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Interrogating the Estrogenic Activity of Sakuranetin, a Phytoalexin Extracted from Rice

Flavonoids are a family of natural plant polyphenols subdivided based on the substituents on the aromatic rings. These compounds are detected in most parts of the plant and in a wide variety of vegetables, legumes, and fruits. While common members (flavones, flavanonols, flavanones) are synthesized naturally, production of these compounds can be enhanced in response to stressors. Identified compounds have been reported to exhibit strong bioactivity, including anticancer, antioxidant, anti-inflammatory, antiviral, and antimicrobial properties. Of these, sakuranetin demonstrates promise as a bioactive natural compound. Sakuranetin was initially discovered in the bark of cherry trees but has since been found in other plant species. While not present in significant quantities in most plant species, sakuranetin production is upregulated in response to physical or biological stressors. Namely, ultraviolet-C irradiation and treatment with CuCl_2 , jasmonic acid, or phytopathogens significantly increases concentration in the leaves of rice.

Many flavonoids have been designated as phytoestrogens based on their ability to interact with estrogen receptors (ER) to elicit strong anti-estrogenic and estrogenic responses. A deeper understanding of ER-modulation, through selective agonists/antagonists, is clinically relevant and is currently under investigation for the prevention and treatment of certain cancers, metabolic and cardiovascular diseases, neurodegeneration, inflammation, and osteoporosis. The antiestrogenic properties of phytoestrogens potentially result from binding competition with endogenous estrogens. The ability of phytoestrogens to display both estrogenic and antiestrogenic properties has led researchers to search for new plant sources and methods for natural production.

The production of sakuranetin occurs through the biosynthetic pathway of naringenin, a known phytoestrogen. Despite growing interest in natural compounds and the ER-agonist activity of sakuranetin precursors, the estrogenic effects of sakuranetin have not been determined. This study aims to evaluate sakuranetin as an estrogenic and antiestrogenic flavonoid with the following objectives: (1) to assess dose-dependent estrogenic and antiestrogenic effects in breast cancer cell lines endogenously expressing the estrogen receptor (ER+ lines: MCF-7 and T-47D); (2) to evaluate impact of sakuranetin on ER-mediated proliferation and colony formation; (3) to compare ER binding affinity to synthetic E2 through model docking with estrogen receptor α ; and (4) to examine the impact of sakuranetin on the cellular transcriptome (RNAseq and PCR).

Phytocompounds provide unique opportunities to enhance commercial crops or their byproducts through increased bioactivity. ER+ cell lines treated with sakuranetin exhibited a dose-dependent response in estrogenic activity, proliferation, and colony formation. Docking simulations showed enantiomer-specific binding to the ER binding domain, with a preference for R conformation; while ER binding affinity was lower than synthetic E2, values for sakuranetin are similar to or higher

than previously evaluated phytochemicals. Sakuranetin significantly increased the ER transcriptome and ER-mediated gene expression for MCF-7 cells, further emphasizing its potential as a natural estrogenic compound. Future studies will expand investigation of potential elicitors and plants that exhibit increased levels of sakuranetin.