP would like to acknowledge the support of the European Commission through a 3-year Marie Curie International Outgoing Fellowship under the 7th Framework Programme (FP7-PEOPLE-2009-IOF-253205).

Notes and references

[Φ] *Dedicated to Prof. Rafael Suau a good friend and master*

[a] *CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain.*
[b] *Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain*
[c] *Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain.*
[d] *Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain. Fax: (+34)934037126; Tel: (+34)934037086; E-mail: mercedes.alvarez@irbbarcelona.org*
† *Current address: Memorial Sloan-Kettering Cancer Center, New York City NY, 10065, USA; E-mail: plaquerd@mskcc.org*

1. D. Pla, F. Albericio and M. Álvarez, *Anti-Cancer Agents Med. Chem.*, 2008, **8**, 746-760.
2. H. Fan, J. Peng, M. T. Hamann and J.-F. Hu, *Chem. Rev.*, 2007, **108**, 264-287.
3. M. Bayet-Robert, S. Lim, C. Barthomeuf and D. Morvan, *Biochem. Pharmacol.*, 2010, **80**, 1170-1179.
4. D. Baunbæk, N. Trinkler, Y. Ferandin, O. Lozach, P. Ploypradith, S. Ruchirawat, F. Ishibashi, M. Iwao and L. Meijer, *Mar. Drugs*, 2008, **6**, 514-527.
5. C. Ballot, J. r. Kluza, A. Martoriati, U. Nyman, P. Formstecher, B. Joseph, C. Bailly and P. Marchetti, *Mol. Cancer Ther.*, 2009, **8**, 3307-3317.
6. D. Pla, A. Sischka, F. Albericio, M. Álvarez, X. Fernàndez-Busquets and D. Anselmetti, *Small*, 2009, **5**, 1269-1272.
7. C. Ballot, J. Kluza, S. Lancel, A. Martoriati, S. Hassoun, L. Mortier, J.-C. Vienne, G. Briand, P. Formstecher, C. Bailly, R. Nevière and P. Marchetti, *Apoptosis*, 2010, **15**, 769-781.
8. T. Ohta, T. Fukuda, F. Ishibashi and M. Iwao, *J. Org. Chem.*, 2009, **74**, 8143-8153.
9. D. Pla, A. Marchal, C. A. Olsen, A. Francesch, C. Cuevas, F. Albericio and M. Álvarez, *J. Med. Chem.*, 2006, **49**, 3257-3268.
10. L. Shen, X. Yang, B. Yang, Q. He and Y. Hu, *Eur. J. Med. Chem.*, 2010, **45**, 11-18.
11. S. Boonya-udtayan, N. Yotapan, C. Woo, C. J. Bruns, S. Ruchirawat and N. Thasana, *Chem. Asian J.*, 2010, **5**, 2113-2123.
12. D. Pla, A. Francesch, P. Calvo, C. Cuevas, R. Aligue, F. Albericio and M. Álvarez, *Bioconjugate Chem.*, 2009, **20**, 1100-1111.
13. D. Pla, M. Marti, J. Farrera-Sinfreu, D. Pulido, A. Francesch, P. Calvo, C. Cuevas, M. Royo, R. Aligue, F. Albericio and M. Álvarez, *Bioconjugate Chem.*, 2009, **20**, 1112-1121.
14. L. Chen and M. H. Xu, *Adv. Synth. Catal.*, 2009, **351**, 2005-2012.
15. J. S. Yadav, K. U. Gayathri, B. V. Reddy, P. Subba and A. R., *Synlett* 2009, 43-46.
16. J. C. Liermann and T. Opatz, *J. Org. Chem.*, 2008, **73**, 4526-4531.
17. J. T. Gupton, B. C. Giglio, J. E. Eaton, E. A. Rieck, K. L. Smith, M. J. Keough, P. J. Barelli, L. T. Firich, J. E. Hempel, T. M. Smith and R. P. F. Kanters, *Tetrahedron*, 2009, **65**, 4283-4292.
18. Q. Li, J. Jiang, A. Fan, Y. Cui and Y. Jia, *Org. Lett.*, 2011, **13**, 312-315.
19. T. Fukuda, E.-i. Sudo, K. Shimokawa and M. Iwao, *Tetrahedron*, 2008, **64**, 328-338.
20. T. Fukuda, T. Ohta, S. Saeki and M. Iwao, *Heterocycles*, 2010, **80**, 841-846.
21. The atom numbering shown corresponds to the system used in the original reference, which does not follow IUPAC rules.
22. T. Fukuda, Y. Hayashida and M. Iwao, *Heterocycles*, 2009, **77**, 1105-1122 .
23. Y. Zhang and S. T. Handy, *Open Org. Chem. J.*, 2008, **2**, 58-64.
24. K. Hasse, A. C. Willis and M. G. Banwell, *Eur. J. Org. Chem.*, 2011, **76**, 88–99.
25. D. Pla, A. Marchal, C. A. Olsen, F. Albericio and M. Álvarez, *J. Org. Chem.*, 2005, **70**, 8231-8234.
26. J. W.-H. Li and J. C. Vederas, *Science*, 2009, **325**, 161-165.
27. M. Chittchang, P. Batsomboon, S. Ruchirawat and P. Ploypradith, *ChemMedChem*, 2009, **4**, 457-465.
28. P. Thipnate, J. Liu, S. Hannongbua and A. J. Hopfinger, *J. Chem. Inf. Model.*, 2009, **49**, 2312-2322.
29. P. Thipnate, M. Chittchang, N. Thasana, P. Saparpakorn, P. Ploypradith, S. Hannongbua, *Monatsh. Chem.*, 2011, **142**, 97-109.

30. M. Chittchang, M. Paul Gleeson, P. Ploypradith and S. Ruchirawat, *Eur. J. Med. Chem.*, 2010, **45**, 2165-2172.
31. See reference 6. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.
32. M.-A. Gallego, C. Ballot, J. Kluza, N. Hajji, A. Martoriati, L. Castera, C. Cuevas, P. Formstecher, B. Joseph, G. Kroemer, C. Bailly and P. Marchetti, *Oncogene*, 2008, **27**, 1981-1992.