First Synthesis of Lissoclimide-Type Alkaloids

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Received February 03, 2009: Revised March 23, 2009: Accepted March 24, 2009

Abstract: The first synthesis of lissoclimide-type alkaloids is described. Starting from commercial (+)-sclareolide, the aldehyde γ-bicyclohomofarnesal is synthesized and coupled, through an aldol reaction, with succinimide. The antitumor activity of several lissoclimide analogues is also reported.

Keywords: Lissoclimide, haterumaimide, diterpene, alkaloid, cytotoxicity, aldol.

Among marine organisms, tunicates (ascidians) and mollusks have become a prolific source of novel bioactive secondary metabolites [1]. As part of a study on the cytotoxic metabolites of a New-Caledonian tunicate, *Lissoclinum voeltzkowi*, the natural product dichlorolissoclimide (**1**) (IC_{50} = 0.001 μg/mL against P388 cells) was isolated in 1991 by Malochet-Grivois and co-workers [2]. The chemical structure of **1** was later confirmed by X-ray crystallographic analysis and an initial misassignment was corrected [3]. In 1994, the same research team isolated the corresponding monochloro-derivative, chlorolissoclimide (**2**) [4]. Structurally, both metabolites share the same labdane carbon framework, and display one or two chlorine atoms and a succinimide ring, which are extremely rare features among marine natural products. In addition, both compounds displayed very high levels of cytotoxicity against a number of different tumor cell-lines.

During the years 2001-2002, Ueda and co-workers described the isolation of eleven related compounds from the ascidian *Lissoclinum* sp., which were named haterumaimides A-K because the ascidian was collected off the coast of Hateruma Island, Okinawa, Japan [5]. All the haterumaimides exhibited cytotoxicity against P388 cells. The same authors have recently described the isolation of four additional compounds of this family, named haterumaimides N-Q [6]. Also recently, Schmitz and co-workers have described three new structures, isolated from the mollusks *Pleurobranchus albiguttatus* and *Pleurobranchus forskalii*, of which two have been renamed as haterumaimides L and M [7]. The latter was the first report of the isolation of *Lissoclinum* metabolites from its predators *Pleurobranchus*. Interestingly, all these compounds displayed potent *in vitro* cytotoxicity against different tumor cell-lines.

Due to the potent cytotoxicity and unique structural features of these molecules, several biological and synthetic studies have been reported. For example, dichlorolissoclimide (**1**) and chlorolissoclimide (**2**) have been studied *in vitro* on a human non-small-cell brochopulmonary carcinoma line at the cell cycle level [8]. Also, both compounds have demonstrated that they inhibit protein synthesis blocking translation elongation by inhibiting translocation [9]. Among the synthetic community, Jung and co-workers have long studied the introduction of chlorine atoms in model molecules with the ring system similar to that of chlorolissoclimides [10]. Owing to the promising activity and low natural abundance of these molecules, synthesis remains an important technique in the production of these compounds in sufficient quantities for further study. However, to the best of our knowledge, no synthesis has been reported in the literature to date.

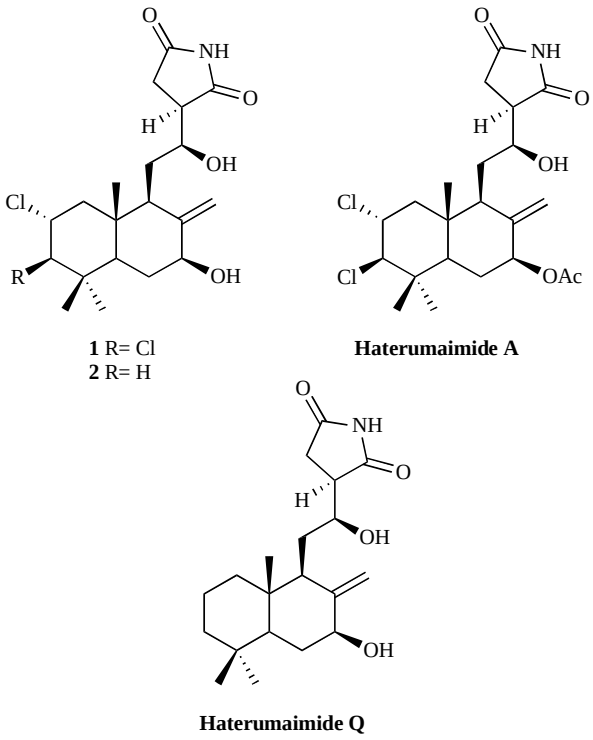

Fig. (1). Some examples of lissoclimide-type alkaloids.

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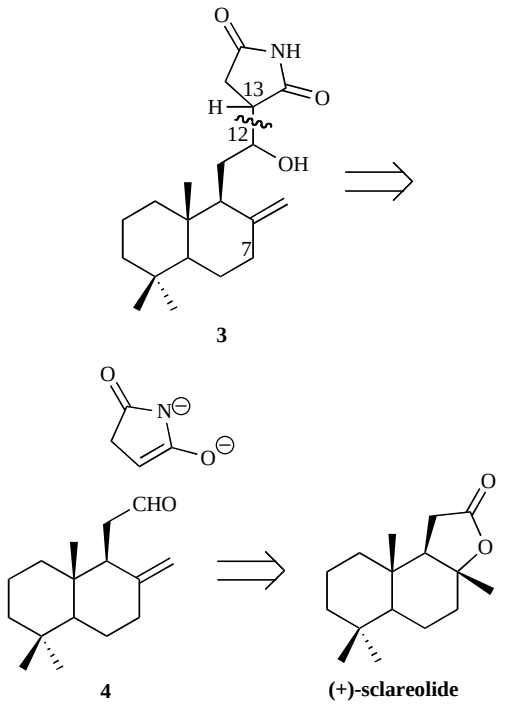


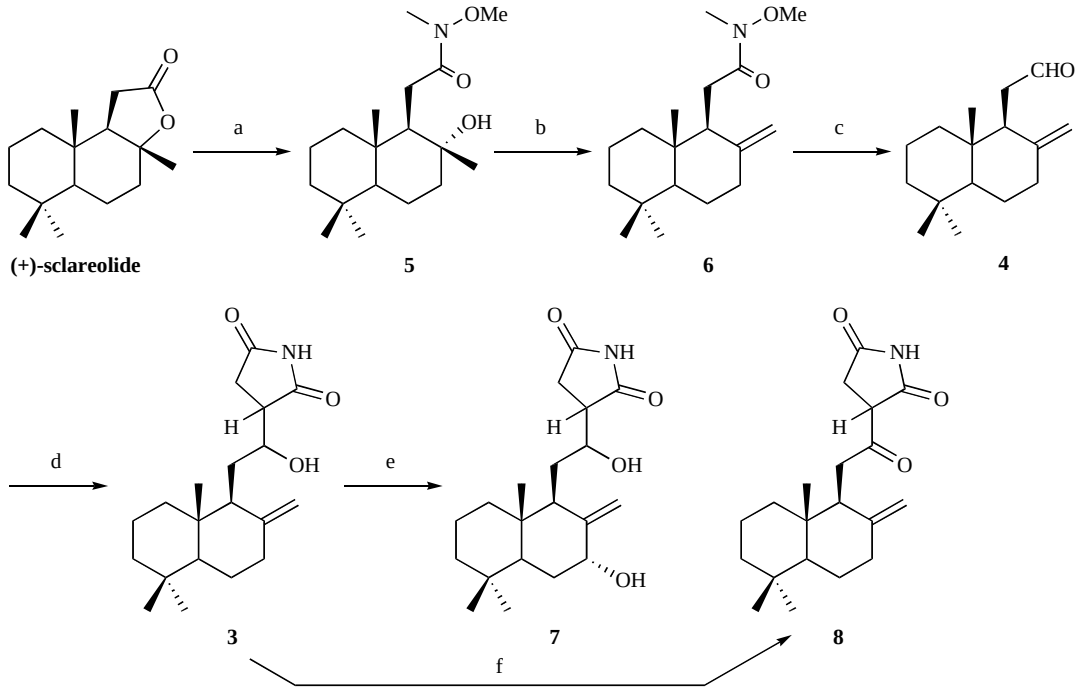
Scheme 1. Retrosynthesis of lissoclimide skeleton.

In this letter, we describe the first synthesis of lissoclimide-type diterpenes. In addition to this synthetic work, we have also carried out *in vitro* analysis of the antitumor activity of the molecules prepared in our laboratory.

Our retrosynthesis towards lissoclimides focused in the aldol condensation between aldehyde **4** and the enolate of succinimide (Scheme **1**). Aldehyde **4** is a known intermediate in the synthesis of different terpenoid systems and can be prepared in three steps starting from commercially available (+)-sclareolide [11]. The key aldol reaction with succinimide has little precedent in the literature, and to the best of our knowledge, only one article reports the aldol condensation between the dipotassium salt of succinimide and aldehydes [12]. Similarly, only one method for the synthesis of the hydroxyalkylated imide moiety present in the lissoclimides has been reported [13]. Thus, the introduction of the succinimide moiety represented a significant and unprecedented synthetic challenge in the context of this family of natural products.

The synthesis began with the preparation of aldehyde **4** from commercially available (+)-sclareolide. Thus, (+)-sclareolide was reacted with the dimethylaluminium amide derived from *N*-methoxy-*N*-methylamine yielding the desired Weinreb´s amide **5** in 85% yield (Scheme **2**). The tertiary alcohol of amide **5** was dehydrated in the presence of $SOCl_2$/pyridine at -78 ºC to give essentially the *exo*-isomer **6** in 90% yield. Finally, compound **6** was treated with DIBAL-H at -78 ºC to give the desired aldehyde **4** in 92% yield [11].

With the aldehyde **4** in hand, our next objective was to carry out the introduction of the succinimide moiety. Thus, a solution of succinimide in THF was added to a solution of LiHMDS at -78 ºC to generate the dianion of succinimide. After 50 min, a solution of aldehyde **4** was added and stirred for 90 min at the same temperature. After quenching with saturated NH_4Cl and extraction with ethyl acetate the resulting residue was chromatographed to afford recovered starting material (20%) and 50% yield of aldol product **3** (Scheme **2**). The aldol product resulted to be by analysis of NMR data, a mixture of four diastereomers (40:35:15:10). All attempts to separate the diastereomers by flash chroma-



Scheme 2. *Reagents and conditions*: (a) MeONHMe·HCl, $AlMe_3$, DCM, 0˚C to rt, 85%; (b) $SOCl_2$/py, DCM, -78˚C, 90%; (c) DIBAL-H, Et_2O, -78˚C to -20˚C, 92%; (d) succinimide, LiHMDS, THF, 70%; (e) SeO_2, t-BuOOH, DCM, 40%; (f) Dess-Martin periodinane, DCM, 70%.

Table 1. Cytotoxic aCtivity of Lissoclimide Derivatives Determined by the MTT Technique Expressed as CC_{50} (μg/mL)[a]

Compound	Cell line[b]				
	Jurkat		Vero		
	CC_{50}	$R^{2,c}$	CC_{50}	R^2	SI[d]
3	4.3 ± 1.0	0.91	29.2 ± 5.3	0.75	6.7
7	48.2 ± 22.2	0.78	69.8 ± 4.3	0.99	1.4
8	14.6 ± 1.3	0.97	67.6 ± 12.0	0.84	4.6
	HeLa		**Vero**		
3	4.5 ± 1.2	0.94	29.2 ± 5.3	0.75	6.5
7	≥200	-	69.8 ± 4.3	0.99	-
8	37.7 ± 4.2	0.89	67.6 ± 12.0	0.84	1.8
Vincristine	0.05±0.01	0.95	1.1±0.2	0,95	22

[a]50% cytotoxic concentration in 48 h. [b]HeLa, human cervix epitheloid carcinoma; Vero, *Cercopithecus aethiops* African green monkey kidney; Jurkat, human acute lymphoblastic leukemia. [c]R^2: coefficient of determination of linear regression. [d]SI, selectivity index is defined as Vero CC50 over HeLa CC50 or Jurkat CC50.

tography were unsuccessful since all the diastereomers possess identical R_f. The stereochemistry at carbons C12 and C13 of the major diastereomers remains unknown. However, the carbon skeleton typical of the lissoclimide-type diterpenes has been successfully contructed following this methodology.

In order to evaluate the cytotoxicity of related oxidized analogues, we carried out the synthesis of several derivatives. For example, functionalization of C7 in **3** by allylic oxidation with SeO_2 gave the alcohol **7** (7-epi-haterumaimide Q), which was isolated as a ca. 1:1 mixture of diastereomers at C12 and C13. The stereochemistry of the hydroxy group at C7 was assigned as depicted based on precedents in the literature for similar oxidations [14].

In addition, we could observe in the ^{1}H NMR spectrum a signal (δ 4.50 ppm) corresponding to the H attached to C7 with very small coupling constants, which agrees with an equatorial orientation. Also, we carried out the oxidation of C12 in **3** with Dess-Martin periodinane [15] to obtain compound **8** (ca. 1:1 mixture at C13) in order to investigate the influence of this functional group in the biological activity of these analogues.

The lissoclimide derivatives **3**,**7** and **8** were tested *in vitro* for potential antitumor and cytotoxic activities determining the concentration of the compound that induces 50% killing (CC_{50}) of the HeLa and Jurkat tumor cell lines; and Vero cell line, respectively (Table **1**). The antitumor activity on HeLa and Jurkat cells, and cytotoxic activity on Vero cells have been carried out using tetrazolium- dye (MTT) assay *in vitro* on cell growth according to the protocol reported by us [16], which was used with a few modifications [17]. All these compounds produced a dose-dependent inhibition on the growth of cell lines, with R^2 (coefficient of linear regression) greater than 0.8. Both lissoclimide derivatives **3** and **8** showed *in vitro* antitumor activity and the lissoclimide derivative **3** showed the highest antitumor activity on the Jurkat tumor line with CC_{50} value of 4.3 ± 1 μg/mL. Also, the lissoclimide derivative with the highest selectivity index (SI) was the lissoclimide derivative **3**. Comparison of the antitumor activity data in the series of natural lissoclimides [6] and our synthetic lissoclimides shows that the stereochemistry at C7, C12 and C13 centers is key to retain the biological activity of this family of compounds.

In conclusion, we have assembled for the first time the lissoclimide carbon skeleton and demonstrated that simple lissoclimides derivatives exhibit moderate cytotoxicity. The identity of the pharmacophore has been confirmed as the hydroxyalkylated imide moiety, which in agreement with the work of Ueda and co-workers [6].

These results encourage us to continue our research of this series by controlling the stereochemistry of the key stereocenters with the aim of obtaining more potent analogues.

ACKNOWLEDGMENTS

Financial support from the Spanish Ministry of Science and Education, under a "Ramón y Cajal" research grant, and also from the Generalitat Valenciana (project GV/2007/007) is gratefully acknowledged. L. B-G. thanks the financial support from Antioquia University of Colombia and COLCIENCIAS, Bogotá, Colombia (Grant RC 432-2004).

REFERENCES

[1] Blunt, J. W.; Copp, B. R.; Hu, W.-P.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.*, **2008**, *25*, 35-94 and previous reports of this series.

[2] Malochet-Grivois, C.; Cotelle, P.; Biard, J. F.; Hénichart, J. P.; Debitus, C.; Roussakis, C.; Verbist, J. F. *Tetrahedron Lett.*, **1991**, *32*, 6701-6702.

[3] Toupet, L.; Biard, J. F.; Verbist, J. F. *J. Nat. Prod.*, **1996**, *59*, 1203-1204.

[4] Biard, J. F.; Malochet-Grivois, C.; Roussakis, C.; Cotelle, P.; Hénichart, J. P.; Debitus, C.; Verbist, J. F. *Nat. Prod. Lett.*, **1994**, *4*, 43-50.

[5] (a) Uddin, M. J.; Kokubo, S.; Suenaga, K.; Ueda, K.; Uemura, D. *Heterocycles*, **2001**, *54*, 1039-1047; (b) Uddin, M. J.; Kokubo, S.; Ueda, K.; Suenaga, K.; Uemura, D. *J. Nat. Prod.*, **2001**, *64*, 1169-1173; (c) Uddin, M. J.; Kokubo, S.; Ueda, K.; Suenaga, K.; Uemura, D. *Chem. Lett.*, **2002**, 1028-1029.

[6] Uddin, J.; Ueda, K.; Siwu, E. R. O.; Kita, M.; Uemura, D. *Bioorg. Med. Chem.*, **2006**, *14*, 6954-6961.

[7] Fu, X.; Palomar, A. J.; Hong, E. P.; Schmitz, F. J.; Valeriote, F. A. *J. Nat. Prod*., **2004**, *67*, 1415-1418.

[8] Malochet-Grivois, C.; Roussakis, C.; Robillard, N.; Biard, J. F.; Riou, D.; Débitus, C.; Verbist, J. F. *Anti-Cancer Drug Des.*, **1992**, *7*, 493-502.

[9] Robert, F.; Gao, H. Q.; Donia, M.; Merrick, W. C.; Hamann, M. T.; Pelletier, J. *RNA*, **2006**, *12*, 717-724.

[10] (a) Jung, M. E.; Gomez, A. V. *Tetrahedron Lett.*, **1993**, *34*, 2891-2894; (b) Jung, M. E.; Wasserman, J. I. *Tetrahedron Lett.*, **2003**, *44*, 7273-7275; (c) Jung, M. E.; Murphy, J. M. *Tetrahedron Lett.*, **2007**, *48*, 8388-8391.

[11] (a) de la Torre, M. C.; García, I.; Sierra, M. A. *Tetrahedron Lett.*, **2002**, *43*, 6351-6353; (b) Boukouvalas, J.; Wang, J. X.; Marion, O.; Ndzi, B. *J. Org. Chem.*, **2006**, *71*, 6670-6673.

[12] Bryant, D. R.; Hauser, C. R. *J. Am. Chem. Soc*., **1961**, *83*, 3468-3474.

[13] Kabata, M.; Suzuki, T.; Takabe, K.; Yoda, H. *Tetrahedron Lett.*, **2006**, *47*, 1607-1611.

[14] (a) Jung, M.; Ko, I.; Lee, S.; Choi, S. J.; Youn, B. H.; Kim, S. K. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 3295-3298; (b) Villamizar, J.; Salazar, F.; Fuentes, J.; Tropper, E.; Alonso, R. *J. Chem. Research (S)*, **2003**, 504-506; (c) Oh, S.; Jeong, I. H.; Shin, W.-S.; Wang, Q.; Lee, S. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 1656-1659.

[15] Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc*., **1991**, *113*, 7277-7287.

[16] Arnó, M.; Betancur-Galvis, L.; González, M. A.; Sierra, J.; Zaragozá, R. J. *Bioorg. Med. Chem.*, **2003**, *11*, 3171-3177.

[17] Cell monolayers were trypsinized and washed with culture medium and then plated at 1.5 x 10^4 and 3.3 x 10^4 cells per well for HeLa and Jurkat, respectively; and 1.25 x 10^4 cells per well for Vero cells in 96-well flat-bottomed plates. After 24 h of incubation, each diluted compound was added to the appropriate wells and the plates were incubated for further 48 h at 37 °C in a humidified incubator with 5% CO_2. Vincristine and Pyrogallol were used as positive controls. The CC_{50} for each compound were obtained from dose–effect curves for linear regression methods and CC_{50} values are expressed as the mean ± S.E.M. of least four dilutions by eight-duplicate.