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Research paper

Synthesis and formulation studies of griseofulvin analogues with improved solubility and metabolic stability

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ABSTRACT

Griseofulvin (**1**) is an important antifungal agent that has recently received attention due to its anti-proliferative activity in mammalian cancer cells. Comprehensive SAR studies have led to the identification of 2′-benzyloxy griseofulvin **2**, a more potent analogue with low micromolar anticancer potency *in vitro*. Analogue **2** was also shown to retard tumor growth through inhibition of centrosomal clustering in murine xenograft models of colon cancer and multiple myeloma. However, similar to griseofulvin, compound **2** exhibited poor metabolic stability and aqueous solubility. In order to improve the poor pharmacokinetic properties, 11 griseofulvin analogues were synthesized and evaluated for biological activity and physiological stabilities including SGF, plasma, and metabolic stability. Finally, the most promising compounds were investigated in respect to thermodynamic solubility and formulation studies. The 2′-benzylamine analogue **10** proved to be the most promising compound with low μM *in vitro* anticancer potency, a 200-fold increase in PBS solubility over compound **2**, and with improved metabolic stability. Furthermore, this analogue proved compatible with formulations suitable for both oral and intravenous administration. Finally, 2′-benzylamine analogue **10** was confirmed to induce G2/M cell cycle arrest *in vitro*.

1. Introduction

Griseofulvin (**1**), a natural product from *Penicillium griseofulvum*, was first discovered in 1939 and has since 1958 been known for its antifungal properties in guinea pigs and man (Fig. 1) [1–3]. Since then, more than 400 analogues have been synthesized and in more recent years the compound class has received renewed interest due to findings of anticancer effects [4–7]. Griseofulvin has been identified as a tubulin binder that disrupts tubulin polymerization and microtubule dynamics [8,9] and was in 2007 shown to inhibit centrosomal clustering *in vitro* [10]. While normal cells have exactly two centrosomes at the onset of mitosis, tumor cells often have supernumerary centrosomes that lead to formation of multiple spindle poles. To avoid lethal asymmetric cell divisions, cancer cells rely on a dynamic process called centrosomal clustering to form pseudo-bipolar spindles and thus ensure appropriate cell division. Consequently, inhibition of centrosomal clustering may constitute a novel therapeutic strategy in oncology, by selective eradication of cancer cells with supernumerary centrosomes [11–13].

Following this discovery, we have published three SAR studies covering more than 60 griseofulvin analogues with structural modifications to the 4, 5, 6, 2′, 3′, and 4′ positions [14–16] that were tested against multiple cancer cell lines. The endeavor led to identification of a new griseofulvin analogue, the 2′-benzyloxy analogue **2**, that exhibited a 25-fold activity increase as a centrosome clustering inhibitor compared to **1** (Fig. 1). *In vivo* studies confirmed analogue **2** to retard tumor growth through inhibition of centrosomal clustering in murine xenograft models of colon cancer and multiple myeloma [14,17].

While griseofulvin analogues show potential as small molecule

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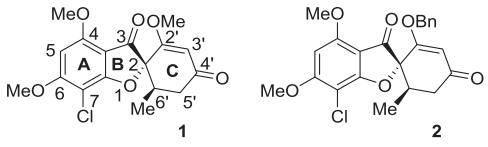


Fig. 1. Chemical structure of griseofulvin (**1**) with IUPAC recommended numbering and ring designation and the 2′-benzyloxy griseofulvin analogue **2**.

therapeutics in cancer treatment, the compounds generally suffer from poor pharmacokinetic properties including low solubility and metabolic instability [16,18]. In order to further our studies of griseofulvin derivatives, we herein report the synthesis and biological evaluation of 11 griseofulvin analogues (6 reported for the first time) with different 2′-substitution patterns. In addition to biological activity, griseofulvin (**1**) and analogues hereof were also subjected to pharmacokinetic studies involving stability in simulated gastric fluid (SGF) and plasma, plasma protein binding (PPB) as well as metabolic stability. Finally, thermodynamic solubility in phosphate buffered saline (PBS) and formulation studies were performed on the most promising analogues.

2. Results and discussion

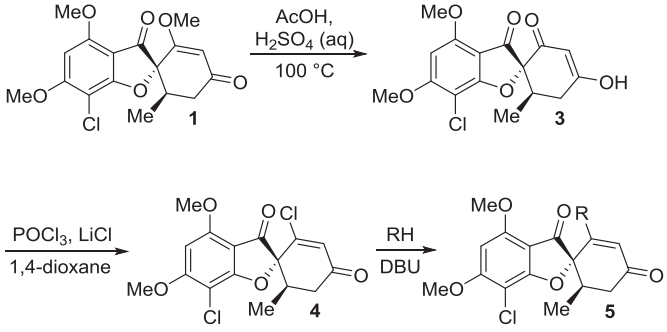
2.1. Synthesis

The strategy used for preparation of the griseofulvin analogues was first described by Stephenson and co-workers [19] and later modified by Rønnest and colleagues (Scheme 1) [14,15]. Griseofulvin (**1**) was hydrolyzed to griseofulvic acid (**3**) and then converted into the 2′-chloro derivative **4** with phosphoryl chloride and lithium chloride in 1,4-dioxane. 2′-Chloro griseofulvin **4** was treated with a suitable amine or alcohol nucleophile (RH) in the presence of 1,8-diazabicycloundec-7-ene (DBU) to yield the desired product with the general structure **5**.

2.2. Compounds

In our previously reported SAR studies, multiple 2′-oxy modified griseofulvin analogues were synthesized and evaluated. Here, a series of *para*-substituted 2′-benzyloxy analogues as well as the 4′-oxime of **2** were found to have activity similar to compound **2** [14,15]. For this reason, the 2′-(4-methylbenzyloxy) analogue **6**, the 2′-(4-fluorobenzyloxy) analogue **7**, the 2′-(4-trifluoromethylbenzyloxy) analogue **8**, and the 4′-oxime analogue **9** were included in the current study (Fig. 2).

In addition to these previously synthesized inhibitors, six new analogues were prepared. To examine the importance of the 2′-heteroatom and potentially enhance solubility, a similar series of



Scheme 1. Overview of the synthetic strategy used for preparation of the 2′-modified griseofulvin analogues with the general structure **5**.

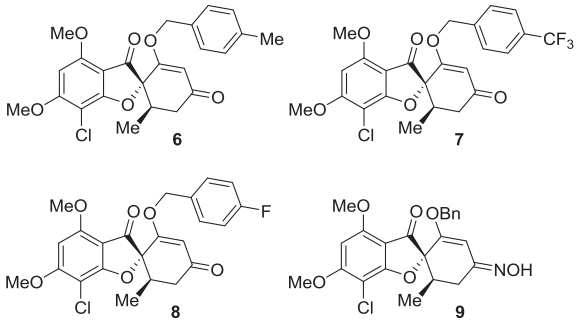


Fig. 2. Previously reported 2′-oxy modified griseofulvin analogues with antiproliferative activity comparable to compound **2**.

2′-amino modified analogues, **10**–**12**, was synthesized. Furthermore, as all previously reported 2′-benzyloxy analogues have contained an unbranched linker, the 2′-tertiary amino analogue **13** as well as the two 2′-phenethyl modified analogues, **14** and **15**, were also prepared (Fig. 3).

In total, 12 compounds consisting of griseofulvin (**1**), five previously reported 2′-oxy modified analogues (**2** and **6**–**9**), and six new analogues (**10**–**15**), were investigated.

2.3. Biological evaluation

The activity of **1** was measured as 25.0 ± 4.9 μM and 22.4 ± 5.4 μM against the human epithelial cancer cell line MDA-MB-231 and the human osteosarcoma cancer cell line U2OS, respectively. As evident from Table 1, all tested analogues exhibited improved biological activity compared to **1** against both cancer cell lines. The previously reported analogues **2**, **6**, and **9** and the two new 2′-amino modified analogues **10** and **13** were the most potent and showed an approximate 10-fold increase in activity over **1**. Except for **6**, *para*-substitution on the 2′-aryl moiety led to a decrease in activity. Both 2′-oxy linker modified analogues **14** and **15** displayed a decrease in activity compared to **2**. As the additional methyl group in these compounds would likely decrease aqueous solubility, **14** and **15** were not included in the following pharmacokinetic studies.

2.4. Plasma stability

Variations within ±20% is considered within the uncertainty of this assay and the numbers indicate high stability. Thus, **1** and all tested analogues exhibited excellent stability in both rat and mouse plasma (Table 2) with values ranging from 87 to 117%.

2.5. Simulated gastric fluid stability

To identify acid sensitive compounds that would be incompatible with oral administration, the analogues were subjected to a SGF stability assay (Table 2). SGF was designed to mimic the fasted stomach and contained HCl, NaCl, and pepsin at pH 1.2. The 2′-oxy modified analogues, **2** and **6**–**9**, generally showed SGF stability similar to **1**. The 2′-amino modified analogues **10**–**12** exhibited slightly lower stability compared to the 2′-oxy analogues. Interestingly, **13** proved highly unstable in SGF with only 15% of the parent compound remaining after 2 h.

2.6. Metabolic stability

Metabolic stability was evaluated using liver microsomes from mice and rats and determined as intrinsic clearance (CL_{int}). As