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Alkyne-tagged analogue of jaspine B: new tool for identifying jaspine B mode of action

Alexandrine Rozié,^[b] Cécile Santos,^[a] Isabelle Fabing,^[a] Patrick Calsou,^[b] Sébastien Britton,^{*,[b]} Yves Génisson^[a] and Stéphanie Ballereau^{*,[a]}

Abstract: The first biologically relevant clickable probe related to the antitumor marine lipid jaspine B is reported. Concise synthetic route to both enantiomers relied on the chiral SCF enantiomeric resolution of racemic materials. The eutomer dextrogyre derivative represents the first jaspine B analogue with enhanced cytotoxicity with IC₅₀ down to 30 nM. These enantiomeric probes revealed a chirality-dependent cytoplasmic imaging of U2OS cancer cells by *in situ* click labelling.

Jaspine B, also known as pachastrissamine (Fig 1), is a natural anhydrophytosphingosine isolated independently from two different marine sponges, *Jaspis* sp.^[1] and *Pachastrissa* sp.,^[2] respectively.

Figure 1. Jaspine B (Pachastrissamine)

This marine lipid exhibits an *in vitro* cytotoxicity with IC₅₀ in the low micromolar range against a large panel of cancer cell lines.^[3] In order to gain insights into jaspine B antitumor behaviour, biological studies aiming at exploring its cellular mechanism of action were undergone. In 2009, Canals *et al.* correlated the cytotoxicity of jaspine B in lung carcinoma cells with dihydroceramide-mediated autophagy.^[4] Simultaneously, our group reported that jaspine B triggers apoptotic cell death in melanoma cells through blockage of sphingomyelin biosynthesis and accumulation of cellular ceramide.^[5] Two years later, Yoshimitsu *et al.* described jaspine B and its stereoisomers as moderate to potent *in vitro* inhibitors of sphingosine kinases (SphK1 and SphK2).^[6] Yoo *et al.* also investigated the antimelanoma activity of jaspine B and proposed that it is a result of the inhibition of ERK-mediated FOXO3 downregulation and decreased Cdk2 levels.^[7] In 2014, we have also reported Jaspine B as a potential inhibitor of the ceramide trafficking protein CERT which mediates ceramides transfer from the

endoplasmic reticulum to the Golgi.^[8] Very recently, Cingolani *et al.* disclosed that jaspine B induces non-apoptotic cell death in gastric cancer cells independently from its inhibition of ceramide synthase.^[9]

Despite this recurrent interest in the likely multi-target jaspine B antitumor mode of action, underlying molecular mechanisms have not yet been deciphered. So as to deepen the understanding of the jaspine B cytotoxic behaviour, novel pharmacological tools are needed. We are reporting here the design, total synthesis and first use for *in cellulo* imaging of unprecedented jaspine B-related chemical probes.

Numerous syntheses of jaspine B and analogues were reported in the literature.^[3, 10] Yet, surprisingly, no molecular probes deriving from this antitumor anhydrophytosphingosine have been disclosed to date. In order to explore this track, we first focused on the development of fluorophore-labelled derivatives. Comparative evaluation of all jaspine B structural analogues allowed delineation of several key structure-activity trends. The all-*S* stereochemistry of the tetrahydrofuran core is crucial for the cytotoxic activity^[11] as is the presence of the 1,2-aminoalcohol moiety.^[9, 12] On the other hand, modifications of the aliphatic chain, including in the terminal position, are reasonably well tolerated.^[12b, 13] On this basis, we envisioned jaspine B-related probes bearing a fluorescent tag at the terminus of the alkyl chain so as to minimize its impact on the biological behaviour. In order to ensure a synthetic flexible access to these probes, we choose to synthesize an ω -ethynyl jaspine B derivatives allowing a late introduction of the fluorophoric moiety through a copper-catalysed azide-alkyne cycloaddition (CuAAC) reaction (Fig 2).

Figure 2. Jaspine B probe.

The advantages of this biorthogonal click reaction have no longer to be demonstrated and its range of applications in chemical biology fully illustrates the scope of this transformation.^[14]

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Besides the fact that several azide-functionalized clickable fluorescent probes are commercially available to be engaged in the CuAAC click reaction with a ω -ethynyl jaspine B, such a strategy allows advantageously to introduce the fluorophoric group either at the end of the chemical synthesis or upon cell treatment with the acetylenic probe itself. The marginal structural modification introduced by the alkyne moiety at the extremity of the alkyl chain is indeed expected to minimize the impact on cell penetration, localization and biological activity as it was already observed for various terminal acetylenic lipids.^[15]

Bearing in mind that the overall length of the aliphatic backbone has a significant impact on jaspine B cytotoxicity,^[12b, 16] we targeted ω -alkyne-tagged analogues with two different lipidic chains: one presenting the same overall length (C14) as the natural compound and another one with the clickable alkyne tag added to the native jaspine B aliphatic tail (C16). These analogues were first prepared in racemic series according to a concise synthetic pathway we initially developed for the total synthesis of jaspine B.^[11b] This approach can also provide a practical access to both enantiomers of the targeted analogues through enantiomeric resolution of racemic materials.

Scheme 1. Synthesis of ω -ethynyl jaspine B analogues (±)-10a and (±)-10b. **Reagents and conditions:** a) Sulfone **3a** or **3b**, LiHMDS, THF then DBU, THF, (±)-**4a**: 43%, (±)-**4b**: 72%, E/Z 2:1; b) H₂, (PPh₃)RhCl, C₆H₆, EtOH, (±)-**5a**: 88%, (±)-**5b**: 90%; c) TBAF, THF, (±)-**6a**: 75%, (±)-**6b**: quant.; d) DMP, CH₂Cl₂, (±)-**7a**: 98%, (±)-**7b**: 84%; e) Bestmann-Ohira reagent, MeOH, (±)-**8a**: 93%, (±)-**8b**: 98%; f) i) CAN, CH₃CN:H₂O, (±)-**9a**: 70%, (±)-**9b**: 99%; ii) KOH, EtOH/H₂O, (±)-**10a**: 60%, (±)-**10b**: 89%.

The lactone oxazolidinone **1** (Scheme 1), obtained from 2,3-aziridino- γ -lactone **2**,^[12c] was submitted to a Julia modified olefination with the ω -functionalized sulfones **3a** or **3b**,^[17] to give **4a** and **4b**, respectively. Diastereoselective hydrogenation delivered in turn the fully protected intermediates **5a** and **5b**. The

terminal alcohol was then deprotected and oxidized into an aldehyde to give **7a** and **7b**. Homologation into the expected terminal alkyne was ensured by means of the Bestmann-Ohira reagent.^[18] Deprotection of the 1,2-aminoalcohol moiety then relied on the oxidative cleavage of the PMB-group (giving (±)-**9a** and (±)-**9b**) followed by the hydrolysis of the oxazolidinone ring to afford the targeted ω -ethynyl jaspine B (±)-**10a** and (±)-**10b**.

In order to assess the influence of the terminal alkyne tag on biological behavior of these new jaspine B analogues, their cytotoxicity was first evaluated and compared to that of the synthetic racemic jaspine B.^[11b]

The viability of U2OS (human osteosarcoma) cells was measured as previously described^[19] in the presence of (±)-jaspine B, (±)-**10a** and (±)-**10b**. The two clickable analogues (±)-**10a** and (±)-**10b** exhibit an IC₅₀ value of 56 nM and 60 nM, respectively (Table 1), significantly lower than that of (±)-jaspine B (IC₅₀ 186 nM). This finding supports the assumption that introducing an alkyne unit at the end of the anhydrophytoshingosine alkyl chain does not indeed significantly alter the biological activity of the resulting probe.

Table 1. Cytotoxicity of (±)-jaspine B, (±)-**10a** and (±)-**10b** on U2OS cells; IC₅₀ are calculated using GraphPad Prism 5.

Compound	(±)-Jaspine B	(±)- 10a	(±)- 10b
IC ₅₀ (μM)	0.186	0.056	0.060
95% confidence interval	0.168-0.207	0.050-0.063	0.057-0.064
n =	4	4	4

We then tackled the preparation of enantiopure materials. The ω -ethynyl jaspine B (±)-**10b** bearing a homologated C16 lipidic chain was selected on the basis of the commercial availability of the corresponding starting pentadecanol. The enantiomeric resolution was carried out on the racemic mixture of protected analogue (±)-**8b** by means of semipreparative supercritical fluid chromatography (SFC) using a Chiralpak IC column (5 mm silica particles immobilized with cellulose tris-(3,5-dichlorophenyl)carbamate) eluted with 20% of a CH₂Cl₂/MeOH mixture (80:20, v/v) as an additive in a CO₂ mobile phase. Both enantiomers were obtained in 88% yield and with an enantiomeric purity > 99.5% according to its re-analysis by chiral SCF (see ESI). The all-S absolute configuration was assigned to the dextrogiere enantiomeric sample on the basis of the parent jaspine B.

Each enantiomer of the protected ω -ethynyl jaspine B analogue (+)-**8b** and (-)-**8b** was in turn individually submitted to the two steps deprotection protocol leading to (+)-**10b** and (-)-**10b** (Scheme 2).

Scheme 2. Synthesis of ω -ethynyl analogues of jaspine B and *ent*-jaspine B. *Reagents and conditions:* a) i) CAN, CH₃CN:H₂O, (+)-**9b**: 67%, (-)-**9b**: 84%; ii) KOH, EtOH/H₂O, (+)-**10b**: 79%, (-)-**10b**: 67%.

The cytotoxicity of each enantiomer of ω -ethynyl jaspine B (+)-**10b** and (-)-**10b** was evaluated in U2OS cells and compared with those of natural (+)-jaspine B and its synthetic enantiomer (-)-jaspine B (Table 2).

Table 2. Cytotoxicity of (+)-jaspine B, (-)-jaspine B, (+)-**10b** and (-)-**10b** on U2OS cells; IC₅₀ are calculated using GraphPad Prism 5..

Compound	(+)-Jaspine B	(-)-Jaspine B	(+)- 10b	(-)- 10b
IC ₅₀ (μ M)	0.109	2.298	0.034	1.532
95% confidence interval	0.102-0.116	2.117-2.494	0.031-0.038	1.394-1.685
n =	8	8	4	4

In coherence with what is observed for the natural product and its enantiomer, the absolute configuration of the ω -ethynyl jaspine B analogues proved to be determinant for the cytotoxicity. The dextrogyre (S,S,S)-**10b** corresponds to the eutomer that, in addition, displays a significantly superior potency than its enantiomer (eudismic ratio 0.025). Of note is also the fact that (+)-**10b** is 3.2 times more cytotoxic than the synthetic sample of natural (+)-jaspine B. There is indeed, to the best of our knowledge, no precedent report of an analogue of jaspine B with enhanced cytotoxicity.

As mentioned earlier, the CuAAC click reaction is orthogonal enough to tolerate many functional groups. We thus envisaged performing this reaction with the totally unprotected analogue **10b**. However, our first attempts to introduce a fluorophore by means of a CuAAC reaction between the terminal alkyne group of **10b** and an azide-tagged fluorescent probe proved troublesome. The reaction under standard conditions (catalytic CuSO₄ with sodium ascorbate as reducing agent) gave no transformation. The use of an over-stoichiometric amount of Cu(I) (2 eq. of CuI) in the presence of a copper ligand tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (2 eq. of TBTA) over a prolonged reaction time (>48h) were necessary to form the expected cycloadducts. As a representative example, **10b** was engaged in a CuAAC reaction with azido derivatives of NBD or

pyrene (Scheme 3 and ESI). Yet, the expected click reaction products were only obtained with moderate yields (<50%) and revealed surprisingly unstable. These disappointing results further prompted us to exploit the *in situ* click labelling approach.

Scheme 3. CuAAC click reaction of **10b**. *Reagents and conditions:* a) CuI (2 eq.), *N,N*-diisopropylethylamine (DIPEA, 2 eq.), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA, 2 eq.), N₃-R (1 eq.), 48 h.

To reveal the subcellular distribution of jaspine B, we analyzed the subcellular distribution of (-)-**10b** and (+)-**10b** in U2OS cells. U2OS cells are derived from a human osteosarcoma, a rare pediatric cancer for which treatments are limited. U2OS are characterized by large nucleus and cytoplasm and have therefore been extensively used for analyzing the subcellular distribution of clickable small molecules in several studies.^[20] Furthermore, reference pictures of U2OS cells subcellular compartments, obtained through the Cell Painting assay,^[21] are available from the Broad Institute Image Cell Library^[22] facilitating the analysis of patterns observed in these cells. U2OS cells were treated 2h with 0.5 μ M (+)-**10b**, (-)-**10b** or with the vehicle alone and fixed the cells with 2% paraformaldehyde. After permeabilization with 0.2% (v/v) Triton X-100, the CuAAC reaction was performed *in situ* with an azido-AlexaFluor 594 fluorophore, as described previously.^[20a, 23] This experiment revealed a different distribution of (+)-**10b** and (-)-**10b** in cells (Fig 3).

Figure 3. Imaging (+)-10b and (-)-10b distribution in U2OS cells. Post-fixation *in situ* CuAAC-mediated ligation of an azido-AlexaFluor 594 was used to reveal the cellular distribution of the clickable probes. White arrows indicate cytoplasmic aggregates. The white scale bar corresponds to 10 microns.

Compound (-)-10b showed a weak cytoplasmic staining evocative of endoplasmic reticulum. In addition to a similar but brighter pattern, the eutomer (+)-10b formed large and intense aggregates in the cytoplasm of treated U2OS cells. A similar subcellular distribution of (-)-10b and (+)-10b was observed using A549 cells (see ESI). A549 cells are derived from a human lung cancer, are characterized by large nucleus and cytoplasm facilitating imaging and their subcellular compartments have also been analyzed through the Cell Painting assay.^[21] To confirm that the pattern observed with the clickable analogue (+)-10b reflects the (+)-jaspline B distribution, we performed a competition experiment on living cells by co-incubating the (+)-10b with a 20-fold excess of (+)-jaspline B. As shown on figure 4, the cytoplasmic aggregates observed with (+)-10b are lost when (+)-10b is incubated with an excess of non clickable natural (+)-jaspline B. Together these results strongly support that (+)-10b can be used as a surrogate to analyze jaspine B subcellular distribution and to clarify the elusive mechanism of action of this potential anticancer agent.

We disclosed here the first design and synthesis of biologically relevant clickable analogues of jaspine B. In contrast to previously reported analogues of jaspine B,^[3] our clickable derivatives present a cytotoxic activity superior to the natural anhydrophytosphingosine. Taking advantage of these clickable probes, we established that jaspine B accumulates in the cytoplasm of human cells in vesicular structures and forms large aggregates. The nature of these aggregates and their relationship with jaspine B cytotoxic activity await further investigations. The potential use of the reported enantiomeric clickable probes in a chemoproteomic target identification approach will also be explored in due course.

Figure 4. Competing (+)-10b staining with (+)-jaspline B. U2OS cells were treated 2h with 0.5 μ M (+)-10b alone, as a reference, or together with 10 μ M (+)-jaspline B, corresponding to a 20-fold excess. Post-fixation *in situ* CuAAC-mediated ligation of an azido-AlexaFluor 594 was used to reveal the cellular distribution of (+)-10b. White arrows indicate cytoplasmic aggregates. The white scale bar corresponds to 10 microns.

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Keywords: JaspineB • Pachastrissamine • Fluorescent probe • Click chemistry • Bioorthogonality

- [1] V. Ledroit, C. Debitus, C. Lavaud and G. Massiot, *Tetrahedron Lett.* **2003**, 44, 225-228.
- [2] I. Kuroda, M. Musman, I. I. Ohtani, T. Ichiba, J. Tanaka, D. G. Gravalos and T. Higa, *J. Nat. Prod.* **2002**, 65, 1505-1506.
- [3] M. Martinková and J. Gonda, *Carbohydr. Res.* **2016**, 423, 1-42.
- [4] D. Canals, D. Mormeneo, G. Fabrias, A. Llebaria, J. Casas and A. Delgado, *Bioorg. Med. Chem.* **2009**, 17, 235-241.
- [5] Y. Salma, E. Lafont, N. Therville, S. Carpentier, M. J. Bonnafe, T. Levade, Y. Génisson and N. Andrieu-Abadie, *Biochem. Pharmacol.* **2009**, 78, 477-485.
- [6] Y. Yoshimitsu, S. Oishi, J. Miyagaki, S. Inuki, H. Ohno and N. Fujii, *Bioorg. Med. Chem.* **2011**, 19, 5402-5408.

- [7] H. Yoo, Y. S. Lee, S. Lee, S. Kim and T. Y. Kim, *Phytotherapy Res.* **2012**, *26*, 1927-1933.
- [8] C. Santos, F. Rodriguez, V. Garcia, D. Moravcikova, D. Berkes, A. Daich, T. Levade, C. Baudoin-Dehoux, S. Ballereau and Y. Génisson, *Chembiochem* **2014**, *15*, 2522-2528.
- [9] F. Cingolani, F. Simbari, J. L. Abad, M. Casasampere, G. Fabrias, A. H. Futerman and J. Casas, *J. Lipid Res.* **2017**, *58*, 1500-1513.
- [10] a) S. Ballereau, M. Baltas and Y. Génisson, *Curr. Org. Chem.* **2011**, *15*, 953-986; b) E. Abraham, S. G. Davies, P. M. Roberts, A. J. Russell and J. E. Thomson, *Tetrahedron: Asymmetry* **2008**, *19*, 1027-1047.
- [11] a) M. Martinková, E. Mezeiová, M. Fabišková, J. Gonda, M. Pilátová and J. Mojžiš, *Carbohydr. Res.* **2015**, *402*, 6-24; b) C. Santos, I. Fabing, N. Saffon, S. Ballereau and Y. Génisson, *Tetrahedron* **2013**, *69*, 7227-7233; c) G. Jayachitra, N. Sudhakar, R. K. Anchoori, B. V. Rao, S. Roy and R. Banerjee, *Synthesis* **2010**, 115-119.
- [12] a) S. Pashikanti, R. Ukani, S. A. David and A. Datta, *Synthesis* **2017**, *49*, 2088-2100; b) Y. Salma, S. Ballereau, S. Ladeira, C. Lepetit, R. Chauvin, N. Andrieu-Abadie and Y. Génisson, *Tetrahedron* **2011**, *67*, 4253-4262; c) S. Ballereau, N. Andrieu-Abadie, N. Saffon and Y. Génisson, *Tetrahedron* **2011**, *67*, 2570-2578.
- [13] a) E. Zhang, S. Wang, J. Gao, X. J. Shi, M. M. Wang, S. M. Xu and H. M. Liu, *Heterocycles* **2016**, *92*, 2018-2031; b) J.-M. Xu, E. Zhang, X.-J. Shi, Y.-C. Wang, B. Yu, W.-W. Jiao, Y.-Z. Guo and H.-M. Liu, *Eur. J. Med. Chem.* **2014**, *80*, 593-604; c) Y. Salma, S. Ballereau, C. Maaliki, S. Ladeira, N. Andrieu-Abadie and Y. Génisson, *Org. Biomol. Chem.* **2010**, *8*, 3227-3243.
- [14] P. Thirumurugan, D. Matosiuk and K. Jozwiak, *Chem. Rev.* **2013**, *113*, 4905-4979.
- [15] A. Gaebler, R. Milan, L. Straub, D. Hoelper, L. Kuerschner and C. Thiele, *J. Lipid Res.* **2013**, *54*, 2282-2290.
- [16] Y. Génisson, L. Lamande, Y. Salma, N. Andrieu-Abadie, C. Andre and M. Baltas, *Tetrahedron: Asymmetry* **2007**, *18*, 857-864.
- [17] J. Pospíšil and H. Sato, *J. Org. Chem.* **2011**, *76*, 2269-2272.
- [18] G. J. Roth, B. Liepold, S. G. Müller and H. J. Bestmann, *Synthesis* **2004**, *2004*, 59-62.
- [19] V. Vichai and K. Kirtikara, *Nat. Protoc.* **2006**, *1*, 1112.
- [20] a) R. Rodriguez, K. M. Miller, J. V. Forment, C. R. Bradshaw, M. Nikan, S. Britton, T. Oelschlaegel, B. Xhemalce, S. Balasubramanian and S. P. Jackson, *Nat. Chem. Biol.* **2012**, *8*, 301; b) E. Zacharioudakis, P. Agarwal, A. Bartoli, N. Abell, L. Kunalingam, V. Bergoglio, B. Xhemalce, K. M. Miller and R. Rodriguez, *Angew. Chem. Int. Ed.* **2017**, *56*, 6483-6487; c) T. T. Mai, A. Hamaï, A. Hienzsche, T. Cañeque, S. Müller, J. Wicinski, O. Cabaud, C. Leroy, A. David, V. Acevedo, A. Ryo, C. Ginestier, D. Birnbaum, E. Charafe-Jauffret, P. Codogno, M. Mehrpour and R. Rodriguez, *Nat. Chem.* **2017**, *9*, 1025.
- [21] M.-A. Bray, S. Singh, H. Han, C. T. Davis, B. Borgeson, C. Hartland, M. Kost-Alimova, S. M. Gustafsdottir, C. C. Gibson and A. E. Carpenter, *Nat. Protoc.* **2016**, *11*, 1757.
- [22] M.-A. Bray, S. M. Gustafsdottir, M. H. Rohban, S. Singh, V. Ljosa, K. L. Sokolnicki, J. A. Bittker, N. E. Bodycombe, V. Dančik, T. P. Hasaka, C. S. Hon, M. M. Kemp, K. Li, D. Walpita, M. J. Wawer, T. R. Golub, S. L. Schreiber, P. A. Clemons, A. F. Shamji and A. E. Carpenter, *GigaScience* **2017**, *6*, giw014-giw014.
- [23] D. Larrieu, S. Britton, M. Demir, R. Rodriguez and S. P. Jackson, *Science* **2014**, *344*, 527-532.

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The synthesis of the first biologically relevant clickable probes related to the natural antitumor jaspine B is reported. These enantiomeric probes revealed a chirality-dependent cytoplasmic imaging by *in cellulo* click labelling.