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Natural product inhibitors of fatty acid biosynthesis: synthesis of the marine microbial metabolites pseudopyronines A and B and evaluation of their anti-infective activities

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Abstract

Total syntheses of the title natural products, pseudopyronines A (**1**) and B (**2**), have been achieved using methyl β-oxo carboxylic ester starting materials. The natural products and a small set of structurally related compounds were evaluated for growth inhibitory activity against a range of pathogenic microorganisms and were found to exhibit good potency ($IC_{50} \geq 0.46$ μg/mL) and selectivity towards *Leishmania donovani*. Several of the compounds inhibited recombinant fatty acid biosynthesis enzymes from both *Plasmodium falciparum* and *Mycobacterium tuberculosis*, validating these targets in the search for new anti-infective agents.

Keywords: Fatty acid biosynthesis; Marine natural product; *Leishmania*; *Mycobacterium*

1. Introduction

Fatty acids play crucial roles in the function and viability of cells providing components for biological membranes, acting as chemical messengers and facilitating the storage of energy. The biosynthesis of fatty acids follows an iterative process of condensation and elongation of acetate (in the form of malonate), which is undertaken by a sequence of enzymatic steps either in the context of a single multifunctional protein (Type I fatty acid synthase) in animals and fungi or a dissociable multienzyme system (Type II FAS) in plants, bacteria and protozoa.[1,2] The lack of homology between mammalian FAS-I and bacterial FAS-II systems makes it possible to discover and develop specific inhibitors that have therapeutic potential in the treatment of a number of diseases.[3]

A number of small molecule inhibitors of fatty acid biosynthesis (FAB) are known. Triclosan (an antiseptic) and isoniazid (an antituberculosis drug) both inhibit bacterial enoyl-ACP-reductase (FabI/InhA), the enzyme responsible for the final reduction step during FAB. New classes of inhibitors have been identified by screening synthetic compound libraries[4] and via bioassay guided studies of natural products. Examples of the latter include cerulenin,[5] thiolactomycin[6] and more recently bischloroanthrabenzoxocinone,[7] phomallenic acids A–C,[8] platensimycin[9] and flavonoids.[10,11] The thiolactomycin scaffold has been the subject of structure–activity studies, in particular focussing on growth inhibition of *Mycobacterium*

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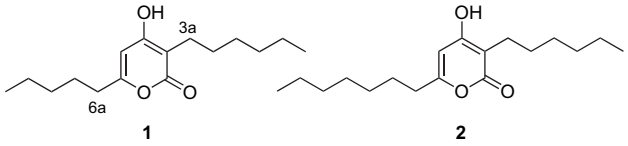
tuberculosis.[12] The presence of Type II fatty acid biosynthesis in the apicoplast of *Plasmodium falciparum* raises the possibility of FAB inhibitors also being useful templates for the discovery of antimalarial agents.[13,14] Studies involving thiolactomycin,[15] triclosan[16] and flavonoid[10] derivatives have been reported.

Arising from a search for new inhibitors of bacterial fatty acid biosynthesis, the 2-pyrone-containing natural products pseudopyronines A (**1**) and B (**2**) were recently reported as mild antibiotics.[17,18] The compounds, detected using a lacZ reporter cell-based assay, were isolated from fermentation of *Pseudomonas* sp. F92S91, which was itself isolated from a sponge collected in Fiji. Pyrone **2** had previously been reported as Sch 419560, identified from fermentation extracts of *Pseudomonas fluorescens*.[19] Pseudopyronines A and B inhibited the growth of Gram-positive bacteria, including *Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus*, *Moraxella catarrhalis* and *Enterococcus faecium* (MIC 1–64 μg/mL) with pseudopyronine B being the more active of the two compounds. Pseudopyronine B (**2**) inhibited the cellular uptake and incorporation of thymidine, uridine and amino acids suggestive of a mode of action related to the disruption of membrane function.[17] Notably, pseudopyronine B showed neither a membrane-damaging effect on human red blood cells nor caused haemolysis, indicating selectivity for bacterial membranes.



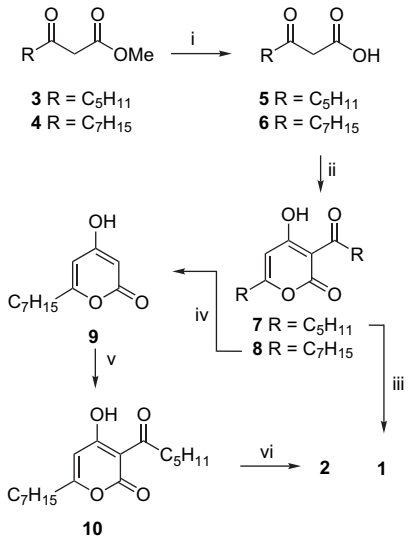
As part of our ongoing interest in the potential offered by natural products to act as leads in the development of new antituberculosis[20] and antimalarial agents,[10,21] we now report the synthesis of pseudopyronines A and B, the preparation of a number of analogues and the results of screening these compounds for activity against whole organisms and purified recombinant enzymes of the FAB pathway.

2. Results and discussion

2.1. Chemistry

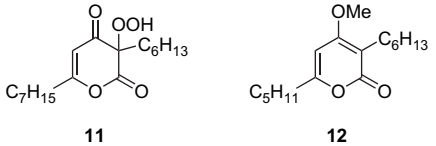
2.1.1. Pseudopyronines A and B

While a number of methodologies have been reported for the preparation of 4-hydroxy-2-pyrones, the carbonyldiimidazole-mediated condensation of β-keto carboxylic acids[22] was particularly attractive for the synthesis of the pseudopyronines as it could also lead to the preparation of a number of related compounds for structure–activity studies. In the case of pseudopyronine A (**1**), the required β-oxo carboxylic acid **5** was prepared via saponification of the known methyl β-oxo ester **3** (Scheme 1).[23] Cyclisation of **5** using 1.1 equiv of carbonyldiimidazole in THF afforded the target acylpyrone skeleton **7**. Reduction of the α-acyl group in **7** utilising $NaCNBH_3$ in THF/aq HCl[24] afforded pseudopyronine A (**1**), which exhibited spectroscopic data identical to those reported for the natural product.[18] The synthesis of pseudopyronine B (**2**) started with ester **4**[23] and progressed to pyrone **8** in similar fashion. Deacylation of **8** by heating at 90 °C in 90% H_2SO_4 yielded 6-heptyl-4-hydroxy-2-pyrone (**9**) with subsequent acylation by hexanoyl chloride in TFA affording the pyrone skeleton with the appropriate length carbon chain at C-3. Reduction with $NaCNBH_3$ in THF/aq HCl afforded pseudopyronine B (**2**). Our sample of **2** exhibited identical spectroscopic data to those reported for the natural product[18] and also co-eluted on analytical HPLC with an authentic sample of pseudopyronine B.



Scheme 1. Reagents and conditions: (i) NaOMe, MeOH, H_2O, rt; (ii) 1,1′-carbonyldiimidazole, THF, rt, 24 h; (iii) $NaCNBH_3$, THF, 2 M HCl, rt, 2.5 h; (iv) 90% H_2SO_4, 130 °C, 1 h; (v) hexanoyl chloride, TFA, reflux, 3 h; (vi) $NaCNBH_3$, THF, 2 M HCl, rt, 19 h.

The original report of **1** and **2** demonstrated the lack of stability of **2** in the $CDCl_3$ NMR solvent, with **2** undergoing reaction with oxygen to form hydroperoxide **11**.[18] We also observed similar instability for pseudopyronine A (**1**), which converted over 7 days standing in $CDCl_3$ in an NMR tube to form the analogous 3-peroxy derivative. New NMR signals were observed at δ 5.68 (s, H-5) and δ 1.92 (m, 2H-3a) consistent with those reported for the 3-peroxy derivative of pseudopyronine B.[18] This instability should not be observed if the 4-hydroxyl group is trapped as the methyl ether. Indeed, 4-*O*-methyl pseudopyronine A (**12**), prepared in 53% yield from **1** by reaction with excess trimethylphosphate, was stable in $CDCl_3$ for many weeks with no observed degradation.



2.1.2. Analogues

To be in a position to study aspects of the structure–activity requirements of pseudopyronines A and B, the preparation