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Simplifying nature: Towards the design of broad spectrum kinetoplastid inhibitors, inspired by acetogenins

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ABSTRACT

The need for new treatments for the neglected tropical diseases African sleeping sickness, Chagas disease and Leishmaniasis remains urgent with the diseases widespread in tropical regions, affecting the world's very poorest. We have previously reported bis-tetrahydropyran 1,4-triazole analogues designed as mimics of the annonaceous acetogenin natural product chamuvarinin, which maintained trypanocidal activity. Building upon these studies, we here report related triazole compounds with pendant heterocycles, mimicking the original butenolide of the natural product. Analogues were active against *T. brucei*, with a nitrofuran compound displaying nanomolar trypanocidal activity. Several analogues also showed strong activity against *T. cruzi* and *L. major*. Importantly, select compounds gave excellent selectivity over mammalian cells with a furan-based analogue highly selective while remaining active against all three cell lines, thus representing a potential lead for a new broad spectrum kinetoplastid inhibitor.

1. Introduction

As defined by the World Health Organization, a neglected tropical disease (NTD) is a tropical infection that affects millions of people worldwide[1] but has seen inadequate investigation into treatment.[2,3] This is principally due to the limited financial incentive in treating such conditions, which is linked to their prevalence in developing countries where healthcare spending is low and health infrastructure is poor. Three such diseases are African sleeping sickness,[4] Chagas disease[5] and Leishmaniasis,[6] spread by the related kinetoplastid parasitic causative agents *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* spp, respectively. These parasites are present in tropical regions across the world; *T. brucei* in sub-Saharan Africa, *T. cruzi* in South America and increasingly in North America[7] and *Leishmania* throughout the tropics and subtropics.[8] All three diseases share a genetic similarity and treatments developed for one specific disease have sometimes proved effective in treating another e.g. nifurtimox was originally developed for Chagas disease but has subsequently found application as a combination therapy for African sleeping sickness.[9,10]

The tropical plants of the Annonaceae species have long been known for their therapeutic properties, with the crude extracts of fruits, leaves, roots and bark used in traditional medicinal practices to treat a range of tropical diseases.[11] Interest in this plant species as a source of potential new drugs intensified with the discovery of a family of fatty acid derived natural products, collectively termed the Annonaceous acetogenins, which now number in excess of 400.[12] Structurally, the acetogenins are C32/C34 fatty acids generally characterised by a central array of one to three tetrahydrofuran (THF) rings with flanking hydroxyl groups and appended with a terminal butenolide ring system as found in squamocin **1** and senegalene **2** (Fig. 1).[13] In contrast the tetrahydropyran (THP) ring system is less common and only seven acetogenins bear this motif including chamuvarinin **3**. Members of this family of secondary metabolites are often highly cytotoxic displaying sub-nanomolar activity towards human cancer cell lines.[14]

By contrast, the biological evaluation of acetogenins for the treatment of NTD has thus far been remarkably limited considering the wide scale use across sub-Saharan Africa of acetogenin-containing crude plant extracts to treat African sleeping sickness.[15] Studies to date have primarily focussed on the phytochemical study of extracts from Annonaceae species for trypanocidal activity, with efforts focused on *T. brucei*,[16] though select compounds such as squamocin **1** have shown encouraging preliminary activity against *T. cruzi* and *Leishmania* strains.[17] In 2003 Laurens et al. reported that the root and bark extracts of *Uvaria chamae* are active towards *T. brucei*, which subsequently led to the isolation of chamuvarinin **3**.[18] Chamuvarinin is one of only seven acetogenins

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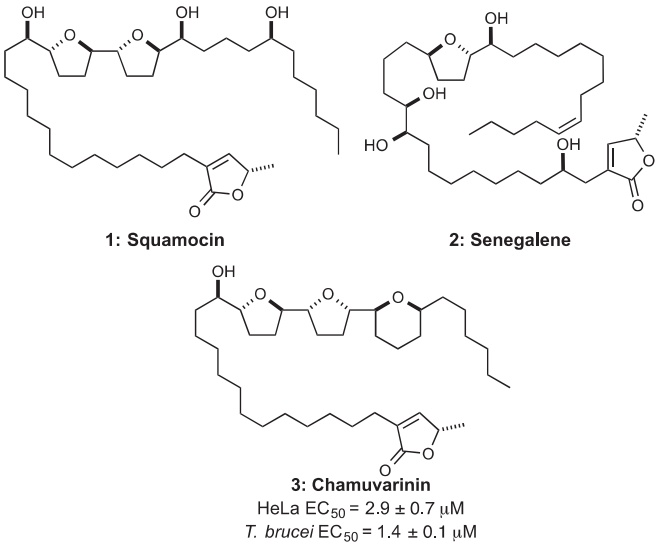


Fig. 1. Annonaceous acetogenins with known anti-kinetoplastid activity.

to contain a tetrahydropyran ring system and the only acetogenin to contain an all adjacently linked bis-THF-THP core ether ring system. In 2011, we reported the first total synthesis of chamuvarinin thereby establishing its absolute stereochemical structure. In addition our synthetic material displayed single figure micromolar activity towards the bloodstream form of *T. brucei brucei*, albeit with low selectivity over the HeLa human cell line.[19]

We built on these studies by designing a series of analogues inspired by chamuvarinin **3** that maintained *T. brucei* activity commensurate to that of the natural product (Fig. 2).[20] These analogues simplified the structure of chamuvarinin principally by replacing the central THF ring of the tricyclic core with a 1,4-triazole. This allowed rapid assembly of enantiomerically enriched THP building blocks via click chemistry, producing initial lead **4**, which displayed low (1.8 μM) trypanocidal activity against bloodstream form *T. brucei*. From this starting point, other features of the natural product were introduced, in particular the pendant butenolide, the orientation of which ('left- or right-hand side' as drawn) was critical to activity with **6** maintaining activity, while **5** was completely inactive. These results encouraged us to explore alternatives to the butenolide, principally on the azide, or 'left-hand side' of the molecule. Inspired by the work of Yamashita and co-workers, we have focused on simple aromatic heterocycles that were found to act as effective butenolide mimics.[21] Our phenotypic screening program has now expanded to the other NTD parasite cell lines, *T. cruzi* and *Leishmania major*, so new potential inhibitors were evaluated against all three protozoa.[22]

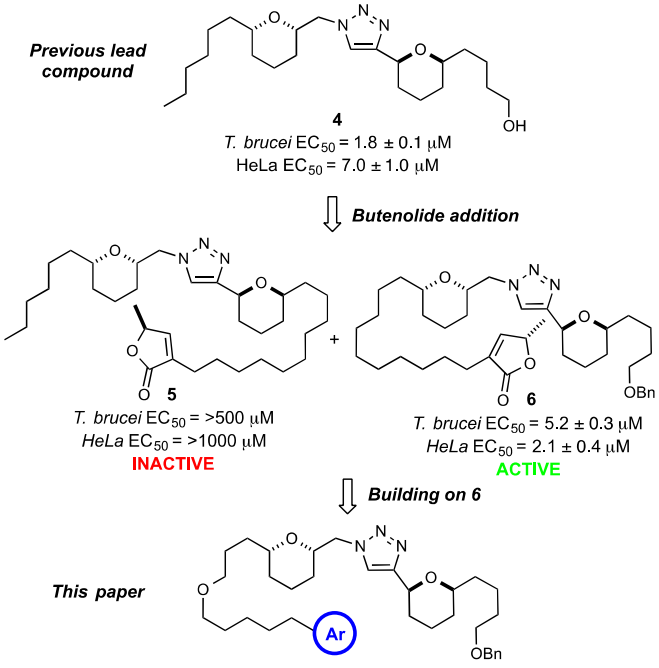


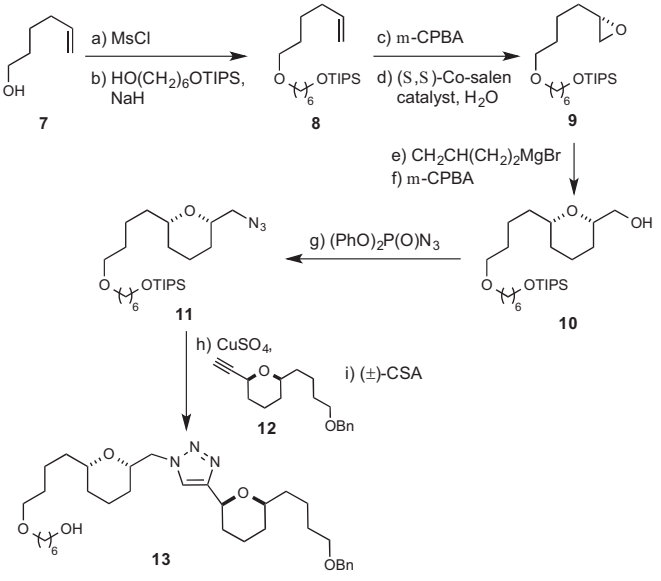
Fig. 2. Directionality in our triazole based *T. brucei* inhibitors.

2. Results and discussion

2.1. Synthesis

In order to rapidly access analogues with varying head groups, we targeted a THP precursor with an extended chain already present. Beginning from alcohol **7**, mesylation followed by S_N2 reaction with 6-((triisopropylsilyl)oxy)hexan-1-ol generated alkene **8** (Scheme 1).[23] After racemic epoxidation, kinetic resolution with Jacobsen salen catalyst produced chiral epoxide **9** with 95% ee.[24] Ring-opening with homoallyl magnesium bromide followed by epoxidation of the resulting double bond and *in situ* cyclisation generated THP alcohol **10** along with its diastereomeric pair (not shown) which were readily separated chromatographically. After conversion of the alcohol to azide **11** with DPPA, a "click" reaction with alkyne **12** generated the key THP-triazole-THP tricyclic core. Straightforward acidic deprotection then gave alcohol **13**.

Alcohol **13** served as a key point for derivatisation. Functional group interconversion of the alcohol via the azide provided amine **16** (Scheme 2). This then allowed the complete set of head group analogues **17–32** to be accessed via straightforward coupling with the appropriate acid in generally good yields. Targeted analogues focused on oxygen, nitrogen and sulfur containing five-membered aromatic heterocycles including furan, pyrrole and thiophene



Scheme 1. Synthesis of starting material: (a) MsCl, NEt_3, CH_2Cl_2, 0 °C → RT; (b) NaH, 6-((triisopropylsilyl)oxy)hexan-1-ol, THF, reflux, 91% (2 steps); (c) 3-chloroperbenzoic acid, CH_2Cl_2, 0 °C → RT, 83%; (d) 0.6 mol% (*S,S*)-Co-salen catalyst, 6 mol% AcOH, H_2O, THF, 49%; (e) $CH_2CHCH_2CH_2MgBr$, CuI, THF, −40 °C → RT, 84%; (f) 3-chloroperbenzoic acid, CH_2Cl_2, 0 °C → RT; then (±)-camphorsulfonic acid (20 mol%), RT, 43% *syn*, 38% *anti*; (g) diphenylphosphoryl azide, K_2CO_3, MeOH, RT, 83%; (h) $CuSO_4{\cdot}5H_2O$, Na ascorbate, H_2O, *t*-BuOH, RT, 89%; (i) (±)-camphorsulfonic acid (20 mol%), CH_2Cl_2:MeOH, RT, 88%.