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Semisynthesis of new aphidicolin derivatives with high activity against *Trypanosoma cruzi*

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ABSTRACT

Chagas disease continues to be a difficult disease to eradicate, largely because of the widespread populations it affects as well as the highly toxic effects of current therapies. Thus, the exploration of innovative scaffolds, ideally with distinct mechanisms of action, is urgently needed. The natural product aphidicolin and its effects on cell cycle division have been widely studied; it is a potent inhibitor of parasitic cells. In the present study, we report for the first time the semisynthesis of a series of aphidicolin derivatives, their unique structural features, and demonstration of their activity against *Trypanosoma cruzi* cells. Two demonstrated high potency and selectivity against parasitic amastigote cells, and thus show promise as new leads for Chagas disease treatment.

Finding new drug candidates for Chagas disease remains challenging. In the past 40 years, many synthetic and natural compounds have been assayed against *Trypanosoma cruzi*;[1] however, only a few compounds have advanced to clinical trials. The recent clinical trials with azoles, specifically posaconazole[2] and the ravuconazole prodrug E1224, were disappointing.[3] Therefore, public–private partnerships together with international research groups set comprehensive and well-considered criteria to ensure that the most promising chemical series are selected for further optimization and development.[4,5] These current requirements include higher activity when compared to benznidazole in an in vitro assay against the parasite, and selectivity index higher than or equal to 50 (cell culture parasite/mammalian cells). Compounds with <50% cell death can progress to screening in animal models.[6,7]

The establishment of this set of criteria, alongside appropriate chemical and molecular properties[8] of new hits and leads, would hopefully accelerate and reduce attrition in drug discovery for Chagas disease and other neglected tropical conditions. However, in order to develop innovative, safe, efficacious, and orally active drug candidates that can overcome the limitations of existing regimens, it is essential to expand the chemical library of natural and synthetic compounds available for biological assays. Only then would we be able to expand outside the flawed classes in current use (see Fig. 1).

Aphidicolin **1**, a widely used human DNA polymerase-α (Pol α) inhibitor, was previously described as a potent antiparasitic compound.[9–11] Despite these prominent activities, combining potency and selectivity for a parasite against human cells is an unsolved challenge. Although some derivatives of **1** demonstrated potential biological activities,[9] the medchem progression of hits and leads is limited by the available library of analogues, as well as the unknown mechanism of action. In this sense, increasing the chemical space of aphidicolin analogues, with diverse functional groups and chemical features, can enhance the potential to find and develop more effective compounds against parasitic cells.

Structurally, **1** is a tetracyclic diterpene with a rigid structure[12] and four hydroxyls with limited reactivity due to steric hindrance, intramolecular interactions, and low solubility in routine organic solvents, which create difficulties when exploring common synthetic strategies. From a mechanistic point of view, **1** inhibits cell division and cell cycle synchronicity.[13,14] However, previous studies show that **1** is rapidly metabolized in vivo by a specific cytochrome P-450, forming 3-ketoaphidicolin as a major and inactive metabolite.

Also, it was demonstrated in vitro that **1** is unable to inhibit parasitic Pol α, especially in trypanosomatids.[15] Recently, our group outlined the development of a small library of **1** derivatives against the *Leishmania* spp. cell lines, increasing the available library of drug-like aphidicolanes and highlighting the importance of this natural product as prototype for the development of drug

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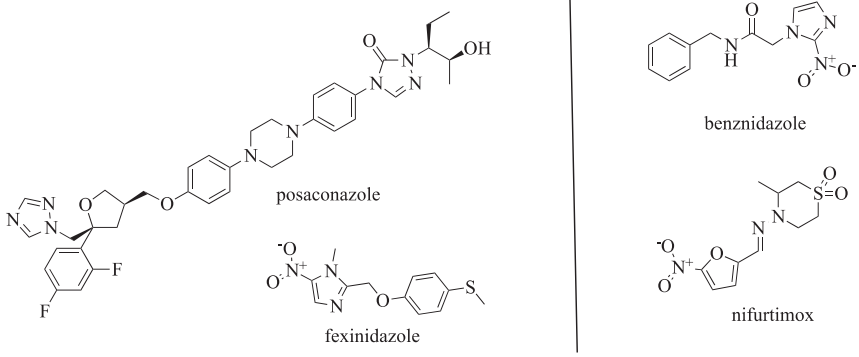
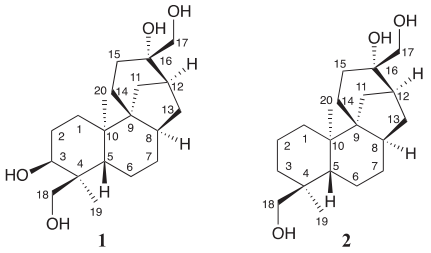
Drugs recently advanced for Clinical trials for Chagas disease **Avaiable drugs for Chagas disease**



Natural antiparasitic compounds: aphidicolin and 3-deoxy-aphidicolin



1, R=H, activity against *T. b. rhodesiens*e (118 nM) and *T. b. brucei* (120 nM)
R= glycinate, prodrug for human polymerase alpha and *L. donovani* at 40 mM

Figure 1. Numbered planar structure of aphidicolin (**1**) and deoxy-aphidicolin (**2**).

candidates for infectious diseases. Motivated by the need to increase therapeutic options for trypanosomiasis, especially Chagas disease, and by the preliminary antiparasitic findings for aphidicolanes, in this study we describe the development of two new potent and safe trypanocidal (amastigote form) aphidicolin derivatives, based on the modification of ring A and ring C, in an attempt to maintain the antiparasitic effect, reduce human toxicity and expand aphidicolanes library in order to help to elucidate the structural requirements for the antiparasitic activity of **1**, whereas introducing appropriate molecular properties for better biological profile. In this work, we chemically manipulated the free hydroxyls in order to help understanding the importance of the hydrogen bonds fraction, and related properties, for the antichagasic activity.

The tetracyclic diterpene aphidicolin **1** and 3-deoxy-aphidicolin **2** were isolated from cultures of the endophytic fungus *Nigrospora sphaerica*, as previously described.[16] The semisynthetic derivatives **3**–**7** were synthesized from **1** using a series of reactions involving protection-deprotection methodologies, oxidation, oximation, acylation, and heterocyclization (Scheme 1). Compounds **2**–**5** were previously reported by our research group (Fig. 2).[17]

The acylation of the hydroxyl groups of **1** was performed with benzoyl chloride and catalyzed by DMAP in pyridine, affording

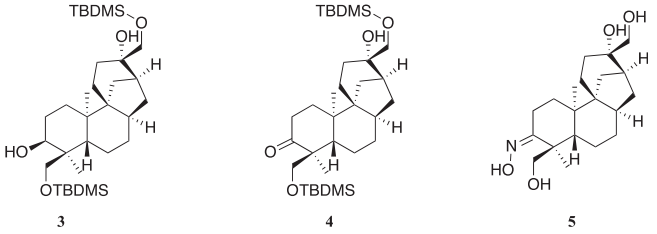
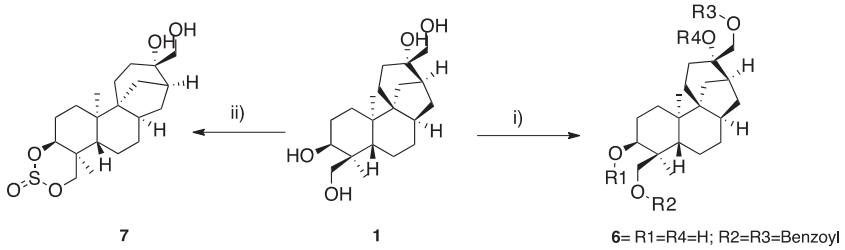


Figure 2. Structures of previously synthesized compounds **3**, **4** and **5**.

compound **6** in order to avoid the CYP-450 access to C-3 hydroxyl group with a steric hindrance at C-18 and create more highly lipophilic derivatives. The structure of the dibenzoate ester **6** was inferred by ^{1}H and ^{13}C NMR data, in which signals of ten aromatic protons at δ_H 8.04 (m, 2H), δ_H 7.98 (m, 2H), δ_H 7.64 (m, 2H), δ_H 7.52 (m, 4H), and two carbonyl carbons were observed (δ_C 165.9 and δ_C 165.8). The esterification at C-17 and C-18 was confirmed from diagnostic HMBC correlations from H-17 (δ_H 4.03) to C-12 (δ_C 41.1), C-16 (δ_C 72.0), and C=O (δ_C 165.9). In addition, H-18 showed key correlation to C=O (δ_C 165.8) and C-3 (δ_C 65.8). The structure



Scheme 1. (i) Benzoyl chloride, DMAP, Pyr, rt 1 h; 75% (ii) $SOCl_2$, rt 3 h; 48%.