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Preparation and antitrypanosomal activity of secochiliolide acid derivatives

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ABSTRACT

Secochiliolide acid (**1**) isolated from the Patagonian shrub *Nardophyllum bryoides*, was used as a scaffold for the preparation of a series of nine derivatives. Compound **1** and its derivatives were tested against *Trypanosoma cruzi* epimastigotes grown in liquid media. It was first observed that secochiliolide acid (**1**) inhibited the proliferation of the parasites, with an IC_{50} of 2 μg/mL. Six of the synthesized derivatives were also active with IC_{50}'s between 2 and 7 μg/mL which are comparable to that of the commercial drug benznidazole (2.5 μg/mL). These results indicate that the carboxyl group is not essential for the bioactivity of **1**, while the presence of the tetrasubstituted exocyclic double bond seems to be important. Moreover, the presence of the furan and spirolactone rings is not essential for the bioactivity *per se*, but is important in combination with other structural fragments present in the molecule.

Trypanosoma cruzi is a monoflagellate parasite that causes Chagas' disease, one of the major parasitic diseases in Latin America. These parasites go through various stages between vertebrate and invertebrate hosts.[1–4] The flagellate non-infective stage epimastigotes have been the most used for initial testing of drugs, because they can be grown *in vitro* and their life cycle is well known.[5] Although many efforts have been made for decades to find effective drugs against this parasite, the results are still frustrating. The main cause of failure for most of the compounds with trypanocidal activity is the occurrence of significant side effects on the patients, making them non-effective during the chronic phase of the disease.[6,7] Benznidazole and Nifurtimox have been compounds widely used against Chagas' disease, although their use is restricted because of their side effects.[7–9] Therefore, the search for new compounds with trypanocidal activity remains in the interest of many laboratories. In this endless search, one of the strategies is the screening of plant natural products, because of their abundance and structural variety in nature, and that in some cases they can be obtained in large quantities.[6,10]

In a previous Letter, we reported the isolation of secochiliolide acid (**1**) and other compounds from the Patagonian shrub *Nardophyllum bryoides*, and a preliminary study on their cytotoxic activity.[11] Secochiliolide acid was first isolated by Bohlmann and co-workers from *Nardophyllum lanatum* and the related species *Chiliotrichum rosmarinifolium*.[12] As was usual at that time, the authors only described the isolation and structure elucidation, and no bioactivity studies were performed on the compounds. Secochiliolide acid has a rare seco ent-halimane structure, with a spirolactone and a furan ring as characteristic moieties, and offers several sites for possible structural modifications. These structural features, together with the fact that **1** is a major metabolite in the ethanolic extract of *N. bryoides* and can be isolated in large amounts, make this compound an interesting scaffold for the preparation of derivatives. In particular the spirolactone moiety is present insome natural trypanocidal compounds such as psylostachyin, a sesquiterpene lactone, identified as one of the active components of *Ambrosia tennuifolia*, and previously isolated from other plants of the same genus,[13,14] which made secochiliolide acid an interesting compound to test for this type of activity. This hypothesis was rewarded by initial promising results, which led to the preparation of a series of derivatives that are described herein, as well as their effects on *T. cruzi* epimastigotes. Some of these compounds were ac-

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tive against the parasites at low IC_{50}'s, comparable to that of benznidazole.

A simplified protocol was established for the isolation of **1** in a preparative scale which took advantage of the acidity of the compound, minimizing the chromatographic steps and avoiding the use ofHPLC for the final purification. As in our previous work, the crude ethanolic extract of fresh *N. bryoides* was concentrated to an aqueous suspension and then partitioned between MeOH:H_2-O (9:1) and cyclohexane to yield lipophilic and polar subextracts. The latter was concentrated under reduced pressure and then partitioned between EtOAc and 10% aqueous NaOH. The basic fraction was then acidified with HCl and extracted with EtOAc. This final organic fraction contained mainly **1** and flavonoids, and the final purification of the desired compound was achieved by Sephadex LH-20 permeation and flash column chromatography (Supplementary data).

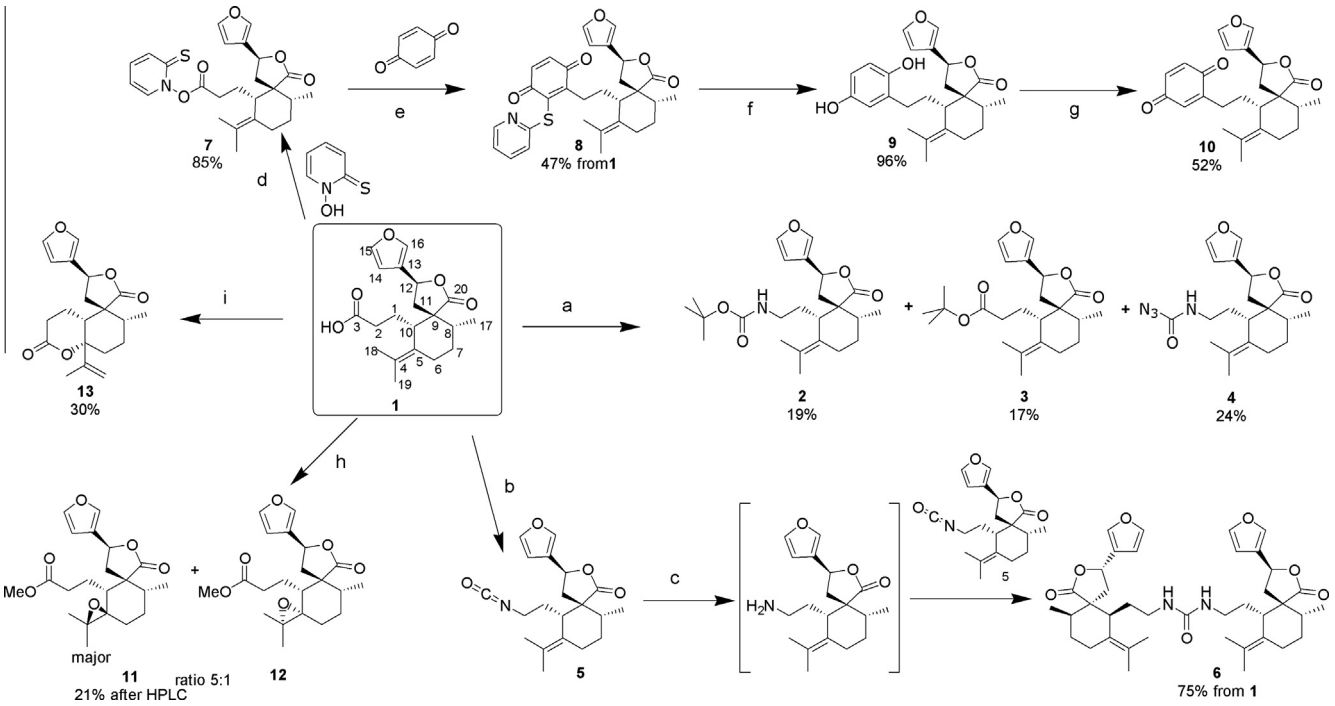
The structural modifications performed on **1** were focused on the carboxyl group and the double bond. The modifications on the carboxyl group include the introduction of functional groups bearingnitrogen atoms by means of the Curtius rearrangement, and the coupling with a benzoquinone moiety by means of a Barton decarboxylation reaction. The modifications performed on the double bond include a diasteroselective epoxidation and a bromocyclization reaction. All these modifications are depicted in Scheme 1.

Transformation of the carboxyl group into an acylazide would lead, by a Curtius rearrangement, to the obtention of the corresponding isocyanate, which in turn could be attacked by a variety of nitrogen nucleophiles, yielding aza-nor derivatives of **1**. Reaction of **1** with diphenylphosphorazidate (DPPA),[15] a mild azide donor, and *t*-butanol in an one-pot attempt to obtain the *t*-butylcarbamate, gave a mixture of three products: the desired product **2** (19%), the *t*-butyl ester **3** (17%) and the carbamoylazide **4** (24%).

The presence of compound **3** as a by-product is interesting since it is not easily prepared by other techniques, and can be explained by elimination of hydrazoic acid from an initially formed acylazide to yield a ketene, which then reacts with *t*-butanol to produce the ester **3**.[16] A possible hypothesis on the formation of compounds **3** and **4** is outlined in Scheme 2 (Supplementary data). In order to prevent the formation of compound **3**, toluene was used as solvent instead of *t*-butanol, which enabled the obtention of the isocyanate **5**. Treatment of crude **5** with base (KOH) in acetonitrile/H_2O yielded, instead of the expected primary amine, the dimeric urea **6** (75%). The formation of this interesting symmetric derivative can be explained by the attack of the initially formed amine, acting as a good nucleophyle, to the isocyanate **5**. This attack is faster than the corresponding by the hydroxyl group.

The incorporation of *p*-benzoquinone as a structural fragment, usually produces compounds with great bioactivity. In a previous publication we reported the preparation of norcholane-*p*-benzoquinone hybrids by means of a Barton decarboxylation reaction, which was inspired in a synthesis of avarone.[17–19] Following the same strategy, the Barton ester of **1** was prepared by reaction with *N*-hydroxy-2-thiopyridone in the usual way. Irradiation of the Barton ester **7** with a 300 W tungsten lamp generated the alkyl radical produced by decarboxylation of **1**, which was trapped by an excess of *p*-benzoquinone to yield the adduct **8**, which was reductively desulfurized with Raney Ni in CH_2Cl_2 to yield hydroquinone **9** in very good yield. Compound **9** was finally oxidized to the quinone **10** with MnO_2.

The epoxidation of the double bond of **1** would give access to a variety of additional derivatives, while the stereochemical relationships of the substituents in the six-membered ring of compound **1** would probably influence the diasteroselectivity of the reaction. Epoxidation with MCPBA was performed both on **1** and its methyl ester, which in turn was prepared by reaction of **1** with CH_2N_2 in ether. Reaction of the methyl ester of **1** with MCPBA in CH_2Cl_2 at 0° proceeded with complete conversion, yielding a 5:1 mixture of diasteromers, **11** and **12**. Both diasteromers could be purified by HPLC, and completely characterized. The major product



Scheme 1. Reagents and conditions: (a) DPPA, Et_3N, *t*BuOH, reflux 2 h; (b) DPPA, Et_3N, dry toluene, reflux 3 h; (c) KOH, CH_3CN:H_2O; (d) DCC, CH_2Cl_2, 0°; (e) CH_2Cl_2, 0°, hν (300 W), 30′; (f) Raney Ni, DME, reflux 20′; (g) MnO_2, Et_2O, rt 20′; (h) (i) CH_2N_2, ether, (ii) mCPBA, CH_2Cl_2, 0°; (i) (i) NBS, $NaHCO_3$, $CHCl_3$:EtOH (3:1), rt, (ii) HCl, acetone, rt.

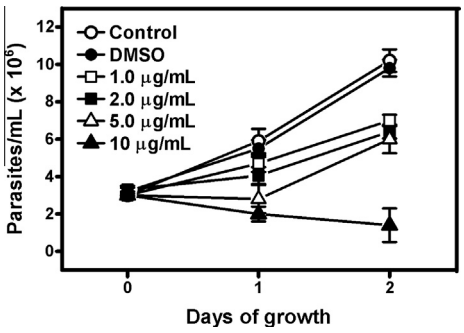


Figure 1. Effect of secochiliolide acid on the growth of *T. cruzi* epimastigotes. Parasites were incubated with the compound at the indicated concentrations. DMSO used as solvent did not affect the growth of parasites.

11 had the epoxide at the β-face, which could be explained by the combined steric effects of the side chain and Me-17, which are both located at the α-face. However, when the same reaction was performed on the acid **1**, the diasterofacial selectivity was lost, yielding a 1:1 mixture of diasteromeric epoxides. This may be explained if the free carboxyl group directs the epoxidation on the α-face, which would counterbalance the above mentioned steric effects. The use of a hydroxyl group to induce stereoselectivity has been previously reported.[20]

Encouraged by the previous results, a bromonium-catalyzed cyclization was performed on compound **1**. In this case, it was expected that the carboxyl side chain on the α-face would attack from the opposite face of a cyclic bromonium ion to give a lactone as final product. Bromination of **1** with NBS/$NaHCO_3$ in $CHCl_3$:MeOH (3:1) gave lactone **13** as the main product. The spectroscopic analysis of **13** (MS, NMR) showed that the molecule had no bromine atoms, and that an isopropenyl group had been formed. This structure is consistent with a reaction mechanism in which aninitially formed bromonium ion on the less hindered β-face was attacked from the opposite face by the carboxylate ion formed in the basic medium to yield an intermediate *cis*-lactone (which could not be detected), which then eliminates HBr to give compound **13**. Compound **13**, named secochiliolide lactone, had been previously isolated as a natural product by Bohlmann together with secochiliolide acid **1** from *N. lanatum*.[12] The easy formation of compound **13** from **1** suggests a possible biosynthetic pathway which can relate both compounds.

The activity of **1** and several derivatives was tested against *T. cruzi* epimastigotes grown in liquid media.[21] It was first observed that secochiliolide acid (**1**) inhibited the proliferation of parasites, partially at concentrations between 1 and 5 μg/mL, and completely

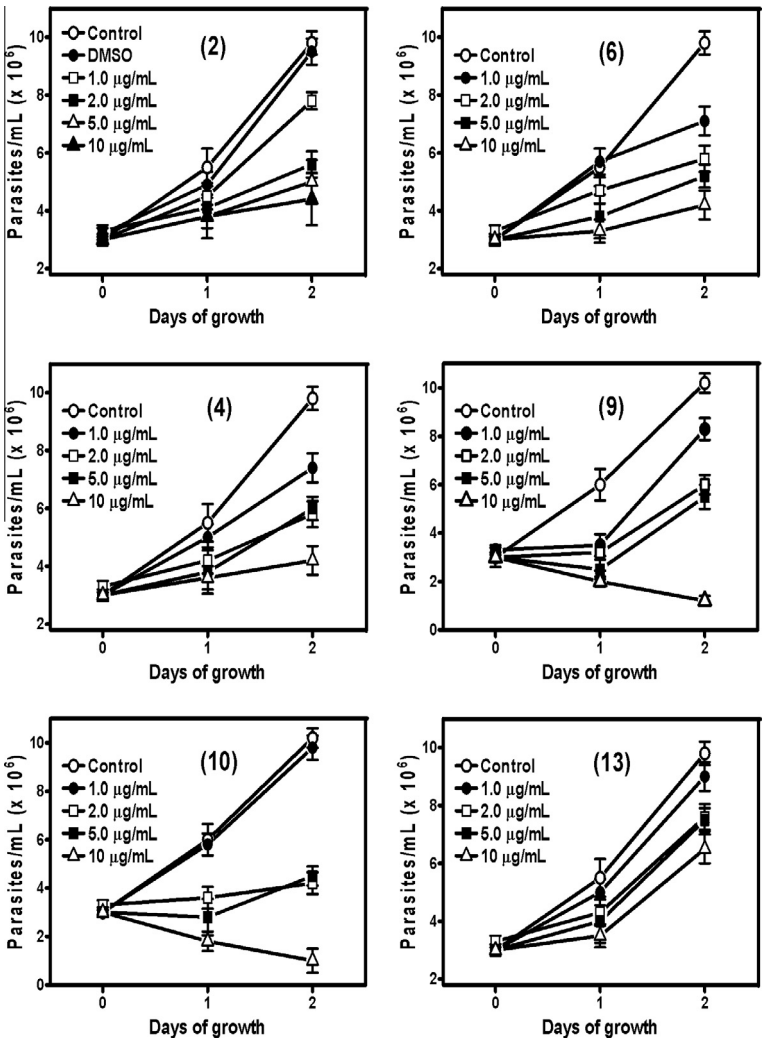


Figure 2. Effect of semisynthetic derivatives of secochiliolide acid on the growth of epimastigotes. The points on each curve represent the means of parasite concentration in three different experiments.

Table 1
Trypanocidal evaluation of secochiliolide acid and derivatives

Compd	1	2	3	4	5	6	9	10	11	13	Benznidazole
IC_{50} (μg/mL)	2	2	N.A.	4	N.A.	5	7	2	N.A.	5	2.5
S.E.M.	0.24	0.3		0.27		0.29	0.22	0.2		0.32	0.24

The values represent the mean ± SEM from three independent experiments.

at 10 μg/mL (Fig. 1). The estimated IC_{50} for compound **1** was 2 μg/mL, which is comparable with that of benznidazole. In order to identify the active groups of this compound, the previously described semi-synthetic derivatives of **1** were tested, (Fig. 2) and their IC_{50}'s are shown in Table 1. Six of the semi-synthetic derivatives (**2**, **4**, **6**, **9**,**10** and **13**) displayed activity against parasites in the same concentration range as benznidazole, while compounds **3**, **5** and **11** did not affect the growth of parasites. Some of the synthetic intermediates such as **7** and **8** were not tested. Among the semi-synthetic derivatives, only compounds **2** and **10** had a similar trypanocidal power (IC_{50} ~2 μg/mL) than secochiliolide acid, while the other compounds were less active (IC_{50} >5 μg/mL).

The results obtained in the present study indicate that secochiliolide acid **1** is a promising model compound against *T. cruzi*, since its IC_{50} was similar to that of benznidazole. The fact that this compound can be easily obtained in fairlylarge amounts from an abundant natural source, together with its relatively easy purification contribute to its importance as a new natural alternative to the highly toxic drugs actually in use. The results obtained from the semi-synthetic derivatives indicate that the carboxyl group is not essential for the bioactivity since its replacement by several other groups (quinone **10**, hydroquinone **9**, *t*-butylcarbamate **2**) yielded products that showed comparable activity or only a slight decrease in IC_{50}. Quite surprisingly, the *t*-butyl ester **3**, which can be metabolically converted to **1** by hydrolysis, was inactive. A significant result is the comparison of the almost identical growth curves for compounds **1**, **9** and **10** obtained at a concentration of 10 μg/mL, which inall cases indicate a complete inhibition of the parasite proliferation at that concentration, in spite of their final different IC_{50}.

On the other hand, the presence of the tetrasubstituted exocyclic double bond seems to be necessary for the bioactivity, since epoxidation produced an inactive derivative (**11**). Compound **13**, which also arises from a transformation on this double bond, is considerably less active than **1**. But, taking into account that the methyl ester group is still present in **11**, and the complex transformation that produced compound **13**, the loss of activity may not be attributed solely to the transformation of the double bond. The fact that the inactive compounds that were prepared still retain other important structural fragments such as the spirolactone and the furan ring, suggest that the observed activity of compound**1** may arise by a combination of the different structural fragments present in the molecule. Further modifications of **1** will be performed in order to validate these observations.

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Supplementary data

Supplementary data (experimental procedures, Scheme 2, spectroscopic data and copies of NMR spectra of all new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.064.

References and notes

1. Moloney, A. *Lancet* **2009**, *374*, 1490.
2. Morris, K. *Lancet Infect. Dis.* **2009**, *9*, 468.
3. The Lancet Editorial *Lancet* **2009**, *373*, 1820.
4. The Lancet Neurology *Lancet Neurol.* **2009**, *8*, 501.
5. De Souza, W. *Curr. Pharm. Des.* **2002**, *8*, 269.
6. Sepúlveda-Boza, S.; Cassels, B. K. *Planta Med.* **1996**, *62*, 97.
7. Duschak, V. G. *Recent Pat. Anti-Infect. Drug Discovery* **2011**, *6*, 216.
8. Amato Neto, V. *Mem. Inst. Oswaldo Cruz* **1999**, *94*, 337.
9. Maya, J. D.; Repetto, Y.; Agosin, M.; Ojeda, J. M.; Tellez, R.; Gaule, C.; Morello, A. *Mol. Biochem. Parasitol.* **1997**, *86*, 101.
10. Salem, M. M.; Werbovetz, K. A. *Curr. Med. Chem.* **2006**, *13*, 2571.
11. Sánchez, M.; Mazzuca, M.; Veloso, M. J.; Fernández, L. R.; Siless, G.; Puricelli, L.; Palermo, J. A. *Phytochemistry* **2010**, *71*, 1395.
12. Jakupovic, J.; Banerjee, S.; Bohlmann, F.; King, R. M.; Robinson, H. E. *Tetrahedron* **1986**, *42*, 1305.
13. Sülsen, V. P.; Frank, F. M.; Cazorla, S. I.; Anesini, C. A.; Malchiodi, E. L.; Freixa, B.; Vila, R.; Muschietti, L. V.; Martino, V. S. *Antimicrob. Agents Chemother.* **2008**, *52*, 2415.
14. Mabry, T. J.; Miller, H. E.; Kagan, H. B.; Renold, W. *Tetrahedron* **1966**, *22*, 1139.
15. Liang, H. *Synlett* **2008**, 2554.
16. Shiori, T. *TCIMail* **2007**, 134.
17. Barton, D. H.; Bridon, D.; Zard, S. *Tetrahedron* **1987**, *43*, 5307.
18. Ling, T.; Poupon, E.; Rueden, E.; Kim, S. H.; Theodorakis, E. A. *J. Am. Chem. Soc.* **2002**, *124*, 12261.
19. Siless, G. E.; Knott, M. E.; Derita, M. G.; Zacchino, S. A.; Puricelli, L.; Palermo, J. A. *Steroids* **2012**, *77*, 45.
20. Hoveyda, A. H.; Evans, D. A.; Fu, G. C. *Chem. Rev.* **1993**, *93*, 1307.
21. Lozano, E.; Barrera, P.; Tonn, C.; Nieto, M.; Sartor, T.; Sosa, M. A. *Parasitol. Int.* **2012**, *61*, 275.