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Inhibition of phosphatidylinositol-3-kinase by the furanosesquiterpenoid hibiscone C

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ABSTRACT

The phosphatidylinositol-3-kinase (PI_3K) pathway regulates cellular metabolism and is upregulated in many cancers, making it an attractive chemotherapeutic target. Wortmannin is a potent inhibitor of PI_3K; however, its potential as a chemotherapeutic is limited due to its instability, lack of selectivity, and lengthy chemical synthesis. In contrast, hibiscone C, a structurally simpler and less studied member of the furanosteroid family, has been expediently prepared by total synthesis. We demonstrate that hibiscone C competitively inhibits PI_3K activity in intact cells, slows proliferation, and induces cell death. Hibiscone C may therefore serve as a productive scaffold for the development of therapeutically relevant PI_3K inhibitors.

The members of the sesquiterpene furanosteroid family of natural products have attracted significant attention as inhibitors of the phosphatidylinositol-3-kinases (PI_3K),[1] enzymes known to play critical roles in cell growth and differentiation. The key structural similarity shared by each member of this family is a furan ring flanked by two conjugated carbonyl groups. The inherent electrophilicity of these diacylheteroaryl systems (Scheme 1) is enhanced in furanosteroids by ring strain caused by the fusion of many contiguous sp^2-hybridized carbons within their polycyclic core structures. As a result, furanosteroids display increased reactivity towards nucleophiles that accounts for their varied and often potent biological activities.[2] The most intensely studied member of this family is wortmannin (Scheme 1), which inhibits multiple kinases, including all isoforms of PI_3K, and mammalian Polo-like kinase, a cell cycle enzyme that has been proposed as a biomarker for certain types of cancers.[3] A crystal structure of wortmannin bound in the ATP binding site of PI_3K reveals the key feature of its mechanism of action; namely, covalent attachment of a lysine residue that results in irreversible inhibition (Scheme 1).[4]

The enhanced electrophilicity of the furanosteroids also contributes to their toxicity and reduces their potential as chemotherapeutic agents. Of particular concern is their lack of enzyme selectivity. For instance, wortmannin is known to inhibit numerous enzymes beyond those that are most important for its antiproliferative activity, which limits its potential as an anticancer agent.[3] Additionally, wortmannin is known to undergo hydrolytic opening of the furan ring in neutral buffer,[3] decomposes to inactive products via lactone hydrolysis under mild conditions, and reacts with a variety of nucleophiles under physiological conditions.[5] Numerous studies aimed at preparing less toxic wortmannin analogs have identified molecules that exhibit similar potencies as wortmannin, and efforts targeting more stable and more potent analogs are ongoing (Fig. 1).[6]

Given their structural similarities, it is reasonable to suggest that all members of this family of natural products may share a similar mechanism of action, and a number of them in addition to wortmannin[7] have succumbed to total synthesis (Fig. 2).[8] Despite intense recent synthetic interest in this family of natural products, surprisingly little is known about the biological activity of one of its structurally simplest members, the furanosesquiterpoenoid hibiscone C.[9] We were attracted to this synthesis target because hibiscone C lacks the lactone functionality that contributes to the instability of wortmannin. Aided by our racemic hibiscone C total synthesis effort (Scheme 2),[8c] herein we describe results of biological assays that suggest the hibiscone C scaffold may be a productive starting point for the discovery of new furanosteroids with desirable biological activity.

To evaluate the biological activity of racemic hibiscone C, we first tested its ability to inhibit PI_3K signaling in T lymphocytes. T lymphocytes (T cells) are white blood cells of the immune system that have multiple functions including facilitating activation of other immune cells, maintaining peripheral tolerance, and killing

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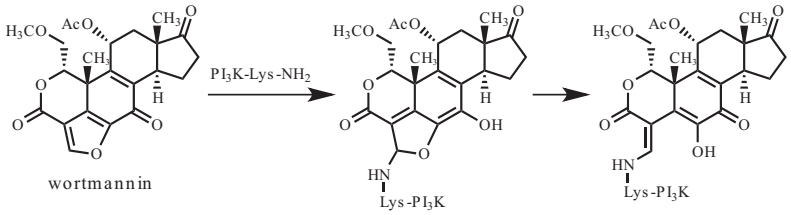


Scheme 1. Mechanism of action of wortmannin.

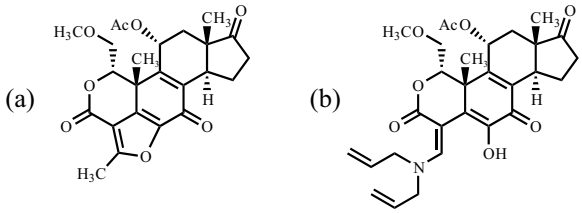


Fig. 1. Previously prepared analogs of wortmannin: (a) showed diminished activity,[6a] whereas (b) proceeded to clinical trials under the name PX-866.[6b]

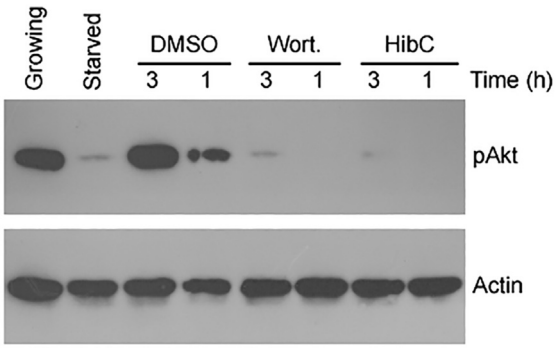


Fig. 3. Hibsicone C inhibits PI_3K pathway activity. Activated T cells were grown in culture (growing), then deprived of IL-2 overnight (starved). Cells were then treated with 100 μM wortmannin (Wort.), 100 μM racemic hibiscone C (HibC), or vehicle (DMSO) for 30 min and then stimulated with 1000 U/mL recombinant human (rh) IL-2 for the times indicated. Phosphorylation of Akt was detected by Western blot. Detection of actin serves as a loading control. Data are representative of three independent experiments.

of infected cells. Metabolically, activated T cells resemble cancer cells in that they engage in aerobic glycolysis.[10] Furthermore, the cellular metabolism of T cells, like cancer cells, is dependent on PI_3K pathway activity.[11] It has been shown that activated T cells can respond to stimulation by the growth factor interleukin-2 (IL-2) by activating PI_3K. Activated PI_3K phosphorylates PIP_2 to generate PIP_3, which subsequently phosphorylates the serine/threonine kinase Akt.[12] Therefore, the phosphorylation of Akt serves as an excellent readout of PI_3K activity. To determine if hibiscone C can inhibit PI_3K pathway activity, activated T cells growing in culture were starved of IL-2, which resets the PI_3K pathway, and were then treated with 100 μM wortmannin, racemic hibiscone C, or vehicle. The extent of Akt phosphorylation was measured in response to IL-2 restimulation by Western blot (Fig. 3). Hibiscone C prevented the phosphorylation of Akt, similar to results obtained with the known PI_3K inhibitor wortmannin.

As the critical diacylfuran functional group responsible for the irreversibility of the PI_3K inhibitory activity of wortmannin is also present in hibiscone C, it is reasonable to hypothesize that hibiscone C, like wortmannin, may irreversibly bind PI_3K and competitively inhibit its enzymatic activity. To test this possibility directly, growing T cells (those not starved of IL-2) were treated with 100 μM wortmannin, hibiscone C, or vehicle, and the extent of Akt phosphorylation was again measured (Fig. 4). We observed no detectable Akt phosphorylation following the administration of hibiscone C, suggesting that hibiscone C competitively inhibits PI_3K. Similar results were observed for wortmannin.

Wortmannin is known to be a potent inhibitor of PI_3K activity. Since the more structurally simple hibiscone C lacks many of the

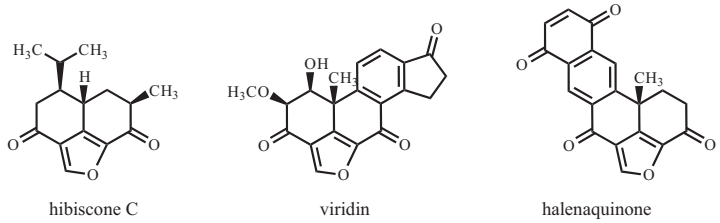
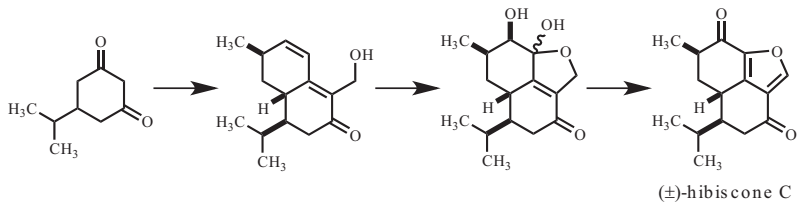


Fig. 2. Some previously synthesized furanosteroids.



Scheme 2. Summary of the synthesis of racemic hibiscone C.[8c] Starting from a commercially available dione, the natural product can be prepared in seven steps through a series of annulations and oxidation state adjustments.