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Research paper

Design, synthesis and anthelmintic activity of 7-keto-sempervirol analogues

Alessandra Crusco [a, b], Cinzia Bordoni [b], Anand Chakroborty [a], Kezia C.L. Whatley [a], Helen Whiteland [a], Andrew D. Westwell [b, **], Karl F. Hoffmann [a, *]

[a] Institute of Biological, Environmental and Rural Sciences (IBERS), Penglais Campus, Aberystwyth University, Aberystwyth SY23 3DA, United Kingdom
[b] School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff CF10 3NB, United Kingdom

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ABSTRACT

The plant-derived, diterpenoid 7-keto-sempervirol was recently reported to display moderate activity against larval stages of *Schistosoma mansoni* ($IC_{50} = 19.1\ \mu M$) and *Fasciola hepatica* ($IC_{50} = 17.7\ \mu M$), two related parasitic blood and liver flukes responsible for the neglected tropical diseases schistosomiasis and fascioliasis, respectively. Here, we aimed to increase the potency of 7-keto-sempervirol by total synthesis of 30 structural analogues. Subsequent screening of these new diterpenoids against juvenile and adult lifecycle stages of both parasites as well as the human HepG2 liver cell line and the bovine MDBK kidney cell line revealed structure-activity relationship trends. The most active analogue, **7d**, displayed improved dual anthelmintic activity over 7-keto-sempervirol ($IC_{50} \approx 6\ \mu M$ for larval blood flukes; $IC_{50} \approx 3\ \mu M$ for juvenile liver flukes) and moderate selectivity (SI ≈ 4–5 for blood flukes, 8–13 for liver flukes compared to HepG2 and MDBK cells, respectively). Phenotypic studies using scanning electron microscopy revealed substantial tegumental alterations in both helminth species, supporting the hypothesis that the parasite surface is one of the main targets of this family of molecules. Further modifications of **7d** could lead to greater potency and selectivity metrics resulting in a new class of broad-spectrum anthelmintic.

1. Introduction

Schistosomiasis, caused by infection with blood fluke schistosomes, is the most devastating human parasitic disease after malaria considering the number of people currently infected and at risk of infection [1]. It is a chronic disease of poverty characterized by pain and disability that, collectively, exacerbates the already compromised healthcare situation of developing tropical countries [2]. The number of people infected is approximately 600 million [3] and, along with 300 thousand deaths per year, schistosomiasis is considered as the most deadly neglected tropical disease (NTD) [4]. No vaccines are available to prevent infection and, therefore, treatment of infected individuals is predominantly facilitated by chemotherapy with praziquantel (PZQ) (Fig. 1A). PZQ is active against adult worms of all *Schistosoma* species, but less effective against the immature forms, leading easily to reinfections [5]; moreover, due to its large scale administration, concerns about drug resistance are increasing [5,6].

Another NTD predominantly controlled by a single drug, triclabendazole, is fascioliasis. Ingestion of liver fluke parasites initiates fascioliasis and leads to up to 17 million human [7] and numerous livestock animal infections per annum, causing enormous economic losses in the global beef, lamb and milk industries [8]. In the United Kingdom alone, an estimated £23 million is lost annually due to fascioliasis [9]. However, recent reports suggest that this figure could rapidly increase due to changes in global climate and extensive animal movement [10,11]. Triclabendazole (Fig. 1B) is the only commercially available drug that is active against both juvenile and adult liver fluke lifecycle stages. Unfortunately, triclabendazole resistant *Fasciola* parasites have been described throughout Asia, Africa, South America, North America, Australia and Europe [12], making the discovery of new drugs for combating this human and animal disease an urgent healthcare priority.

For these reasons, research into the discovery of new anti-flukicidal drugs is increasing with natural products being an

* Corresponding author.
** Corresponding author.
E-mail addresses: WestwellA@cf.ac.uk (A.D. Westwell), krh@aber.ac.uk (K.F. Hoffmann).

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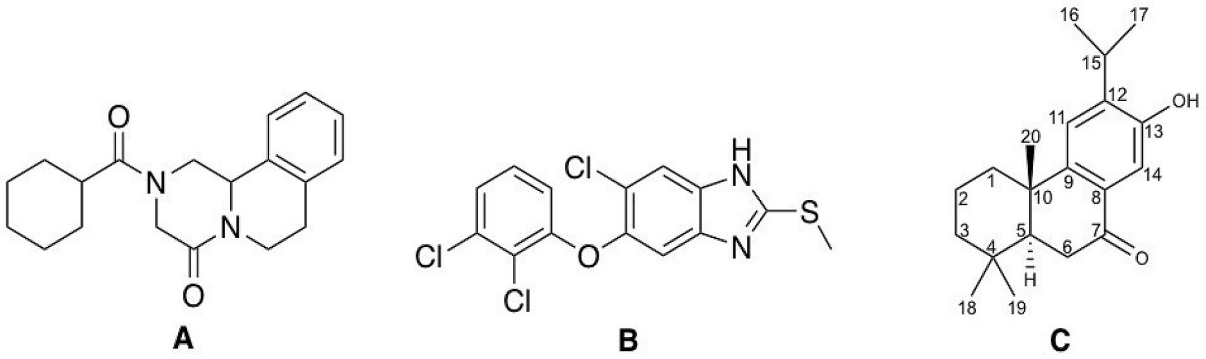

Fig. 1. Anthelmintic compound structures discussed in this study. Illustrated are the structures used for treating schistosomiasis, Praziquantel (A), and for fascioliasis, Triclabendazole (B). The anthelmintic diterpenoid 7-keto-sempervirol and the scaffold numbering system are also shown (C).

exceptionally attractive resource for beginning such a process [13]. Indeed, plant-derived natural products are a well-recognised source of anti-infective compounds due to natural selection shaping the production of more toxic and protective compounds to facilitate survival in a microorganisms-rich environment [14]. One example of a plant-derived compound isolated from *Lycium chinense* with bioactivity against both *Schistosoma mansoni* and *Fasciola hepatica* is the diterpenoid 7-keto-sempervirol (Fig. 1C) [15]. While this diterpenoid was only moderately potent, it was effective in killing juvenile and adult forms of both fluke species. Considering the fact that some endemic areas for schistosomiasis are co-endemic for fascioliasis and that, in these areas, it is common to find people with both infections [16–18], a compound with potent dual anthelmintic activity against multiple lifecycle stages would be highly desirable.

In this study, 7-keto-sempervirol was subjected to medicinal chemistry optimisation, with the aim of exploring whether different substitutions could increase the dual anthelmintic potency of this natural product. To achieve this, structural related analogues were obtained by total synthesis using the different synthetic pathways reported here. The compounds were subsequently screened against larval (schistosomula) and adult *S. mansoni* blood flukes as well as against newly excysted juvenile (NEJs), immature and adult *F. hepatica* liver flukes. The newly synthesised compounds were also screened against HepG2 liver human cells and MDBK kidney bovine cells to assess potency and selectivity as well as to deduce preliminary structure activity relationships (SARs). The results of compound synthesis and bioactivities are presented and discussed here.

2. Results and discussion

2.1. Chemistry

Based on the previously reported anthelmintic activity of the diterpenoid 7-keto-sempervirol, we investigated the impact of chemical structure modification on activity to obtain more potent and selective analogues. To achieve this, six families of compounds containing the basic scaffold of 7-keto-sempervirol were synthesised with each family containing distinct aromatic substitutions (Fig. 2).

Diverse synthetic strategies were adopted for each family. Non-cyclised analogues lacking bond C9-C10 were obtained from the coupling between commercially available beta-cyclocitral and substituted benzyl chlorides, as described by Huang et al. [19], to generate the first set of analogues as secondary alcohols (**1a-f**) (Scheme 1). Corresponding keto-analogues (**2a-f**) were then

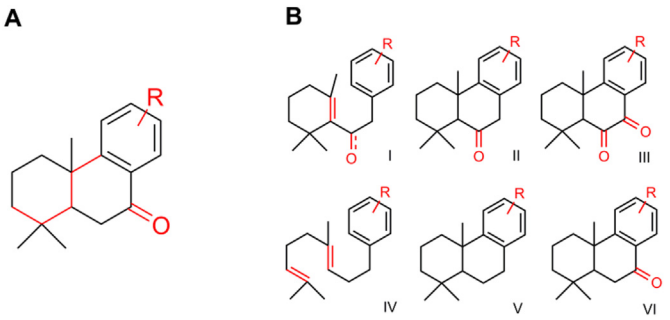

Fig. 2. Description of the 7-keto-sempervirol analogues. The scheme shows the basic scaffold of 7-keto-sempervirol (A) and the six families (I-VI) of analogues (B) designed from it. Each family differs for modification of the scaffold (in red) and includes several analogues differing for the R group.

obtained by oxidation in the presence of tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO) (Scheme 1).

From the synthesised keto-analogues, two different cyclization methods were performed to obtain the tricyclic diterpenoid scaffold with a keto-group in position six (Scheme 2). The first method (Scheme 2A) used the Lewis acid BBr_3 to facilitate the synthesis of the trans diastereoisomers (**3a-c**) as described by Huang et al. [19], while the second method (Scheme 2B) used $RuCl_3$ and AgOTf to obtain the cis diastereoisomer (**4a-c**), according to the method of Youn et al. [20]. However, this set of compounds showed instability when exposed to oxygen at room temperature and, therefore, their biological activity was not measured. Indeed, changes in colour and composition of the 6-keto compounds were observed as quickly as two (and up to seven) days post synthesis (See Supplementary information). Analysis of the resulting mixtures revealed high levels of autoxidation (cis > trans) that, upon subsequent purification and characterisation, revealed the presence of a keto group in positions 6 and 7 (Scheme 2C). These 6,7-diketo derivatives (**5a-c**) were more stable than the original 6-keto compounds and, for this reason, are considered a new family of analogues. Literature precedent for spontaneous oxidation in terpene-based systems, including mechanistic studies, is known [21].

The other three families of compounds were obtained from one synthetic pathway according to the method described by Surendra and Corey [22] (Scheme 3). Substituted Grignard compounds were added to geraniol acetate in the presence of Li_2CuCl_4 (Scheme 3A) and the products obtained (**6a-c**) were then cyclised through an enantioselective reaction catalysed by the complex (R)-BINOL-$SbCl_5$; the resulting hydrophobic compounds (**7a-d**) lacked a keto