Bioorganic & Medicinal Chemistry 17 (2009) 235–241

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological properties of Pachastrissamine (jaspine B) and diastereoisomeric jaspines

Daniel Canals [a,†], David Mormeneo [a,b,†], Gemma Fabriàs [a], Amadeu Llebaria [a], Josefina Casas [a,*], Antonio Delgado [a,b,*]

[a] Research Unit on BioActive Molecules (RUBAM), Departament de Química Biomèdica, Institut de Química Avançada de Catalunya (IQAC-C.S.I.C), Jordi Girona 18-26, 08034 Barcelona, Spain
[b] Universitat de Barcelona, Facultat de Farmàcia, Unitat de Química Farmacèutica (Unitat Associada al CSIC), Avgda. Juan XXIII s/n, 08028 Barcelona, Spain

ARTICLE INFO

Article history:
Received 29 September 2008
Revised 30 October 2008
Accepted 3 November 2008
Available online 18 November 2008

Keywords:
Phytosphingosine
Dihydroceramide
Ceramide
Cytotoxicity
Stereoisomers

ABSTRACT

The synthesis of isomeric jaspines (anhydro phytosphingosines), arising from intramolecular cyclization of the corresponding phytosphingosines with different configurations at C3 and C4 positions of the sphingoid backbone, is reported. Natural jaspine B is the most cytotoxic isomer on A549 cells and it induces cell death in a dose-dependent manner. The cytotoxicity of jaspine B has been correlated with a significant increase of intracellular dihydroceramides, which seem to play an active role in autophagy.

1. Introduction

Sphingolipids (SLs) comprise ubiquitous natural products with essential roles in cell biology. From a chemical perspective, mammalian SLs derive mainly from (*E*,2*S*,3*R*)-2-amino-4-octadecen-1,3-diol or sphingosine (Sph), although a minor portion arise from the saturated analogue sphinganine or its 4*R*-hydroxy derivative phytosphingosine.

Some unusual sphingolipids exhibiting potent antitumor activity have been described from marine organisms.[1] In 2002, Kuroda and co-workers reported on the isolation and characterization of Pachastrissamine, an anhydro phytosphingosine isolated from the marine sponge *Pachastrissa* sp.[2] This compound is also known as jaspine B (Fig. 1) after its isolation from the sponge *Jaspis* sp.[3–5] Other structurally related analogues have also been isolated from the same species, including jaspine A and jaspine B,[4] whose C2 epimer (Fig. 1) has also been reported.[5] This compound, initially described as a plant metabolite,[6] was first characterized by total synthesis of a shorter analogue,[7–9] and later reported as a chemical entity, both in the form of free amine[10–13] or as the corresponding *N*-benzoyl amide in the course of a series of chemical transformations from *N*-substituted D-*ribo* phytosphingosine derivatives.[14]

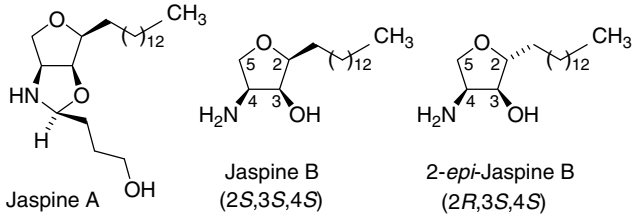


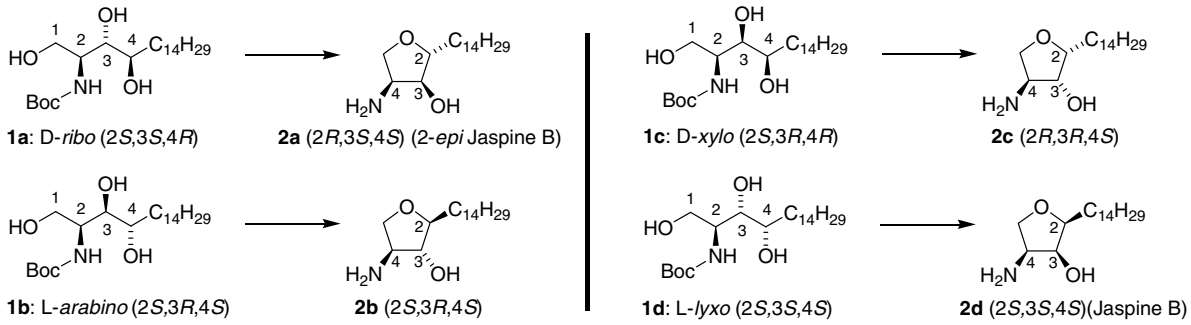
Figure 1. Structure of natural jaspines.

The structure of jaspine B has been unambiguously confirmed by total synthesis from L-serine,[15] Garner's aldehyde,[11] D-*ribo* phytosphingosine,[12,13] D-xylose,[16] (*R*)-glycidol,[17] D-(−)-tartaric acid,[18] and a galactal derivative,[19] among others.[20,21]

Jaspine B shows cytotoxicity against several cell lines at a nanomolar level.[2,4] However, despite its remarkable biological activity, little is known about the structure–activity relationships in jaspine B and, in particular, on the role of the stereochemistry of the tetrahydrofuran substituents on the biological profile of this interesting family of compounds. Based on these premises, we undertook the synthesis and biological evaluation of all four jaspine stereoisomers (**2a–2d**) arising from the intramolecular cyclization of *N*-Boc phytosphingosines (**1a–1d**) with different configuration around the C3 and C4 positions of the sphingoid backbone (Scheme 1).[22] To

* Corresponding authors. Tel.: +34 934006108; fax: +34 932045904.
E-mail address: adelgado@cid.csic.es (A. Delgado).
† These authors contributed equally to this work.

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.11.026



Scheme 1. Stereoisomeric *N*-Boc phytosphingosines and their corresponding anhydro derivatives (see also Ref. 4).

our knowledge, **2b** and **2c** are unprecedented, whereas no biological data have been reported in the literature for **2a**.

2. Results and discussion

2.1. Chemistry

Jaspines **2a**–**2d** were obtained by intramolecular cyclization of *N*-Boc phytosphingosines (**1a**–**1d**), resulting from Sharpless asymmetric dihydroxylation of the corresponding olefins.[23] Treatment of **1a–1c** with *p*-toluenesulfonyl chloride in CH_2Cl_2/pyridine led to **4a–4c**, presumably through in situ cyclization of the transient tosylates **3a–3c** arising from selective tosylation of the primary alcohol, as described in related systems (Scheme 2).[24,12] However, treatment of **1d** under similar reaction conditions allowed the isolation of tosylate **3d**, which was further cyclised to **4d** following reported protocols.[17]

In all cases, deprotection of the *N*-Boc group in **4a–4d** under standard acidic conditions (TFA/CH_2Cl_2 5:1, rt, 1 h) led to the expected jaspines **2a**–**2d** in good yields (74–82%). These were fully characterized by NMR methods, by comparison with reported data, for **2a**[5,10,12] and **2d**,[2,4,5,17,12,13] and also by 2D experiments for unreported **2b** and **2c**.

2.2. Biological results

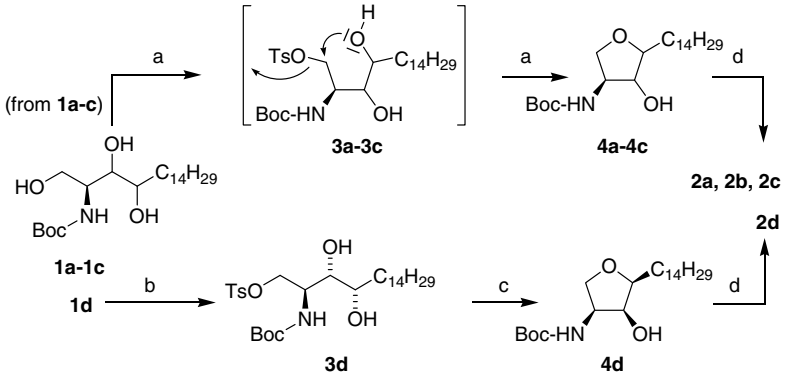
2.2.1. Cytotoxic effects of 2a–2d on A549 cells

Cytotoxicity of jaspines **2a–2d** was examined in A549 human alveolar cells by the MTT test. Jaspine B (**2d**) was the most cytotoxic compound in this assay (LD_{50} = 0.13 ± 0.01 μM), whereas diastereomeric jaspines **2a–2c** were about 10–20 times less toxic, as judged by their LD_{50} values (**2a**, 1.25 ± 0.03 μM; **2b**, 1.50 ± 0.03 μM; **2c**, 2.5 ± 0.04 μM). These data point out the stereoselectivity of jaspine isomers in this assay, since the less toxic isomer **2c** differs from jaspine B (**2d**) in both C3 and C4 stereocenters. Interestingly, phytosphinogosines derived from *N*-Boc removal of **1a**–**1d** were 7–140 times less toxic than the configurationally equivalent cyclic analogues **2a–2d**.[25]

Since the MTT test is a mere measure of living cells, the toxic effects of jaspine stereoisomers were investigated by flow cytometry analysis after double labelling with annexin V (AV) and propidium iodide (PI). The results on A549 cells cultured in the presence of **2a**–**2d** at different concentrations are shown in Figure 2. In control experiments, the percentage of AV-stained cells (2%) was not significantly increased after treatment with jaspine B (**2d**) or stereoisomeric jaspines **2a–2c**. However, the percentage of AV-PI double positive cells increased moderately at high concentrations of **2a–2c** (1 and 2 μM), whereas this effect was less remarkable for jaspine B (**2d**). Finally, a concentration-dependent increase in the percentage of PI positive cells was observed in all cases (Fig. 2). These results demonstrate that apoptosis only accounts for a minor percentage of cell death and, therefore, a different cell death mechanism must be implicated.

2.2.2. Cytotoxicity of compounds 2a–2d decreases in the presence of 3-methyladenine

Since it has been recently demonstrated that some agents used in antitumor therapy are able to induce autophagy, but not apoptosis, in several cancer cells,[26] we explored the ability of the jaspine analogues to promote this type of programmed cell death in A549 cells. Among the different described methods for monitoring autophagy, inhibition of cell death by 3-methyladenine (3-MA) is a frequently used proof to confirm the autophagy process.[27,28] Under our assay conditions, no significant differences ($p \geqslant 0.05$) were observed between cells treated with 3-MA and those treated with the vehicle only. Conversely, a marked decrease in toxicity of jas-



Scheme 2. Reagents and conditions: (a) TsCl (1.1 equiv/mol), pyr/CH_2Cl_2 (1:1), 25 °C, 20 h; (b) TsCl (3 equiv/mol), TEA, cat DMAP, CH_2Cl_2; (c) K_2CO_3, MeOH, 20 h, 0–25 °C; (d) TFA/CH_2Cl_2 5:1, rt, 1 h.

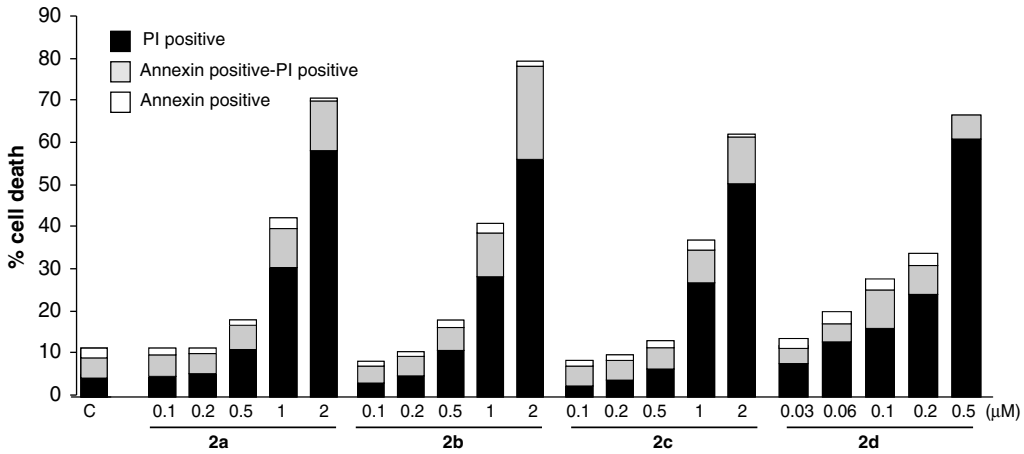


Figure 2. Flow cytometry analysis of A549 control (C) and treated cells for 24 h at the indicated concentrations of compounds **2a**–**2d**. *Y* axis indicates the percentage of cell population stained with annexin V, propidium iodide or both.

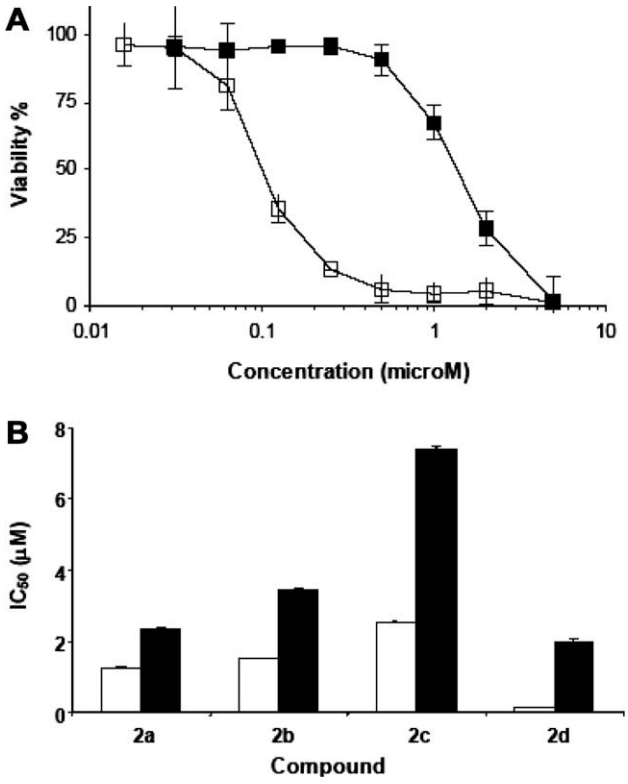


Figure 3. (A) Cytotoxicity in A549 cells of **2d** in the presence (full symbols) or in the absence (empty symbols) of 10 mM 3-MA. (B) Cytotoxicity (LD_{50} values) of **2a**–**2d** in A549 cells in the presence (full bars) or in the absence (empty bars) of 10 mM 3-MA. Values are means ± SD of three independent experiments with triplicates.

pine B in the presence of 3-MA was found (Fig. 3A). Thus, the LD_{50} value calculated for jaspine B in the presence of 3-MA increased around 15 times in comparison with that found in the absence of 3-MA (Fig. 3B). Similar results, albeit less remarkable, were found for compounds **2a–2c**, where an increase in the LD_{50} values by a factor of 2 (for **2a–2b**) and 3 (for **2c**) were observed. Interestingly, the presumed autophagic activity profile shown by jaspine B seems related to its cyclic nature, since the configurationally equivalent open chain L-*lyxo*-phytosphingosine exhibited similar cytotoxicity both in the absence or the presence of 3-MA (28.2 ± 0.42 vs 29.4 ± 0.54 μM, $p \geqslant 0.05$). These results are indicative of a hitherto unreported mode of action for jaspine B and its stereoisomers.

2.2.3. Jaspine B modifies ceramide and dihydroceramide levels in A549 cells

Recent studies have established that endogenous sphingolipids are involved in autophagy. Lavieu et al.[29] reported that ceramide (Cer) and sphingosine-1-phosphate (Sph1P) are able to trigger autophagy with opposing outcomes on cell survival. On the other hand, an increase of endogenous dihydroceramides (DHCer's) was detected in cells treated with fenretinide,[26] a compound that has been reported to cause autophagy.[30] In order to elucidate if sphingolipids are involved in the 3-MA-inhibited cytotoxicity induced by jaspine B, its effect on the ceramidome of A549 cells was investigated.

The concentration-dependent effects of jaspine B on endogenous Cer and DHCer levels at 10 h are shown in Figure 4 and the time-course variation of Cer and DHCer amounts in A549 cells cultured with 50 and 200 nM of jaspine B are shown in Figure 5. Cells treated with 5 and 10 nM of jaspine B exhibited the same levels of Cer's and DHCer's than controls. Regarding Cer levels, a 1.2- to 1.4-fold increase in C16Cer was observed when A549 cells were treated at concentrations $\geqslant$25 nM of jaspine B. Conversely, levels of C24Cer and C24:1Cer remained unaffected up to 200 nM jaspine B concentration, in which a slight but significant increase in both species was found.

Jaspine B produced both a concentration and time-dependent effect on the DHCer production. Thus, an increase of C24DHCer and C24:1DHCer was already detected after 2 h incubation with 50 nM (a non-cytotoxic concentration) of jaspine B. After 10 h incubation (Fig. 4), C24DHCer exhibited the highest build up as jaspine B concentrations raised, reaching a 2.5-fold increased at 200 nM. A similar trend was observed for C16DHCer, although only a 1.7-fold increase took place for this species at the same concentration. Finally, levels of C24:1DHCer attained a 1.3-fold increase at 25 nM, which remained stable up to 100 nM.

The time-course increase in DHCer's followed different profiles depending on the molecular species (Fig. 5). Thus, both C24DHCer and C24:1DHCer levels rose up after 10 h treatment (2.6- and 1.8-fold increase at 200 nM, respectively), followed by a decrease at 24 h. However, C16DHCer levels remained increasing up to 24 h, when a 2.7-fold increase at 200 nM occurred.

3. Discussion

The marine environment is a source of extremely potent compounds that have demonstrated, among many other properties, significant biological activities as antitumor, and cytotoxic

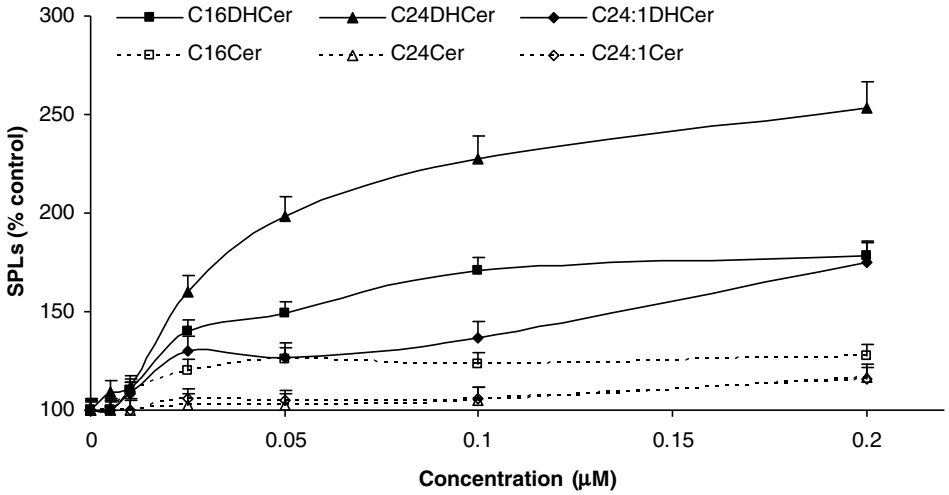


Figure 4. Concentration-dependent effects of jaspine B on endogenous ceramide (Cer) and dihydroceramide (DHCer) species at 10 h of treatment. C16, C24, and C24:1 indicate the *N*-acyl chain length. Data are means ± SD of three experiments with triplicates.

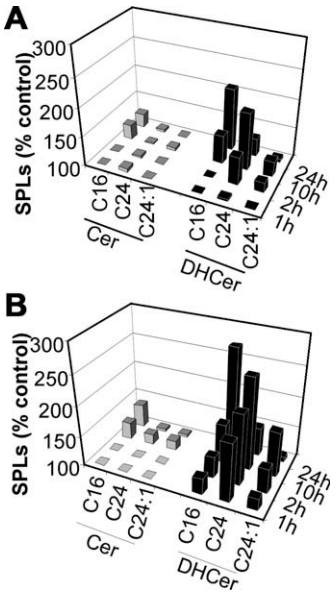


Figure 5. Time-course effects of jaspine B on endogenous ceramide (Cer) and dihydroceramide (DHCer) species at 50 nM (A) and 200 nM (B). C16, C24, and C24:1 indicate the *N*-acyl chain length. Data are means ± SD of three experiments with triplicates.

drugs.[31–33] In particular, marine sponges are the source of a wide variety of sphingosine-related compounds.[1] Among them, jaspine B represents the first natural anhydro phytosphingosine reported so far. It is cytotoxic against several cell lines at a nanomolar level through a yet unknown mechanism.

The tetrahydrofuran backbone is an ubiquitous heterocyclic unit found in a number of biologically active natural products.[34] It is essential for the cytotoxic activity of jaspine B, since the related open-chain phyosphingosines were 10–100 times less toxic. Nevertheless, a systematic survey of the biological stereoselectivity of jaspine B isomers has not yet been reported. Our data show that inversion of C2 and/or C3 stereocenters provides less toxic compounds.

It has been reported that phytosphingosine induces apoptosis, which is the most common mechanism of programmed cell death in human cancer cells.[35] When A549 cells were cultured with jaspine B at different concentrations, flow cytometry analysis after double staining with propidium iodide and annexin V (Fig. 2, **2d**) showed that less than 5% of the cells were annexin V positive and around a 20% were annexin V positive/propidium iodide positive. These results demonstrate that apoptosis can only account for a minor percentage of cell death and, therefore, most of the treated cells must die by a different mechanism.

Autophagy, also named as type II programmed cell death, is a dynamic process in which proteins and altered organelles are sequestered inside cytoplasmic membrane structures for further degradation and recycling. An increasing number of studies report that autophagy is activated in cancer cells in response to various anticancer therapies, such as tamoxifen in breast cancer cells,[36] or fenretinide in prostate cancer cell lines.[37] As for natural products, resveratrol, a phytoalexin that is present in grape nuts and red wine, induced autophagy in ovarian cancer cells[38] and soybean B group triterpenoid saponins caused autophagy in colon cancer cells.[39] Genistein, an isoflavonoid abundant in soy products, induced apoptosis and autophagy in a dose-dependent response.[40] A similar dual effect has been reported for curcumin[41] and for *Solanum nigrum*, an herbal plant whose extracts are used in traditional oriental medicines.[42] In the present study, an autophagy inhibitor (3-MA) has been used to explore whether autophagy may play a role in the cytotoxicity of jaspine B (**2d**) and that of the stereoisomeric jaspines **2a-2c**. Although compound 3-MA inhibits class I and class III phosphatidylinositol 3-kinases, two enzymes with opposed functions in autophagy, the overall effect of this inhibitor is typically to block autophagy.[27] As shown in Figure 3A, 500 nM jaspine B caused a 95% cell death, whereas no cytotoxicity was detected at the same concentration in the presence of 3-MA. A similar, although less remarkable, cytoprotective effect of 3-MA was observed for related stereoisomers (**2a–2c**).

Scarlatti et al.[36] have established that Cer's could mediate the autophagy induced by tamoxifen in MCF-7 cells, whereas an increase of endogenous DHCer's, but not Cer's, were observed in DU145 cells treated with fenretinide, a compound that also causes autophagy.[30] In addition, exogenously added cell permeable Cer can induce autophagy in malignant glioma cells[43] and in HT-29 cells by increasing the intracellular pool of long chain Cer's.[36] On the other hand, the effect of cell permeable DHCer seems to be cell specific, since it has been reported to be ineffective toward autophagy in HT-29,[36] whereas Zheng et al. reported that exogenous DHCer was able to induce autophagy in DU145 cells.[30] Our results in cultured A549 cells indicated that jaspine B caused a slight increase in Cer contents with a substantial concentration-dependent

and time-course-dependent effect on DHCer production. DHCer levels in A549 cells increased up to 3-fold after treatment with 200 nM jaspine B. Since DHCer's have traditionally been considered as inert lipids, reports on variations of their intracellular levels are scarce in the literature. Increases on DHCer levels have been reported in different cell types after incubation with DHCer desaturase inhibitors,[44] fenretinide,[37,26] or γ-tocopherol.[45] These increases were higher than those found in this work with jaspine B, although the different cell models and compound used in the several studies can account for these differences. Accumulation of DHCer suggests a reduced DHCer desaturase activity upon treatments. While the activity of fenretinide as a DHCer desaturase inhibitor has been documented[37,26] and Jiang et al. suggested that DHCer desaturase is likely to be inhibited as a result of γ-tocopherol treatment, neither jaspine B nor its stereoisomers **2a–c** inhibit DHCer desaturase.[25] Whether the protein expression is downregulated by this compound is under investigation.

4. Conclusion

In summary, we have demonstrated that jaspine B and its diastereoisomers **2a–2c** caused cell death in A549 cells in a dose-dependent manner, and that this effect was prevented by 3-MA, an autophagy inhibitor. Moreover, cell death was accompanied by a concentration- and time-course dependent build-up of DHCer's, but not Cer's. Collectively, these results suggest that DHCer-mediated autophagy might be involved in the cytotoxicity of jaspine B. This possibility, along with the cell signalling pathways implicated will be investigated in the near future and will be reported in due course.

5. Experimental

5.1. Cytotoxicity assay in A549 cells

Human alveolar epithelial A549 cells were obtained from the American Type Culture Collection (ATCC) and grown in HAM F12 with glutamine medium supplemented with 10% foetal bovine serum (FBS). Cells were kept at 37 °C in 5% CO_2/95% air. At the time of the experiments, cells were seeded in medium with 10% FBS at 105 cells per well in 96-well plates. Twenty-four hours later, media were replaced with fresh medium and compounds were added to give final concentrations of 0.02–400 μM. Cells were incubated at 37 °C in 5% CO_2/95% air for 24 h. The number of viable cells was quantified by the estimation of its dehydrogenase activity, which reduces 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to water-insoluble formazan, which was dissolved in DMSO and measured at 570 nm with a Multiskan plate reader (Labsystems). All compounds were dissolved in ethanol and control experiments were performed with ethanol (0.1%). For suppression of autophagy, cells were treated with the inhibitor 3-methyladenine (3-MA) at a concentration of 10 mM for 2 weeks[46] on a 25 mL flask, changing the medium every 2 days. Then, 10^5 cells were seeded in 96-well plates in media with/without 3-MA containing compounds **2a–2d** at the above-mentioned concentrations and cultured for 24 h. The number of viable cells was estimated with the MTT test.

5.2. Flow cytometry analysis

A549 cells cultured with different concentration of compounds **2a–2d** for 24 h. Cells were collected by brief trypsinization, stained with an Alexa Fluor 488 annexin V/propidium iodide staining kit (Molecular Probes, Inc. Oregon), and evaluated by FACS (Coulter XL) with Coulter EPLDS.[47] Compounds **2a–2c** were tested at 0.125, 0.25, 0.5, 1, and 2 μM, whereas jaspine B (**2d**) at 0.033, 0.066, 0.125, 0.25, and 0.5 μM.

5.3. LC–MS analysis of sphingolipids

A549 cells were seeded in 1 mL of medium with 10% FBS in a 6-well plates. Twenty-four hours later, media were replaced with fresh medium containing jaspine B at different concentrations (5, 10, 25, 50, 100, and 200 nM) and cultured for different times (1, 2, 10, and 24 h) at 37 °C/5% CO_2. Then, cells were washed in PBS, collected by brief trypsinization and 10^6 cells transferred to glass vials. An aliquot of cells was taken for protein measurements. Sphingolipid extracts, fortified with internal standards (*N*-dodecanoylsphingosine, *N*-dodecanoylglucosylsphingosine and N-dodecanoyl sphingosylphosphorylcholine, 0.5 nmol each), were prepared as described[30] and analysed. The liquid chromatography–mass spectrometer consisted of a Waters Aquity UPLC system connected to a Waters LCT Premier orthogonal accelerated time of flight mass spectrometer (Waters, Millford, MA), operated in positive electrospray ionisation mode. Full scan spectra from 50 to 1500 Da were acquired and individual spectra were summed to produce data points each 0.2 s. Mass accuracy and reproducibility were maintained by using an independent reference spray via the LockSpray interference. The analytical column was a 100 mm × 2.1mm id, 1.7 μm C8 Acquity UPLC BEH (Waters). The two mobile phases were phase A: MeOH/H_2O/HCOOH (74:25:1 v/v/v); phase B: MeOH/HCOOH (99/1 v/v), both also contained 5 mM ammonium formate. A gradient was programmed—0.0 min, 80% B; 3 min, 90% B; 6 min, 90% B; 15 min, 99% B; 18 min, 99% B; 20 min, 80% B. The flow rate was 0.3 mL min^{-1}. The column was held at 30 °C. Quantification was carried out using the extracted ion chromatogram of each compound, using 50 mDa windows. The linear dynamic range was determined by injecting standard mixtures. Positive identification of compounds was based on the accurate mass measurement with an error <5 ppm and its LC retention time, compared to that of a standard (±2%).

5.4. Chemistry

Solvents were distilled prior to use and dried by standard methods. FT-IR spectra are reported in cm^{-1}. ^1H and ^{13}CNMR spectra were obtained in $CDCl_3$ solutions at 300 MHz (for ^1H) and 75 MHz (for ^{13}C), respectively, unless otherwise indicated. Chemical shifts (δ) are reported in ppm relative to the solvent ($CDCl_3$) signal. ESI/HRMS spectra were recorded on a Waters LCT Premier Mass spectrometer.

5.4.1. (2′*S*,3′*S*,4′*S*)-[2-(*tert*-Butoxycarbonylamino)-3,4-dihydroxyoctadecyl]-*p*-toluenesulfonate (3d)

A suspension of triol **1d** (250 mg, 0.60 mmol) in 20 mL of CH_2Cl_2 under Ar was cooled to 0 °C and treated with 0.45 mL (3.2 mmol) of freshly distilled TEA, 6 mg of DMAP and 343 mg (1.8 mmol) of TsCl. The reaction was stirred at 25 °C for 6 h, while the white suspension became slowly a clear solution. Solvents were removed at reduced pressure and the resulting residue was purified by flash chromatography (Hexane/EtOAc 8:2) to afford **4d** in 66% yield; mp 63–64 °C; $[\alpha]_D^{25}$ −3.4 (*c* 0.82, $CHCl_3$); IR (film): 3381, 2923, 2853, 1684, 1522, 1456, 1366, 1288, 1252, 1175, 1096, 1051, 979. ^1H NMR ($CDCl_3$, 500 MHz): 7.79 (d, *J* = 8 Hz, 2H), 7.35 (d, *J* = 8.5 Hz, 2H), 5.11 (d, *J* = 9 Hz, 1H, NH), 4.43 (dd, *J* = 10, 3.5 Hz, 1H, H1a), 4.23 (dd, *J* = 10, 3 Hz, 1H, H1b), 3.65 (m, 1H, H2), 3.54 (m, 1H, H3), 3.23 (d, *J* = 9.5 Hz, 1H, H4), 2.45 (s, 3H), 1.61 (m, 1H), 1.44 (s, 9H), 1.39 (m, 1H), 1.22–1.34 (m, 24H), 0.87 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 157.1, 145.4, 132.4, 130.2, 128.1, 81.1, 71.9, 69.9, 68.9, 51.8, 32.6, 32.0, 29.8, 29.8,

29.7, 29.6, 29.5, 28.3, 26.2, 22.8, 21.8, 14.3. ESI-MS *m/z* 472.2 $[M-Boc+H]^+$.

5.4.2. Synthesis of tetrahydrofuran derivatives 4a–4c from 1a to 1c

General method. The corresponding *N*-Boc protected phytosphingosine **1a–1c** (0.20 mmol) and 41 mg (0.22 mmol) of TsCl were weighted in a 10 mL flask. Over these solids, 0.5 mL of anhydrous CH_2Cl_2 and 0.5 mL of freshly distilled pyridine were added using a syringe. After stirring the resulting yellow solution at 25 °C for 20 h, MeOH (0.3 mL) and EtOAc (5 mL) were next added and the resulting mixture was washed several times with aqueous saturated $CuSO_4$ solution. The organic phase was dried over $MgSO_4$, filtered and the solvent removed to give a residue that was purified by flash chromatography (Hexane/EtOAc 8:2) to afford *N*-Boc jaspines **4a–4c**.

5.4.3. (2*R*,3*S*,4*S*)-4-(*tert*-Butoxycarbonyl)amino-3-hydroxy-2-tetradecyltetrahydrofuran (4a)

Isolated yield: 70%; mp 80–81 °C; $[\alpha]_D^{25}$ +5.0 (*c* 0.97, $CHCl_3$); IR (film): 3414, 3370, 2927, 2850, 1691, 1523. ^1H NMR ($CDCl_3$, 500 MHz): 5.02 (d, *J* = 6 Hz, 1H, NH), 4.13 (m, 2H, H2, H1a), 3.92 (m, 1H, H3), 3.70 (ddd, 7.0, 5.9, 4.3 Hz, 1H, H4), 3.50 (t, *J* = 10 Hz, 1H, H1b), 2.39 (br s, 1H), 1.53 (m, 1H), 1.53 (ddd, *J* = 8.8, 6.3, 4.3 Hz, 1H), 1.45 (s, 9H), 1.24–1.35 (m, 24H), 0.87 (t, *J* = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): 156.1, 85.3, 80.1, 75.0, 70.3, 53.0, 33.7, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 28.5, 25.9, 22.8, 14.3. ESI-MS *m/z*, 422.3 [M+Na].

5.4.4. (2*S*,3*R*,4*S*)-4-(*tert*-Butoxycarbonyl)amino-3-hydroxy-2-tetradecyltetrahydrofuran (4b)

Isolated yield: 10%; mp 94–96 °C; $[\alpha]_D^{25}$ −31.7 (*c* 1.09, $CHCl_3$); IR (film): 3346, 2915, 2849, 1689, 1550, 1523, 1468, 1390, 1357, 1175. ^1H NMR ($CDCl_3$, 500 MHz): 4.83 (d, *J* = 5.5 Hz, 1H, NH), 4.04 (dd, *J* = 9.5, 6.5 Hz, 1H, H1a), 3.91 (m, 1H, H2), 3.76 (dd, *J* = 5.5, 3.5Hz, 1H, H3), 3.65 (dd, *J* = 9.5, 3.5 Hz, 1H, H1b), 3.63 (m, 1H, H4), 1.65 (m, 1H), 1.58 (m, 1H), 1.44 (s, 9H), 1.38 (m, 1H), 1.20–1.32 (m, 23H), 0.87 (t, *J* = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 156.7, 85.1, 82.8, 80.4, 77.4, 70.5, 60.4, 33.8, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 28.5, 26.1, 22.8, 14.3. ESI-MS *m/z* 422.2 [M+Na].

5.4.5. (2*R*,3*R*,4*S*)-4-(*tert*-Butoxycarbonyl)amino-3-hydroxy-2-tetradecyltetrahydrofuran (4c)

Isolated yield: 50%; mp 106–108 °C; $[\alpha]_D^{25}$ −22.3 (c 0.82, $CHCl_3$); IR (film): 3385, 3354, 2918, 2847, 1682, 1506, 1465, 1404, 1366, 1343, 1252, 1163. ^1H NMR ($CDCl_3$ + 1 drop CD_3OD, 500 MHz): 5.11 (d, *J* = 6 Hz, 1H, NH), 4.04 (dd, *J* = 9.5, 6 Hz, 1H, H1a), 3.93 (d, *J* = 3 Hz, 1H, H3), 3.90 (m, 1H, H2), 3.76 (dt, *J* = 6.8, 3.8 Hz, 1H, H4), 3.43 (dd, *J* = 9.5, 3 Hz, 1H, H1b), 1.63 (m, 2H), 1.44 (s, 9H), 1.38 (m, 1H), 1.20–1.32 (m, 23H), 0.87(t, *J* = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD, 100 MHz): 156.1, 81.5, 80.4, 76.7, 70.3, 59.9, 59.8, 32.0, 29.9, 29.8, 29.7, 29.7, 29.7, 29.4, 28.5, 28.4, 26.5, 22.8, 14.2. ESI-MS *m/z* 422.2 [M+Na], 821.6 [2M+Na].

5.4.6. (2*S*,3*S*,4*S*)-4-(*tert*-Butoxycarbonyl)amino-3-hydroxy-2-tetradecyltetrahydrofuran (4d)

A cooled (0 °C) solution of tosylate **4d** (0.40 mmol) in 5 mL of anhydrous MeOH was treated with 278 mg (2 mmol) of K_2CO_3 and stirred for 20 h at 25 °C. The solvent was next removed and the residual white solid was purified by flash chromatography (Hexane/EtOAc 8:2) to afford **3d** in 50% yield. Mp 109–111 °C; lit.[17] mp 110–111 °C; $[\alpha]_D^{25}$ +6.7 (*c* 0.89, $CHCl_3$); Lit[17] $[\alpha]_D^{25}$ +6.7 (*c* 0.89, $CHCl_3$); IR (film): 3365, 2955, 2917, 2849, 1687, 1526, 1470, 1364, 1328, 1241, 1173, 1135. ^1H NMR ($CDCl_3$, 500 MHz): 5.07 (br s, 1H, NH), 4.32 (m, 1H, H2), 4.06 (m, 2H, H1a, H3), 3.70 (td, *J* = 6.9, 2.8 Hz, 1H, H4), 3.57 (t, *J* = 8.2 Hz, 1H, H1b), 1.62 (m, 1H), 1.53 (m, 1H), 1.45 (s, 9H), 1.24–1.35 (m, 24H), 0.88 (t, *J* = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 156.0, 82.3, 80.0, 77.5, 72.0, 54.3, 32.1, 29.9, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 29.1, 28.5, 26.2, 22.8, 14.3. ESI-MS *m/z* 422.3 [M+Na], 300.3 [M−Boc].

5.4.7. Deprotection of *N*-Boc jaspines 3a–3d

A solution of 0.08 mmol of the corresponding *N*-Boc jaspine in 1.8 mL of CH_2Cl_2/TFA (5:1) was stirred for 1 h at 25 °C. The solvent was next removed under a stream of N_2 and the resulting oil was taken up in aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was dried over MgSO4, filtered and the solvent was evaporated under vacuum. The resulting white solid was purified by flash chromatography ($CH_2Cl_2/CH_3OH/NH_4OH$ 96:4:1) to afford the final compound.

5.4.8. (2*R*,3*S*,4*S*)-4-Amino-2-tetradecyltetrahydrofuran-3-ol (2a)

Obtained in 82% yield (19 mg, 0.06 mmol) as a white solid from 31 mg (0.08 mmol) of **3a**; mp 101–102 °C; $[\alpha]_D^{25}$ +14.8 (*c* 0.97, CH_3OH); IR (film): 3334, 3281, 3135, 2914, 2853, 1596, 1470, 1367, 1310. ^1H NMR ($CDCl_3$, 500 MHz)[5]: 4.12 (dd, *J* = 9, 6.5 Hz, 1H, H1a), 3.60 (m, 2H, H3, H4), 3.47 (m, 1H, H2), 3.40 (dd, *J* = 9, 7 Hz, 1H, H1b), 1.58 (m, 1H), 1.53 (m, 1H), 1.43 (m, 1H), 1.39 (m, 1H), 1.21–1.32 (m, 22H), 0.88 (t, *J* = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 85.4(C4), 74.9(C3), 73.3 (C1), 52.7 (C2), 33.9, 32.1, 29.8, 29.7, 29.7, 29.5, 26.0, 22.8, 14.3. ESI-MS *m/z* 300.2 $[M+H]^+$ 298.2 $[M-H]^-$; HRMS: calculated for $C_{18}H_{38}NO_2$ $[M+H]^+$: 300.2903; found: 300.2921. HPLC >95% H_2O/CH_3CN 10:90 (0.1% TFA) rt = 5.81 min.

5.4.9. (2*S*,3*R*,4*S*)-4-Amino-2-tetradecyltetrahydrofuran-3-ol (2b)

Obtained in 82% yield (24 mg, 0.08 mmol) as a white solid from 39 mg (0.10 mmol) of **3b**; mp 77–78 °C; $[\alpha]_D^{25}$ −3.8 (*c* 0.71, $CHCl_3$); IR (film): 3359, 3278, 3060, 2924, 2873, 2850, 1586, 1471, 1360, 1243. ^1H NMR ($CDCl_3$, 500 MHz): 4.00 (dd, *J* = 9.5, 6 Hz, 1H, H1a), 3.55–3.61 (m, 3H, H1b, H3, H4), 3.30 (ddd, J = 6, 4.5, 4 Hz, 1H, H2), 2.16 (br s, 3H), 1.56–1.68 (m, 2H), 1.46 (m, 1H), 1.21–1.40 (m, 23H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD, 100 MHz): 84.7(C4), 82.5(C3), 72.8(C1), 59.9(C2), 33.9, 32.0, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 26.1, 22.8, 14.2. ESI-MS *m/z* 300.2 $[M+H]^+$, 322.1 [M+Na]; HRMS: calculated for $C_{18}H_{38}NO_2$ $[M+H]^+$: 300.2903; found: 300.2899. HPLC >95% H_2O/CH_3CN 10:90 (0.1% TFA) rt = 5.00 min.

5.4.10. (2*R*,3*R*,4*S*)-4-Amino-2-tetradecyltetrahydrofuran-3-ol (2c)

Obtained in 78% yield (30 mg, 0.10 mmol) as a white solid from 52 mg (0.13 mmol) of **3c**; mp 87–88 °C; $[\alpha]_D^{25}$ −2.5 (*c* 0.71, $CHCl_3$); IR (film): 3370, 3303, 2960, 2913, 2853, 1607, 1475, 1293, 1070. ^1H NMR ($CDCl_3$, 500 MHz): 4.20 (dd, *J* = 9, 6 Hz, 1H, H1a), 3.88 (ddd, *J* = 7.5, 6.3, 3.5 Hz, 1H, H4), 3.81 (dd, *J* = 3, 1.5 Hz, 1H, H3), 3.44 (m, 1H, H2), 3.39 (dd, *J* = 9, 3.5 Hz, 1H, H1b), 1.60 (m, 5H), 1.43 (m, 1H), 1.20–1.38 (m, 23H), 0.87 (t, *J* = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 80.9 (C4), 79.6 (C3), 73.4 (C1), 59.9 (C2), 32.0, 29.9, 29.8, 29.7, 29.5, 28.6, 26.5, 22.8, 14.3. ESI-MS *m/z* 300.2 $[M+H]^+$, 322.1 [M+Na]; HRMS: calculated for $C_{18}H_{38}NO_2$ $[M+H]^+$: 300.2903; found: 300.2905. HPLC >95% H_2O/CH_3CN 10:90 (0.1% TFA) rt = 5.02 min.

5.4.11. (2*S*,3*S*,4*S*)-4-Amino-2-tetradecyltetrahydrofuran-3-ol (2d) (jaspine B)

Obtained in 74% yield (25 mg, 0.08 mmol) as a white solid from 45 mg (0.11 mmol) of **3d**; mp 90–91 °C; $[\alpha]_D^{25}$ +8.7 (*c* 1.10, $CHCl_3$), lit.[17] $[\alpha]_D^{25}$ +9.5 (*c* 1.5, $CHCl_3$); IR (film): 3340, 2919, 2854, 1585, 1471, 1365, 1240. ^1H NMR[4] ($CDCl_3$, 500 MHz)[5]: 3.92 (dd, *J* = 8.5, 7.5 Hz, 1H, H1a), 3.86 (dd, *J* = 4, 3.5 Hz, 1H, H3), 3.73 (ddd, *J* = 7, 6.7, 3.5 Hz, 1H, H4), 3.65 (m, 1H, H2), 3.50 (dd, *J* = 8.5, 7 Hz, 1H,

H1b), 1.65 (m, 2H), 1.20–1.42 (m, 24H), 0.87 (t, J = 6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 83.3 (C4), 72. 5(C1), 71.9 (C3), 54.4 (C2), 32.1, 29.9, 29.8, 29.8, 29.7, 29.5, 29.5, 26.4, 22.8, 14.3. ESI-MS m/z 300.3 $[M+H]^+$, 298.2 $[M-H]^-$. HRMS: calculated for $C_{18}H_{38}NO_2$ $[M+H]^+$: 300.2903; found: 300.2931. HPLC >95% H_2O/CH_3CN 10:90 (0.1% TFA) rt = 5.87 min.

Acknowledgments

Financial support from the Ministerio de Educación y Ciencia, Spain (Project MCYT CTQ2005-00175/BQU), Fondos Feder (EU), Fundació La Marató de TV3 (Projects UB-040730 and CSIC-040731) and Generalitat de Catalunya (2005SGR01063) is acknowledged. D.M. is acknowledged to the Ministerio de Educación y Ciencia, Spain, for a predoctoral fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.11.026.

References and notes

1. Padron, J. M. *Curr. Med. Chem.* **2006**, *13*, 755.
2. Kuroda, I.; Musman, M.; Ohtani, I. I.; Ichiba, T.; Tanaka, J.; Gravalos, D. G.; Higa, T. *J. Nat. Prod.* **2002**, *65*, 1505.
3. Jaspine B can be considered as the result of the formal cyclodehydration between C1 and C4 hydroxyl groups of L-*lyxo*-phytosphingosine. The term 'jaspine' is used in this work to refer to this kind of anhydro phytosphingosine derivatives. For the sake of homogeneity, jaspine derivatives are named and numbered along the text as tetrahydrofuran derivatives.
4. Ledroit, V.; Debitus, C.; Lavaud, C.; Massiot, G. *Tetrahedron Lett.* **2003**, *44*, 225.
5. Abraham, E.; Davies, S. G.; Roberts, P. M.; Russell, A. J.; Thomson, J. E. *Tetrahedron: Asymmetry* **2008**, *19*, 1027.
6. O'Connell, P. W.; Tsien, S. H. *Arch. Biochem. Biophys.* **1959**, *80*, 289.
7. Sugiyama, S.; Honda, M.; Komori, T. *Liebigs Ann. Chem.* **1988**, 619.
8. Sugiyama, S.; Honda, M.; Komori, T. *Liebigs Ann. Chem.* **1990**, 1069.
9. Birk, R.; Sandhoff, K.; Schmidt, R. R. *Liebigs Ann. Chem.* **1993**, 71.
10. Jo, S. Y.; Kim, H. C.; Jeon, D. J.; Kim, H. R. *Heterocycles* **2001**, *55*, 1127.
11. Sudhakar, N.; Kumar, A. R.; Prabhakar, A.; Jagadeesh, B.; Rao, B. V. *Tetrahedron Lett.* **2005**, *46*, 325.
12. van den Berg, R. J.; Boltje, T. J.; Verhagen, C. P.; Litjens, R. E.; van der Marel, G. A.; Overkleeft, H. S. *J. Org. Chem.* **2006**, *71*, 836.
13. Lee, T.; Lee, S.; Kwak, Y. S.; Kim, D.; Kim, S. *Org. Lett.* **2007**, *9*, 429.
14. van den Berg, R.; Korevaar, C. G. N.; Overkleeft, H. S.; van der Marel, G. A.; van Boom, J. H. *J. Org. Chem.* **2004**, *69*, 5699.
15. Bhaket, P.; Morris, K.; Stauffer, C. S.; Datta, A. *Org. Lett.* **2005**, *7*, 875.
16. Du, Y.; Liu, J.; Linhardt, R. J. *J. Org. Chem.* **2006**, *71*, 1251.
17. Ribes, C.; Falomir, E.; Carda, M.; Marco, J. A. *Tetrahedron* **2006**, *62*, 5421.
18. Prasad, K. R.; Chandrakumar, A. *J. Org. Chem.* **2007**, *72*, 6312.
19. Reddy, L. V. R.; Reddy, P. V.; Shaw, A. K. *Tetrahedron: Asymmetry* **2007**, *18*, 542.
20. Genisson, Y.; Lamande, L.; Salma, Y.; Andrieu-Abadie, N.; Andre, C.; Baltas, M. *Tetrahedron: Asymmetry* **2007**, *18*, 857.
21. Yakura, T.; Sato, S.; Yoshimoto, Y. *Chem. Pharm. Bull.* **2007**, *55*, 1284.
22. Since jaspine diastereomers can be also viewed as anhydro phytosphingosine derivatives, we were also interested in their evaluation as fungal growth inhibitors by their analogy with phytosphingosine, a key sphingolipid in fungal metabolism. (Thevissen, K.; Francois, I. E.; Aerts, A. M. B.; Cammue, P. *Curr. Drug Targets* **2005**, *6*, 923–928.) Preliminary studies carried out on *Saccharomyces cerevisiae* indicated that jaspine B (**2d**) was inactive as growth inhibitor at 400 μM, whereas its stereoisomers **2a**–**2c** were moderately inhibitors, with LD_{50} values around 400 μM (**2a**), 80 μM (**2b**), and 102 μM (**2c**).
23. Imashiro, R.; Sakurai, O.; Yamashita, T.; Horikawa, H. *Tetrahedron* **1998**, *54*, 10657.
24. This tosylation–cyclization step is strongly dependent on the stereochemistry of the starting phytosphingosines. Thus, **4a**–**4c** were obtained in 70%, 10%, and 50% yield from **1a** to **1c**, respectively (see Section 4). The low yield from **1b** (10%) might be related to energy constrains associated to the transition state of the cyclization process. Mechanistic studies along this line are in currently underway to account for these differences.
25. Unpublished results from our group.
26. Kraveka, J. M.; Li, L.; Szulc, Z. M.; Bielawski, J.; Ogretmen, B.; Hannun, Y. A.; Obeid, L. M.; Bielawska, A. *J. Biol. Chem.* **2007**, *282*, 16718.
27. Klionsky, D. J.; Cuervo, A. M.; Seglen, P. O. *Autophagy* **2007**, *3*, 181.
28. Klionsky, D. J.; Abeliovich, H.; Agostinis, P.; Agrawal, D. K.; Aliev, G.; Askew, D. S.; Baba, M.; Baehrecke, E. H.; Bahr, B. A.; Ballabio, A., et al *Autophagy* **2008**, *4*, 151.
29. Lavieu, G.; Scarlatti, F.; Sala, G.; Levade, T.; Ghidoni, R.; Botti, J.; Codogno, P. *Autophagy* **2007**, *3*, 45.
30. Merrill, A. H., Jr.; Sullards, M. C.; Allegood, J. C.; Kelly, S.; Wang, E. *Methods* **2005**, *36*, 207.
31. Mayer, A. M.; Gustafson, K. R. *Int. J. Cancer* **2003**, *105*, 291.
32. Mayer, A. M.; Gustafson, K. R. *Eur. J. Cancer* **2004**, *40*, 2676.
33. Mayer, A. M.; Gustafson, K. R. *Eur. J. Cancer* **2006**, *42*, 2241.
34. Faul, M. M.; Huff, B. E. *Chem. Rev.* **2000**, *100*, 2407.
35. Park, M. T.; Kang, J. A.; Choi, J. A.; Kang, C. M.; Kim, T. H.; Bae, S.; Kang, S.; Kim, S.; Choi, W. I.; Cho, C. K.; Chung, H. Y.; Lee, Y. S.; Lee, S. J. *Clin. Cancer Res.* **2003**, *9*, 878.
36. Scarlatti, F.; Bauvy, C.; Ventruti, A.; Sala, G.; Cluzeaud, F.; Vandewalle, A.; Ghidoni, R.; Codogno, P. *J. Biol. Chem.* **2004**, *279*, 18384.
37. Zheng, W.; Kollmeyer, J.; Symolon, H.; Momin, A.; Munter, E.; Wang, E.; Kelly, S.; Allegood, J. C.; Liu, Y.; Peng, Q.; Ramaraju, H.; Sullards, M. C.; Cabot, M.; Merrill, A. H., Jr. *Biochim. Biophys. Acta* **2006**, *1758*, 1864.
38. Opipari, A. W., Jr.; Tan, L.; Boitano, A. E.; Sorenson, D. R.; Aurora, A.; Liu, J. R. *Cancer Res.* **2004**, *64*, 696.
39. Ellington, A. A.; Berhow, M. A.; Singletary, K. W. *Carcinogenesis* **2006**, *27*, 298.
40. Gossner, G.; Choi, M.; Tan, L.; Fogoros, S.; Griffith, K. A.; Kuenker, M.; Liu, J. R. *Gynecol. Oncol.* **2007**, *105*, 23.
41. Aoki, H.; Takada, Y.; Kondo, S.; Sawaya, R.; Aggarwal, B. B.; Kondo, Y. *Mol. Pharmacol.* **2007**, *72*, 29.
42. Lin, H. M.; Tseng, H. C.; Wang, C. J.; Chyau, C. C.; Liao, K. K.; Peng, P. L.; Chou, F. P. *J. Agric. Food Chem.* **2007**, *55*, 3620.
43. Daido, S.; Kanzawa, T.; Yamamoto, A.; Takeuchi, H.; Kondo, Y.; Kondo, S. *Cancer Res.* **2004**, *64*, 4286.
44. Munoz-Olaya, J. M.; Matabosch, X.; Bedia, C.; Egido-Gabas, M.; Casas, J.; Llebaria, A.; Delgado, A.; Fabrias, G. *ChemMedChem* **2008**, *3*, 946.
45. Jiang, Q.; Wong, J.; Fyrst, H.; Saba, J. D.; Ames, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17825.
46. Petiot, A.; Ogier-Denis, E.; Blommaart, E. F.; Meijer, A. J.; Codogno, P. *J. Biol. Chem.* **2000**, *275*, 992.
47. Villorbina, G.; Canals, D.; Carde, L.; Grijalvo, S.; Pascual, R.; Rabal, O.; Teixido, J.; Fabrias, G.; Llebaria, A.; Casas, J.; Delgado, A. *Bioorg. Med. Chem.* **2007**, *15*, 50.