ABSTRACT

SYNTHESIS OF HETEROCYCLE-CONTAINING GENISTEIN ANALOGUES AS ANTI-PROSTATE CANCER AGENTS

Prostate cancer is the second-leading cause of cancer-related deaths in the United States. There is no effective therapy when prostate cancer becomes metastatic and refractory to conventional treatments. For this reason, the identification and exploration of new agents that reduce prostate cancer cell growth are of paramount importance. The increased risk of prostate cancer in the first generation of Asian men emigrating to the United States suggests a chemopreventive effect from traditional Asian food. Genistein, a phytoestrogen isolated from soybeans, has been identified in clinical trials as a candidate for prostate cancer prevention and treatment. However, its efficacy in clinical studies has been limited by the poor bioavailability.

The hypothesis of this research project is that various heteroaromatic rings can serve as potential bioisosteres for phenyl rings in genistein and the incorporation of a basic nitrogen can improve the bioavailability of the genistein analogs. The researchers in this study have synthesized six new pyrazole genistein analogs and three known analogs without a N-containing heteroaromatic ring through a four-step reaction sequence with Suzuki-Miyaura coupling reaction as a key step. The cytotoxic and anti-proliferative effects of these analogs were evaluated against three prostate cancer cell lines (LNCaP, DU-145, and PC-3) and one aggressive cervical cancer cell line (HeLa). The synthesis, cytotoxicity, and structure-activity relationship of genistein analogs will be presented.

Pahoua Xiong
August 2015
SYNTHESIS OF HETEROCYCLE-CONTAINING GENISTEIN ANALOGUES AS ANTI-PROSTATE CANCER AGENTS

by
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A thesis
submitted in partial
fulfillment of the requirements for the degree of
Master of Science in Chemistry
in the College of Science and Mathematics
California State University, Fresno
August 2015
APPROVED

For the Department of Chemistry:

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Dean, Division of Graduate Studies
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ACKNOWLEDGMENTS

Writing this master’s thesis was not what I anticipated it to be and it would not have been a great success without the support and encouragement of many great people in my life. First and foremost, I want to thank Dr. Chen for letting me explore the research world of medicinal natural products and the bioorganic chemistry. The incredibly educational critiques and guidance you have provided has enlightened me with skills and thoughts I would not have discovered by myself.

Secondly, I am grateful for the times and help from my research committee members Dr. Person and Dr. Gandler for taking time from their busy schedule to assist me in completing this master’s thesis and providing me with knowledge.

To the faculty, colleagues, and friends of the Chemistry Department, thank you for making the master’s in Chemistry program a friendly environment where I have been given the opportunity to build and strengthen not only my research techniques, but also my communication skills and chemistry knowledge. I also owe my sincerest gratitude to Doug and Alan for the work in always providing assistance with lab supplies to troubleshooting technical problems with the instruments, and so much more.

To my parents and my siblings, you all never hesitate to and always push me to do well. This is the greatest love I have been blessed with. I want to thank you all for giving me the strength to complete this chapter of my life.

Lastly, I want to send out a big thank you from the bottom of my heart to my daughters, Faith and Grace Yang, and my husband, Bee Yang, for believing in me and for making the impossible possible. Thank you for letting me takes a lot of
times away from you all to pursue my passions and studies. Because of your love, support, and encouragement, we will be successful.
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1. GENERAL INTRODUCTION OF PROSTATE CANCER

1.1 The Health Problem Caused by Prostate Cancer

Prostate cancer is the second leading cause of death for men in the United States. Unlike other cancers, it is slow growing and men rarely experience symptoms during the early stages when the disease is more treatable. When men feel symptoms, the cancer may have already become too advanced that effective treatments are limited. According to the 2015 estimation of the American Cancer Society, approximately 220,000 men would be diagnosed with prostate cancer in 2015 as well as 27,540 deaths. Thus, prostate cancer is the most prevalent malignancy in men in the United States and is also one major health problem in other countries.

The mortality and incidence rates of prostate cancer vary in different countries around the world. The United States, Canada, Sweden, Australia, and France have the highest incidence rate of prostate cancer; whereas European countries have a moderate incidence rate; and Asian along with North African countries have the lowest incident rate of prostate cancer. Hass et al. reported that men in the United States have the highest incidence rates, but lower mortality rate as compare to other countries. The advantage of detecting prostate cancer earlier, which is frequently done in the United States through screening, is that the cases are more curable and may correspond to a lower mortality rate. Whereas in other countries such as Asia and Africa, men are less aware of the early prostate screening programs and have a low incidence rates of prostate cancer and a higher mortality rate. This may imply that men in these countries were diagnosed with an advanced cancer through the occurrence of pains that are untreatable, suggesting
that advanced hormone-refractory prostate cancer contributes to most of the mortality rates of prostate cancer cases.

1.2 Risk Factors of Prostate Cancer

1.2.1 Age

Several epidemiology studies have found the correlation between prostate cancer and the risk factors including age, races, and diet. The data from the National Cancer Institute Surveillance Epidemiology and End Results (NCISEER) indicate that the men aged 65-74 years are more prone to have prostate cancer than those under the age of 50 years. The association of the incidence of prostate cancer with age has also been investigated by Yin et al. They concluded that prostate cancer was linked to donors with prostate adenocarcinoma and high-grade prostate intraepithelial neoplasia over the ages of 50 to 81-years-old.

1.2.2 Races

Aside from age, the (NCISEER) study also concluded that African Americans have a slightly higher risk of prostate cancer. African American is the racial group in the United States that have the highest incidence and mortality rate for prostate cancer when compares to black men in other countries. The high cancer incidence in African Americans in the United States may be due to their typical diet.

1.2.3 Diet

Diet is another factor that plays a significant role in the prostate cancer incidence. A high dietary intake of fruits and vegetables is associated with a decrease in the risk of developing prostate cancer, which was evidenced in the first generation of Asian men immigrating to the United States. They have an
increased risk of prostate cancer related to diet change as they assimilate to the American culture. The rich dietary intake of soy products and vegetables in Asian countries may be the reason for the lower incidence of prostate cancer. On the other hand, Americans have a higher incidence of prostate cancer, which may be due to the fact that they typically consume more red meat and less vegetables.

A case study, conducted by Saxe on 14 patients with recurrent prostate cancer, indicated that the adoption of a plant-based diet could reduce the Prostate Specific Antigen (PSA) levels of the patients. This implies that a plant-based diet has potential for not only prevention but also therapeutic effects for prostate cancer.

1.3 Hormone-Dependent Prostate Cancer and Current Treatment

The etiology of prostate cancer is not clearly understood. However, androgens and androgen receptors (AR) are crucial to the development of prostate cancer. The initial diagnoses of prostate cancers are dependent on androgen, while the later diagnoses of invasive prostate cancer are androgen–independent. The serum concentration of PSA, digital rectal examination (DRE), transrectal ultrasonography (TRUS) and ultrasound-guided biopsy are used as biomarkers in prostate cancer screening. These prostate cancer screenings help with early detection and the treatment options. Treatments for prostate cancer depend on the conditions, stages of the cancer, and the patient’s preference.

Early stage prostate cancer is hormone-dependent and localized in the prostate gland. Its initial treatment options are radical prostatectomy, radiotherapy, and hormonal ablation therapy. Radical prostatectomy is to treat prostate cancer by surgically removing the prostate and seminal vesicles. After prostatectomy, the prostatic tissues will be used to further study and obtain information on histology of the prostatic tissues. The downside of this treatment
is the impact on the quality of life due to patients’ sexual dysfunction experience. An alternative treatment is radiotherapy, which utilizes high radiation energy to kill prostate cancer cells. The radiotherapy for prostate cancer can cause rectal problems, such as diarrhea, rectal pain, and severe rectal bleeding, as well as bladder problems, such as dysuria and hematuria. However, the symptoms of these side effects rely on the radiation intensity and the duration of treatment. Most of these treatments can often cure patients with localized prostate cancer.

Patients with recurrent prostate cancer after prostatectomy and radiotherapy treatments will have elevated PSA levels that are localized (within the prostate gland). This recurrent cancer can also become a locally advanced hormone-dependent prostate cancer, where the cancer has spread outside of the prostate. In this case, androgen deprivation therapy (ADT) is the next treatment. ADT is a hormonal therapy used to reduce androgens with drugs or surgery. The side effects of androgen deprivation therapy includes hot flashes, loss of bone mass, and decreased libido. The use of hormonal therapy will become ineffective for patients with androgen-dependent prostate cancer when the cancer no longer rely on androgen for cancer growth.

1.4 Hormone-independent Prostate Cancer and Current Treatment

Patients with hormone-independent prostate cancer are those who have castrate levels of testosterone and that are no longer responsive to hormonal therapy. Hormone-independent prostate cancers can metastasize to the bone, and to other organs. Most often, about 80% of hormone-refractory prostate cancer patients experienced bone metastases based on a study conducted by Small et al. At this stage of the cancer, chemotherapy is one of the treatments available for treating castrate-resistant prostate cancer patients. The U.S. Food and Drug
Administration (FDA) have approved two chemotherapeutic agents, docetaxel and cabazitaxel, to treat patients with metastatic castrate-resistant prostate cancer. These drugs have shown survival benefits with improved quality of life. The side effects associated with chemotherapeutic drugs are hair loss, nausea, diarrhea and more.

1.4.1 Docetaxel

Before docetaxel, mitoxantrone was the first chemotherapeutic agent that was approved in 1996 to treat acute myeloid leukemia and in 2000 to treat advanced prostate cancer with improvements in the quality of life and a reduction in pain. Nonetheless, studies conducted on docetaxel in combination with prednisone proved to be the first chemotherapeutic agent to prolong life for patients with metastatic hormone-refractory prostate cancer. The U.S. FDA approved docetaxel with prednisone as the first line treatment for patients with metastatic hormone-refractory prostate cancer in 2004. Docetaxel, its structure as shown in Figure 1, is a chemotherapeutic agent from the taxoid family that binds to microtubule in mitotic cells and induces apoptosis. The combination of docetaxel and prednisone has been tested in a randomized human trial study (TAX 327) conducted by Tannock et al, in which this treatment was compared to mitoxantrone and prednisone. In this research, chemotherapy combinations were given to men with castrate-resistant prostate cancer. One group of patients was given the treatment every three weeks and the other group was given weekly. The results of the study showed that men who received docetaxel with prednisone had an increased survival rate of 18.9 months more than those who received mitoxantrone plus prednisone (16.5 months). However, the limitation of docetaxel therapy is drug resistance that is mainly caused by its high substrate affinity for the
multidrug resistance (MDR) P-glycoprotein (p-gp) efflux pump; which plays a significant role in pumping the anticancer drugs out of the malignant cancer cells.\textsuperscript{20-20b}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{mitoxantrone_and_docetaxel}
\caption{Structures of mitoxantrone and docetaxel}
\end{figure}

1.4.2 Cabazitaxel

Cabazitaxel (Figure 2) is a recent FDA-approved chemotherapeutic drug for the treatment of patients with metastasized androgen-independent prostate cancer. As reported by the literature, cabazitaxel has a lower substrate affinity to MDR proteins in comparison to docetaxel, which can overcome docetaxel resistance of malignant cancer cells.\textsuperscript{21} Cabazitaxel is the second line (after docetaxel) treatment approved by the FDA in 2010 for patients with castrate-resistant prostate cancer. In the TROPIC study, cabazitaxel with prednisone was given to men with castrate-resistant prostate cancer after docetaxel treatment. The result with the cabazitaxel treatment showed that patients had a median survival of 2.4 months which is more than patients who received mitoxantrone.\textsuperscript{22} Apparently, the cabazitaxel treatment showed little benefit to the overall survival of patients, however, it causes deaths related to neutropenia and diarrhea.
In summarizing the first and second-lines of chemotherapeutic treatment for men with castrated prostate cancer, the studies reflects that the use of current available chemotherapy has both negative and positives outcomes. In helping patients with advanced prostate cancer with benefits to overall survival and improved quality of life, but more work is needed for reducing side effects. Consequently, there is currently no effective therapy for advanced, metastatic, and hormone-refractory prostate cancer. Thus, it is needed to develop novel chemotherapy agents that are more effective and have minimal side effects.
2. GENISTEIN

2.1 Genistein: A Bioactive Component of Soybeans

Currently, many efforts are being made by scientists in developing new effective chemotherapeutic agents from dietary natural products for the prevention and treatment of prostate cancer. Nutritional substances such as phytochemicals have medicinal benefits in the prevention of chronic diseases. These phytochemicals are found in a small quantity in fruits, vegetables, and grains. Based on the National Cancer Institute (NCI) Experimental Food Program, soybeans have been listed as one of the top 6 cancer-preventive foods in the food pyramid with preventive cancer properties, which have been confirmed in animal and epidemiological studies. Soybeans belong to the *Leguminosae* family and are native to Southeast Asian Countries.

The isoflavone content in soybean seeds varies geographically due to the climate and environmental factors. Three aglycones (daidzein, geinstien, and glycitein) and three 7-\(O-\beta\)-glucosides (daidzein, geinstin, and glycitin) that have been isolated from soybeans are shown in Figure 3. The other six isoflavones extracted from soybeans are 6”-\(O\)-malony-7-\(O-\beta\)-glucosides: (malonyl daidzein, malonyl genistin, and malonyl glycitin), and 6”-\(O\)-acetyl-7-\(O-\beta\)-glucosides: (acetyl daidzein, acetyl genistin, acetyl glycitin). The major isoflavones found in unprocessed soybeans are -7-\(O-\beta\)-glucosides and 6”- \(O\)-malony-7-\(O-\beta\)-glucosides.

Whereas the predominant isoflavones that occur in unprocessed soybeans are isoflavones in their glucoside form.

Genistein (4’,5,7-trihydroxyisoflavone) is a bioactive substance that is correlated to the cancer prevention properties as indicated by epidemiological and
animal studies. Genistein is one well-studied and non-toxic natural product that exhibits inhibitory effects on prostate cancer and other human cancer cell lines.\textsuperscript{26} Genistein has also been identified as having many other biological activities including cell antiproliferation\textsuperscript{26}, antioxidation\textsuperscript{27}, antimicrobial\textsuperscript{28} and estrogenic\textsuperscript{29}. Genistein is also considered as a phytoestrogen chemical. The three-hydroxyl groups attached to the phenyl A and B rings play a major role in antioxidant and estrogenic activities. In Figure 4, the hydroxyl groups at C7 and C4’ of genistein is similar to the 17-\(\beta\)-estradiol that give genistein the ability to bind to both the \(\beta\) and \(\alpha\) isoforms of estrogen receptors through estrogen- and androgen-mediated signaling pathways.\textsuperscript{2} In addition, genistein has different binding affinities to the \(\beta\)
and α isoforms of estrogen receptors, giving genistein its anti-estrogenic activity. The binding affinity difference to the β and α isoforms of estrogen receptors suggests that genistein has an influence on the growth and function in the urogenital tract of males and females. This weak estrogenic activity may emphasize the importance of anticancer potential activity. This may also explains the significance of a rich diet of soybeans (genistein) can result in a low risk of prostate cancer observed in Asian countries.

Figure 4. Chemical structures of estradiol and genistein

2.2 Epidemiology Studies

Geographic epidemiology studies have shown that a low risk of prostate cancer in the East Asian countries correlated to a high consumption of dietary food that includes soy food. On the other hand, the American and European men who have a low intake of soy consumption have a higher risk in the development of prostate cancer. One such study conducted by the Pumford SL group showed that plasma samples of Japanese men and women contain higher contents of isoflavonoid than British men and women. Soybean is a healthy alternative source of meat protein, which is a diet low in saturated fat. The content of genistein varies in different sources of soy foods that are consumed in Asian countries and in countries in the west.
Most soy foods consumed by Asian populations are traditional soy foods such as roasted soybeans, instant soy beverage powder, tofu, tempeh, bean paste, fermented bean curd, and miso. The Asian immigrants consume more soy proteins than Americans. Unlike Asian countries, the Western countries mainly consume new generation soy products, which are mainly processed or soy-added foods that contain a lower content of phytochemicals than the amount found in traditional soy foods. This supports the idea that the dietary intake of phytochemicals from traditional soy foods might be at least one of the pivotal factors for the low incidence of prostate cancer in Asian men.

A higher incidence rate of prostate cancer is observed for first generation Asian men immigrating to the United States. This supports the concept that diet is a factor that influences the risk of prostate cancer. Collectively, Asian countries have a lower incidence of prostate cancer may probably due to their rich dietary intake of soy products and vegetables. The higher incidence rate of prostate cancer in Americans is likely a result of a poor diet of nutritional vegetables and a high consumption of protein from red meat.

2.3 Cytotoxicity of Genistein Towards Various Cancer Cell Lines

*In vitro* cytotoxicity assay is a method used for analyzing cell viability and determining the ability of test compounds to inhibit the growth of different cell lines. Generally, this method is a crucial beginning process in the study of drug discovery and development to screen and identify chemical agents with desired activity against a selected cell culture. There are a variety of cytotoxicity assay that are used to predict the potency of different chemical agents. For instance, trypan blue dye exclusion (TBE) assay will be briefly discussed, it is one of the method used to generate toxicity data of the genistein. TBE is a traditional and
common method that is still widely used today for measuring cell viability and to determine the cytotoxic effect of tested chemical agents. In this method, cytotoxicity of the chemical reagent is measured based on the cell membrane integrity. The cell membranes of the dead cells are not intact as a result of its being stained the trypan blue dye where the color of the dead cells becomes blue. On the other hand, the cell membrane of live cells is intact and prevents the dye from staining it blue.

There are a variety of cell-based studies that show genistein has ability to inhibit the growth and proliferation of various cancer cell types in dose- and time-dependent manners as summarized in Table 1 with IC$_{50}$ of genistein reported in micromolar. IC$_{50}$ is the concentration of a drug required for fifty percent of the cancer cells growth inhibition. These in vitro cancer cell data showed that genistein is a cytotoxic agent that can inhibit a wide range of cancer cell lines at different concentrations of genistein. These in vitro studies show that genistein exhibited potential in vitro anti-cancer activity for different type of cancer cell lines.

2.4 Mechanisms of Action of Genistein as Anti-Cancer Agents

Cancer metastasis is the major cause of death for patients with advanced cancer, and is also a disease that has no effective treatment. The metastatic processes of advanced cancers involve the migration of malignant cells from the primary tumor into the lymphatic or peripheral bloodstream where it then travels to a new site within the body to form the secondary tumor. Most often, tumor cells that detach from the primary tumor do not undergo apoptosis, a programmed pathway of cellular death. This is a crucial cellular pathway to the study because in a normal body cell, the normal cells will recognize cells that do not function
Table 1. Cell Growth Inhibition of Genistein Against Various Cancer Cell lines

<table>
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<tr>
<th>Cell Line</th>
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<td>HL-60</td>
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<td>Cell growth Inhibition</td>
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<td>TBE</td>
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* Acute Myeloid Lukemia (AML)

MTT: [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] Cell proliferation assay
SRB: [sulforhodamine B] cytotoxicity assay
TBE: Tryphan Blue Exclusion cytotoxicity assay
WST-1: [4-[3-(4-Idophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate] Cell proliferation assay and cytotoxicity assay
properly, so they undergo apoptosis. Meanwhile, with cancer, detached primary tumor cells have the potential to successfully metastasize through the cellular pathways of proliferation and angiogenesis to form the secondary tumor. The processes of metastasis are not well understood and are currently being investigated to further understand its complex cellular mechanisms. Tumor cell proliferation, loss of apoptosis, the invasion of tissue and metastasis, and angiogenesis are some of the main key mechanisms of many metastatic cancers. Of these key mechanisms, the main focus of this section will discuss results from studies on the mechanism of genistein related to its ability to inhibit the growth of primary tumors (cancer progression) and its ability to induce apoptosis in cancer cells. The mechanisms of these two cellular pathways are targets of interest for the development of effective treatment against metastatic diseases.

Studies have shown genistein to inhibit cell proliferation in cancer progression by arresting the G2/M cell cycle phase in various cancer cell lines, including breast\textsuperscript{47}, prostate\textsuperscript{48}, and other cancer cells\textsuperscript{49}. The effect of cell cycle arrest at the G2/M phase involves the down-regulation of several cyclins and cyclin dependent kinase (Cdks) by genistein. Based on a study by Li et al., genistein arrests the G2/M of the cell cycle of the hormone-independent breast cancer cell line, MDAMB-231, by reducing the protein level of cyclins B1, CdK1, and Cdc25c in a concentration-dependent manner.\textsuperscript{50} Similarly, a decrease of cyclin B1 activity was also observed in PC-3-M cells when treated with genistein as evidenced in a study conducted by Choi et al.\textsuperscript{48} The cell proliferation inhibited by genistein through blocking the progression of cells by arresting the G2/M phase in both breast and prostate cancer cell lines. This suggests that genistein deactivates several proteins from the family of the cyclins and the cyclin-
dependent kinase, which are essential to mediating the G2/M phase of the cell cycle in cancer progression (primary tumor cell proliferation).

Moreover, genistein has been reported to inhibit the growth of various cancer cell lines through the mechanism of inducing apoptosis. Apoptosis is a cellular mechanism that programmed cell death pathways in normal cells. Genistein induced apoptosis in breast cancer\textsuperscript{51}, colon cancer\textsuperscript{52}, head and neck squamous carcinoma\textsuperscript{53}, ovarian cancer\textsuperscript{54}, and prostate cancer\textsuperscript{55}. Majority of the studies reported the effects of genistein on inducing apoptosis through regulating the Bcl-family of proteins, and including the anti-apoptotic proteins and proapoptosis proteins. Some of the anti-apoptotic proteins identified as inactivators for apoptosis are in the Bcl-2 family such as the Bcl-xL and Bcl-2 proteins. Whereas the pro-apoptotic proteins, consists of the Bax, Bak, and Bad promotes apoptosis.\textsuperscript{56} In one study conducted by Chen et al., results from the RT-PCR and the western blot showed that genistein induced apoptosis in MCF-7 cells with an increased of Bax mRNA expression and a decreased of Bcl-2 mRNA levels when treated with 80 uM of genistein.\textsuperscript{51} Genistein also downregulates the anti-apoptotic proteins (Bcl-2 and Bcl-xL) and upregulates pro-apoptotic proteins (Bax) in human hepatoma cells as reported by Su et. al.\textsuperscript{57} This supports the idea that genistein induced apoptosis by down-regulating the Bcl-2 (anti-apoptotic protein) and up-regulating Bax (proapoptosis protein). In addition, genistein was reported to be a potent inhibitor of protein tyrosine kinases (PTK)\textsuperscript{58}, inhibitor of DNA topoisomerases I and II\textsuperscript{59}. PTK and DNA topoisomerases II are intracellular enzymes that may mediate the transducing-signaling pathways of cell proliferations and apoptosis.
2.5 Anti-tumor Efficacy of Genistein on Animal Models

In addition to genistein’s inhibition of cell proliferation and induction of apoptosis, cancer research in animal models including mice and rat models also proved genistein as an effective anti-tumor agent. Herein, I summarized the outcomes of several animal studies on the chemotherapeutic properties of genistein based on its ability to reduce the volume of tumor size, incidences of tumor, the growth rate of the tumor, and other anti-tumor effects.

The xenograft mouse tumor model is the most widely used animal experiment for studying a variety of cancer when examining the chemotherapeutic effects of genistein in vivo. In this cancer research, a human cancer cell line is selected and implanted into either athymic nude mice or severely compromised immunodeficient (SCID) mice. Genistein has shown in several studies to have antitumor effect against bladder cancer, breast cancer, ovarian cancer and prostate cancer. According to a study conducted by Vantyghem, a reduction of tumor volume and an inhibition of metastatic problem in the lungs and lymph node metastasis were observed in female mice fed with a genistein-supplemented diet of 750ug/g genistein, following surgical resection of primary mammary tumors. In another study by Lakshman et al., genistein was shown to lower the prostate cancer mortality of male athymic nude mice xenografted with PC3-M prostate cancer cells. Implanting PC3-M prostate cancer cells into the mice formed lung micrometastasis of >80% of the mice, in which genistein decreased metastases by 96%.

Another animal study involved the use of 7,12-dimethyl-benz[a]anthracene (DMBA), a carcinogen to induce cancer in rodent cancer model. DMBA is used to initiate tumor in skin and mammary gland. In in vivo study reported by Tanaka et al., when dietary administration of genistein occurs for 50 weeks, it can inhibit
DMBA-induced ovarian carcinogenesis in rats. In this particular study, DMBA were injected into the left ovary of female Sprague-Dawley (SD) rats to induce ovarian neoplasms. The SD rat was fed with 25 to 250 ppm of genistein after one week of its DMBA injection. The results showed that the SD rat receiving an experimental diet of 25 ppm of genistein showed 86% reduction in the incidence of ovarian adenocarcinoma. Whereas SD rats with treatment of 250 ppm genistein showed a 100% reduction of tumor incidence. These results suggest that dietary feeding of genistein can inhibit ovarian carcinogenesis of DMBA-induced rat.

In summary, the animal studies indicate that genistein (a soy isoflavone) has chemopreventive effects. Genistein has also shown to be able to decrease the volume size of the tumor and decrease the incidence of tumor cancer in different animal models.

### 2.6 Clinical Studies of Genistein

With extensive support from *in vitro* and *in vivo* studies for the roles of genistein as a potential agent to prevent and treat cancer, the chemotherapeutic effects of genistein has also been evaluated in phase II clinical trials for patients with prostate cancers.

Researchers have tested the effect of soy isoflavones supplement on the PSA level of prostate cancer patients in different clinical design studies. The designs of these clinical study were based on *in vitro* and *in vivo* data that suggest genistein to inhibit the secretion of PSA expression and growth of prostate cancer cells. According to a study by Kumar et al., a reduction in total serum PSA and testosterone was observed among 76 early stage prostate cancer patients who were given soy isoflavones (containing 60 mg of genistein) for a 12 weeks period. In a phase II clinical trial by Hussain et al., 41 prostate cancer patients with rising
PSA levels received 100 mg of soy isoflavones over a median of 5.5 months, leading to a decrease in the rate of the rise of serum PSA among 39 patients.\textsuperscript{70} Similarly, Dalais et al. observed a significant change in the total PSA levels between the groups of patients who received soy prior to surgery than the group of patients who received wheat bread.\textsuperscript{71} Overall, these clinical data suggests that soy isoflavones may have chemotherapeutic effects for patients with prostate cancer.

2.7 Safety Profile of Genistein

The toxicity of genistein were assessed in healthy patients by Ullmann et al. to investigated the safety and tolerability of synthetic genistein aglycone in a phase I clinical study in which 40 healthy volunteers were given single oral doses of 30, 60, 150, or 300 mg.\textsuperscript{72} In this study, the doses of genistein given to the volunteers were shown to be safe and well tolerated. In contrast, Lazarevic et al. found that 30 mg of synthetic genistein given to patients with early prostate cancer were also well tolerated with no adverse effects of clinical significance.\textsuperscript{73} These clinical results suggested that genistein has no toxic effects on humans. This may also be supported by epidemiological data of soy consumption that may possibly be correlated to the lower prostate cancer incidence in Asian countries, which have the highest soy consumption.

2.8 Genistein Analogs as Anticancer Agents

Poor bioavailability and moderate potency are major factors that hinder the development of genistein as a chemotherapeutic drug. These are some factors that have been targeted by researchers for developing new genistein derivatives and genistein analogs with enhanced bioavailability and potency. Some genistein analogues or derivatives showed better potency than genistein and will be
discussed in hope to help the further design of new genistein analogues or
derivatives as potential anti-cancer drugs.

A few amine complexes (Figure 5) were synthesized by Polkowski et al. to
examine the cytotoxicity of the genistein complexes with piperazine), against
human promyelocytic leukemia cell line (HL-60). The piperazine nitrogen of the
complex is bound at the OH group of C-4’ via hydrogen bonding. The results
showed that the genistein piperazine complex has better solubility than genistein.74
The in vitro anticancer activity of this genistein complex was evaluated against
HL-60 human cells. The proliferation rate decrease in a dose- and time-dependent
manner with IC₅₀ of 37, 28, 21, and 17 µM for 2, 3, 4, and 5 days treatment,
respectively.74 The results indicated that bounding a piperazine at the C-4’ OH
group of genistein can improve its water solubility and retain its in vitro anticancer
potency.

Figure 5. Piperazine genistein complex (1)75

A study on the synthesis of genistein derivatives with an improved
biological activity conducted by Meng et al. involves altering the OH group at the
C-4’ and C-7 of the genistein with fatty acid ester. The design of the derivatives
was to increase the lipophilicity of genistein, which was aimed at increasing
cellular drug concentrations via the passive diffusion through biological
membrane.76 Genistein-7-oleate (2) and genistein-4’7-dioleate (3) , shown in
Figure 6, effectively inhibited incorporation of radioactive thymidine into DNA in
U937 cells (human leukemia) better than genistein. Overall, the results from the studies show these derivatives have better bioavailability than genistein. Compound 2 reduced the cell proliferation by 36% and compound 3 reduced 43% at 3µM. The results indicate the introduction of lipophilic groups is an approach used for improving the cytotoxic activity.

Zhang et al. synthesized some genistein derivatives with heterocyclic substituents at 7-OH group through two or three carbon spacers. Their cytotoxic activities were evaluated against a human chronic myeloid leukemia cell line (K562) and a human nasopharyngeal epidermoid tumor cell line (KB). Compound 4 (Fig. 7) exhibited optimal cytotoxicity against K562 and Kb cells with IC$_{50}$ values of 6.77µM to 15.33µM, which are lower than that of the positive control 5-Fluorouracil. The results also showed that three carbon linker derivatives were more active than two carbon linker derivatives.
Figure 7. Structure of C₃-bridged heterocyclic substituent at the C-7 OH group of genistein⁷⁷
3. SYNTHESSES AND EVALUATION OF GENISTEIN ANALOGUES AS ANTICANCER AGENTS

3.1 Design of the Synthetic Targets

The problem encountered with genistein in clinical studies is the poor bioavailability and moderate potency. The aim of this research project is to develop genistein analogues with improved bioavailability and potency for potential clinical use to treat advanced hormone-refractory prostate cancer. The hypothesis of this project is that heteroaromatic rings can serve as potential bioisosteres of phenyl ring B of genistein and that incorporation of basic nitrogen can improve bioavailability of genistein analogs. The nitrogen can be protonated under acidic or physical conditions of the stomach and the compounds can be converted into salts that can maintain higher water solubility. Our first batch of synthetic targets (5 to 11), as illustrated in Figure 8, have no hydroxyl groups on ring A, which allows us to start with much more practical synthesis. We envision that the bioisosteres replacement of phenol ring B of genistein with N-containing heteroaromatic rings will be able to create a moiety with the capability to increase water solubility and eventually improve bioavailability. To investigate the effects of hydroxyl groups of genistein on cytotoxicity of cancer cell lines, daidzein and compound 3 and compound 4 were also included as target compounds of this project.

3.2 Exploration of the Most Efficient Synthetic Methods

In search of a more efficient and practical synthetic strategy to genistein analogues, Suzuki-Miyaura coupling reaction of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4H-chromen-4-one (21) with an appropriate aryl iodide (Scheme 1) was first explored. The advantages are that 1) compound 21
Figure 8. Chemical structures of genistein, daidzein, and synthesized genistein analogues (3-11)

is a more advanced intermediate to our desired products; and 2) most aryl halides are commercially available. However, the first attempt to synthesize boronic ester 21 by Miyaura borylation of 3-iodochromone, using potassium acetate as base, (Scheme 2) was not successful. The $^1$H NMR spectrum of the crude product indicated the absence of desired product 21. We then replaced potassium acetate with potassium carbonate, a stronger base, to prepare the arylboronic esters from 3-iodochromone according to the procedure described in the literature. The $^1$H NMR spectrum of the purified product from this reaction did show the existence of the borylation product. Unfortunately, the desired compound 21 was obtained accompanying with some impurities, which cannot be removed by conventional purification, such as PTLC and column chromatography.
Scheme 1: Attempted synthesis of genistein analog (22) via an arylboronic ester (21)

### 3.3 Synthesis of Genistein Analogues

The genistein analogues (3-11) were synthesized by Suzuki-Miyaura coupling reactions of 3-iodochromone (12) with the appropriate boronic acid (13-15) (Scheme 2) or aryl boronic ester (16a-f) (Scheme 3). The boronic acids (13-15) employed to make genistein analogues 3-5 by Suzuki-Miyaura coupling reaction, as described in Scheme 2, are commercially available. The 3-iodochromone (12) was readily synthesized in an excellent yield from commercially available 2-hydroxy-4,5-dimethoxyacetophenone (17) according to the procedure illustrated in the literature (Scheme 4). Specifically, condensation of 17 with N,N-dimethylformamide dimethyl acetal gave acrylophenone 18, which was treated with I$_2$ to generate 3-iodochromone 12.

Scheme 2: Synthesis of genistein analogues, 3-5
Scheme 3: Synthesis of pyrazole analogues of genistein, 6-11

Scheme 4: Synthesis of 3-iodochromone, 12

Synthesis of the pyrazole analogues of genistein (6-11) commenced with preparation of appropriate aryl boronic esters (16a-f) from the corresponding aryl iodides (20a-f) and diboronic pinacol ester through the palladium-catalyzed Miyaura borylation (Scheme 5). The corresponding aryl iodides (20a-f) were prepared by N-alkylation of pyrazole (19) followed by iodination. The N-alkylation was achieved by treating pyrazole in DMF with alkyl bromide using sodium hydride as base. The crude products achieved from this step through aqueous work-up are pure enough for the next step reaction. Green iodination of 1-alkylpyrazoles with iodine and hydrogen peroxide in water provided the corresponding iodinated products 16a-f with good yields.
Scheme 5: Synthesis of 1-alky-1H-pyrazole-4-boronic acid, 16a-f

3.4 Cytotoxicity Evaluations

The cytotoxicity of analogues 3-4, as well as daidzein, was evaluated to explore the effects of different phenolic hydroxyl groups of genistein on the potency. The synthetic genistein analogues 5-11 with N-containing heteroaromatic rings as replacements of phenol ring B of genistein were investigated to explore the possibility of these heteroaromatic rings as suitable bioisosteres. The cytotoxicity of all these compounds was evaluated against three prostate cancer cell lines (LNCaP, DU-145, and PC-3) and one aggressive cervical cancer cell line (HeLa) using trypan blue exclusion assay.

The percentages of inhibitory rates for the tested compounds are summarized in Table 2. The cytotoxicity evaluations of compounds 1-4 show that the phenolic hydroxyl groups of genistein in ring A is important for the cytotoxicity against three prostate cancer cell lines, but not HeLa cells. The phenolic hydroxyl group in ring B of genistein is not effective for the cytotoxicity against prostate and HeLa cancer.

3.5 Antiproliferative Activity of Genistein Analogues Against Prostate and Cervical Cancer Cell Lines

The in vitro anticancer potential of the genistein analogues were further evaluate using WST-1 assay to assess the cell proliferation activity following the manufacturer’s instruction. This assay is based on the cleavage of the water-
### Table 2. Cytotoxicity of genistein analogues against prostate and cervical cancer cells

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>% Inhibitory rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LNCaP&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>100 μM</td>
</tr>
<tr>
<td>1</td>
<td>72.4</td>
</tr>
<tr>
<td>2</td>
<td>56.7</td>
</tr>
<tr>
<td>3</td>
<td>33.6</td>
</tr>
<tr>
<td>4</td>
<td>44.3</td>
</tr>
<tr>
<td>5</td>
<td>24.6</td>
</tr>
<tr>
<td>6</td>
<td>9.3</td>
</tr>
<tr>
<td>7</td>
<td>58.2</td>
</tr>
<tr>
<td>8</td>
<td>51.4</td>
</tr>
<tr>
<td>9</td>
<td>54.8</td>
</tr>
<tr>
<td>10</td>
<td>47.7</td>
</tr>
<tr>
<td>11</td>
<td>61.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Human androgen-dependent prostate cancer cell line  
<sup>b</sup> Human androgen-independent prostate cancer cell line  
<sup>c</sup> Human androgen-independent prostate cancer cell line  
<sup>d</sup> Human aggressive cervical cancer cell line

Genistein was used as a positive control for comparison in the parallel experiments and the results were summarized in Table 3. Genistein has potential to attenuate proliferation in three prostate and one cervical cancer cell lines. Compared to androgen-independent PC-3 and DU145 cells, the androgen-
dependent LNCaP cells were more sensitive to genistein and its analogues. These findings are in agreement with those reported in the literature. The structure-activity relationships will be discussed in detail in the next section.

Table 3. Anti-proliferative activity of genistein analogues

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>IC\textsubscript{50} (µM)\textsuperscript{a}</th>
<th>LNCaP\textsuperscript{b}</th>
<th>DU-145\textsuperscript{c}</th>
<th>PC-3\textsuperscript{d}</th>
<th>HeLa\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein</td>
<td>37.4 ± 2.6</td>
<td>92.2 ± 3.2</td>
<td>68.6 ± 3.18</td>
<td>10.0 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>63.1 ± 6.1</td>
<td>134.5 ± 11.6</td>
<td>202.2 ± 21.4</td>
<td>5.4 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>218.0 ± 45.9</td>
<td>305.2 ± 43.7</td>
<td>327.7 ± 34.6</td>
<td>88.2 ± 15.2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>107.9 ± 30.6</td>
<td>267.2 ± 15.9</td>
<td>277.2 ± 45.0</td>
<td>51.3 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>71.9 ± 2.9</td>
<td>137.5 ± 13.3</td>
<td>108.3 ± 30.0</td>
<td>75.9 ± 9.0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>116.1 ± 23.7</td>
<td>221.3 ± 35.9</td>
<td>208.1 ± 45.2</td>
<td>130.3 ± 24.1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>49.3 ± 9.0</td>
<td>121.0 ± 12.3</td>
<td>132.8 ± 20.4</td>
<td>73.9 ± 11.0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>29.1 ± 5.9</td>
<td>118.0 ± 22.4</td>
<td>248.1 ± 7.0</td>
<td>106.8 ± 10.8</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}IC\textsubscript{50} is the drug concentration effective in inhibiting 50% of the cell viability measured by WST-1 cell proliferation assay after 3 days exposure

\textsuperscript{b}Human androgen-dependent prostate cancer cell line

\textsuperscript{c}Human androgen-independent prostate cancer cell line

\textsuperscript{d}Human androgen-independent prostate cancer cell line

\textsuperscript{e}Human aggressive cervical cancer cell line

3.6 Structure-Activity Relationship

The structure-activity relationship of genistein analogues can be summarized as below:

As shown in Table 2, compared with genistein, daidzein lacks the 5-hydroxyl group. Daidzein exhibits decreased cytotoxicity than genistein towards the three prostate cancer cell lines, but a slightly better cytotoxicity against the
HeLa cancer cell line. This indicates that the 5-hydroxyl group in ring A of genistein is important for the cytotoxicity against prostate cancer cells, but not for the HeLa cell line.

A comparison of the structures and cytotoxicity of daidzein and compound 3 led to the conclusion that the 7-hydroxyl group of daidzein is also beneficial to the cytotoxicity against androgen-refractory PC-3 and DU145 prostate cancer cells and the HeLa cervical cancer cells. The cytotoxicity of compound 3 against the LNCaP cells has a better inhibitory effect at a lower concentration (50µM), suggesting that compound 3 has a biphasic action similar to genistein reported in the literature.84

The substitutions of the 4’hydroxyphenyl moiety in compound 3 with 1-alkyl-1H-pyrazol-4-yl (compounds 7-11) show increased cytotoxicity against three prostate cancer cell lines, but not in the HeLa cell line. This indicates that 1-alkyl-1H-pyrazol-4-yl can serve as a good bioisosteres for the 4’-hydroxyphenyl moiety in genistein.

Based on the IC\textsubscript{50} values and the antiproliferative activity of genistein and its analogues, the LNCaP and HeLa cell lines were greatest inhibited by the tested agents in comparison to the PC-3 and DU145 cells. Of the tested genistein analogues, genistein possessing hydroxyl groups at C-5 and C-7 showed lower IC\textsubscript{50} values (higher potency) than compound 3, suggesting that these hydroxyl groups or their bioisosteres on ring A of the isoflavones scaffold are important for cell growth inhibition. This notion is supported by the findings that compound 3, lacking the 5,7-OH substitution pattern, is far less potent than genistein. Replacement of the 4’hydroxyphenyl moiety (ring B) in compound 3 or the phenyl moiety in compound 4 with a nitrogen-containing heteroaromatic ring (compounds 5-11) significantly enhances the anti-proliferative activity against three prostate
cancer and one cervical cancer cell lines. This implies that pyridine-3-yl and 1-alkyl-1H-pyrazol-4-yl can serve as good bioisosteres for the 4’-hydroxyphenyl moiety in genistein.

3.7 Research Summaries and Future Direction

I have successfully synthesized nine genistein analogues, including six new pyrazole analogues and three known compounds, through the chemical synthesis using the Suzuki-Miyaura coupling reaction as the key reaction. The structures have been confirmed by their $^1$H-NMR and $^{13}$C-NMR data, as well as their HRMS data. Trypan blue exclusion method and WST-1 cell proliferation assay were used to evaluate the in vitro anticancer activity of the synthesized genistein analogues against androgen-sensitive and androgen-refractory prostate cancer cell lines, along with one aggressive cervical cancer cell line. The acquired structure-activity relationship data indicated that the two-hydroxyl groups on ring A of genistein are crucial for the cytotoxicity. The preliminary data of the in vitro screening indicates the substitution of ring B of compound 4 with 1-alkyl-1H-pyrazol-4-yl at ring B increases potency in three prostate cancer cell lines.

Consequently, future target 1 as illustrated below would be a more optimal target. Another research direction in the future is to explore the derivatives as described as future target 2 below (Fig. 9).

Figure 9. Chemical structures of future targets
3.8 Experimental

3.8.1 Chemical Synthesis

3.8.1.1 Generals Methods

HRMS were obtained on an Orbitrap mass spectrometer with electrospray ionization (ESI). NMR spectra were obtained on a Bruker Fourier 300 MHz Spectrometer or a Varian NMR 400 MHz Spectrometer in CDCl₃, CD₃OD, or DMSO-d₆. The chemical shifts are given in δ ppm referenced to the respective solvent peak, and coupling constants are reported in Hz. Anhydrous THF and dichloromethane were purified by PureSolv MD 7 Solvent Purification System from Innovative Technologies (MB-SPS-800). All other reagents and solvents were purchased from commercial sources and were used without further purification. Silica gel column chromatography was performed using silica gel (32-63 µ). Preparative thin-layer chromatography (PTLC) separations were carried out on 1000 µ AnalTech thin layer chromatography plates (Lot No.13401). TLC was carried out on aluminum-backed silica gel GF plates (250 µM thickness), and the compounds were visualized by charring with KMnO₄ and/or short wavelength UV light. 3-Iodochromone (12) was synthesized in 88% yield based on the procedure as described in the literature. 1-AlkI-4-iodopyrazoles (19a-f) were synthesized according to the procedure as illustrated in the literature.

Synthesis of 3-Iodochromone (12)

2-Hydroxyacetophenone (447 mg, 3.3 mmol) was added to DMF/DMA (0.65 mL, 4.9 mmol) at 0 °C, and the reaction mixture was stirred at 95 °C for 3 h. To the solution of the obtained solid in the mixture of chloroform (0.53 mL) and pyridine (0.3 mL, 3.3 mmol) was added iodine (1.67 g, 6.6 mmol). The subsequent mixture
was stirred at room temperature for 12 h prior to being quenched with saturated aqueous sodium thiosulfate solution. The mixture was extracted with dichloromethane (100 mL x 3), and the organic extracts were combined and dried over anhydrous MgSO₄, filtrated, and concentrated under reduced pressure. The obtained crude product was subjected to column chromatography, eluting with 10% EtOAc in hexanes, to yield 3-iodochromone (11) (0.789 g, 88 % yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 8.29 (s, 1H), 8.26-8.23 (m, 1H), 7.73-7.68 (m, 1H), 7.48-7.43 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 173.3, 157.7, 156.1, 134.1, 126.6, 126.0, 121.8, 118.0, 86.9.

Synthesis of Genistein Analogues (3-11)

General procedure for the synthesis of 1-alkyl pyrazoles:
To a mixture of sodium hydride (1040 mg, 60%, 26 mmol) in DMF (8 mL) at 0°C was added dropwise a solution of pyrazole (1360 mg, 20 mmol) in DMF (2 mL), and the subsequent mixture was kept stirring at room temperature for 1 h. To this mixture was added alkyl bromide (30 mmol), and the reaction was allowed to proceed at room temperature (25 °C) for 16 h. The reaction mixture was diluted with diethyl ether, which was rinsed with brine. After removing the diethyl ether, the crude product was directly used for the next step reaction without further purification.

General procedure for the synthesis of 1-alkyl-4-iodopyrazoles (20a-f):
To a mixture of 1-alkylpyrazole (20 mmol) in water (14 mL) was sequentially added iodine (14 mmol) and 35% hydrogen peroxide (16.8 mmol), and the mixture was kept stirring for 24 h at room temperature. The reaction was quenched by the addition of a cold solution of saturated sodium thiosulfate (60 mL), and the
subsequent mixture was extracted with ethyl acetate (100 mL × 3). The combined extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. The residue obtained was chromatographed over silica gel, eluting with 5% ethyl acetate in hexanes, to furnish the respective 4-iodopyrazole (20a-f) (see Appendix).

4-Iodo-1-methyl-1H-pyrazole (20a):

This compound was prepared as a colorless oil from the crude product of 1-isopropyl-1H-pyrazole in 53% overall yield for two steps. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.49 (s, 1H), 7.40 (s, 1H), 3.92 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$)$\delta$ 144.2, 134.2, 55.9, 39.2.

4-Iodo-1-isopropyl-1H-pyrazole (20b):

This compound was prepared as a colorless oil from the crude product of 1-isopropyl-1H-pyrazole in 53% overall yield for two steps. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.50 (s, 1H), 7.45 (s, 1H), 4.56-4.43 (m, 1H), 1.49 (d, $J = 6.6$ Hz, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 143.7, 131.0, 55.4, 54.5, 22.9.

1-sec-Butyl-4-iodo-1H-pyrazole (20c):

This compound was prepared as a colorless oil from the crude product of 1-sec-butyl-1H-pyrazole in 53% overall yield for two steps. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.50 (s, 1H), 7.43 (s, 1H), 4.28-4.17 (m, 1H), 1.95-1.73 (m, 2H), 1.47 (d, $J = 6.9$ Hz, 3H), 0.81 (t, $J = 7.5$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 143.8, 131.9, 60.6, 55.4, 30.2, 20.9, 10.7.
4-Iodo-1-(isobutyl)-1H-pyrazole (20d):

This compound was prepared from the crude product of 1-(isobutyl)-1H-pyrazole in 41% overall yield for two steps. Pale yellow oil; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.48 (s, 1H), 7.37 (s, 1H), 3.88 (d, $J = 7.2$ Hz, 2H), 2.20 - 2.07 (m, 1H), 0.86 (d, $J = 6.6$ Hz, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 144.1, 133.9, 60.1, 55.6, 29.7, 19.9.

4-Iodo-1-(pentan-2-yl)-1H-pyrazole (20e):

This compound was prepared from the crude product of 1-(pentan-2-yl)-1H-pyrazole in 60% overall yield for two steps. Pale yellow oil; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.48 (s, 1H), 7.41 (s, 1H), 4.34-4.26 (m, 1H), 1.90-1.83 (m, 1H), 1.71-1.64 (m, 1H), 1.45 (d, $J = 6.8$ Hz, 3H), 1.25-1.13 (m, 2H), 0.87 (t, $J = 7.6$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 143.7, 131.7, 58.8, 55.3, 39.2, 21.3, 19.3, 13.7.

4-Iodo-1-(pentan-3-yl)-1H-pyrazole (20f):

This compound was prepared from the crude product of 1-(pentan-3-yl)-1H-pyrazole in 60% overall yield for two steps. Pale yellow oil; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.46 (s, 1H), 7.36 (s, 1H), 3.90-3.80 (m, 1H), 1.86-1.68 (m, 4H), 0.71 (t, $J = 7.5$ Hz, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 143.9, 132.7, 67.1, 55.2, 28.4, 10.7.

General procedure for the synthesis of 1-alkyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (16a-f):

A 25mL flask charged with pyrazole (272 mg, 1 mmol), palladium catalyst (245 mg, 0.03 mmol), KOAc (294 mg, 3 mmol), and bis(pinacolato) diboron (381 mg, 1.5 mmol) was flushed with argon. DMSO (6 mL) and the appropriate 1-alkyl-4-iodopyrazole (1 mmol) were added and the reaction mixture was stirred for 12 h at 80 °C prior to being extracted with ethyl ether (100 mL x 3). The combined
extracts were rinsed with brine (20 mL x 3), dried over anhydrous MgSO$_4$, filtered, and concentrated under reduced pressure. The residue obtained was chromatographed over silica gel, eluting with 20% ethyl acetate in hexanes, to furnish the respective 1-alkyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (16a-f). A boron-containing impurity is very harsh to be completely removed from boronates 16a-f even after multiple times of column chromatography purification. We therefore directly used compounds 16a-f contaminating with minor impurity (less than 10%) for the next step reaction.

General Procedure for the synthesis of genistein analogues (3-5)

3-Iodochromone (12, 272 mg, 1 mmol) and the appropriate boronic acid (13, 14, or 15, 1.5 mmol) were dissolved in THF (30 mL), and then aqueous Na$_2$CO$_3$ (3.0 mL of 2 M) followed by tetrakis(triphenylphosphine)palladium (35 mg, 0.03 mmol) were added. The reaction was reflux for 5-6 h, cooled to 25 °C, water was added (50 mL), and the mixture was extracted with EtOAc (100 mL × 3). The combined EtOAc extracts were washed with water (30 mL × 3), the organic fraction was dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo to afford the crude product. Purification of the product by preparative TLC using 30% ethyl acetate in hexanes as eluent furnished the respective title compound (3-5) (see Appendix).

3-(4-Hydroxyphenyl)-4H-chromen-4-one (3)

This compound was prepared from the Suzuki coupling of 3-iodochromone (11) and (4-hydroxyphenyl) boronic acid (12) in 21% yield as a brown solid: mp. 221-223°C; $^1$H NMR (400 MHz, CD$_3$COCD$_3$) $\delta$ 8.44 (d, J = 1.6 Hz, 1H), 8.27 (d, J = 2.0 Hz, 1H), 8.22 (dd, J = 8.0, 1.6 Hz, 1H), 7.79 (tt, J = 7.2, 2.0 Hz, 1H), 7.61-7.58 (m,1H), 7.51-7.48 (m, 3H), 6.93-6.89 (m, 2H); $^{13}$C NMR (75 MHz,
CD$_3$COCD$_3$) $\delta$ 174.8, 156.8, 155.6, 152.4, 133.1, 129.6, 125.2, 124.5, 124.1, 123.9, 122.7, 117.6, 114.4; IR (KBr) 3296, 1621, 1607, 1594, 1582, 1568, 1509, 1477 cm$^{-1}$. HRMS (ESI) $m/z$: calcd for C$_{15}$H$_{11}$O$_3$ [M+H]$^+$: 239.0708; Found 239.0709.

**3-Phenyl-4H-chromen-4-one (4)**

This compound was prepared from the Suzuki coupling of 3-iodochromone (12) and phenylboronic acid (14) in 38% yield as a pink needle: mp. 130-131$^\circ$; $^1$H NMR (400 MHz, CD$_3$COCD$_3$) $\delta$ 8.39 (s, 1H), 8.27 (dd, $J$ = 8.0, 1.6 Hz, 1H), 7.70-7.69 (m, 1H), 7.68-7.67 (m, 1H), 7.66 (d, $J$ = 8.4 Hz, 1H), 7.55 (tt, $J$ = 7.2, 1.2 Hz, 1H), 7.50-7.42 (m, 3H); $^{13}$C NMR (75 MHz, CD$_3$COCD$_3$) $\delta$ 174.8, 155.8, 153.5, 133.5, 132.0, 128.6, 127.7, 127.5, 125.4, 124.9, 124.4, 124.2, 117.8; IR (KBr) 1639, 1615, 1463 cm$^{-1}$; HRMS (ESI) $m/z$: calc for C$_{15}$H$_{11}$O$_2$ [M + H]$^+$: 223.0759. Found 223.0760.

**3-(Pyridin-4-yl)-4H-chromen-4-one (5)**

This compound was prepared from the Suzuki coupling of 3-iodochromone (12) and pyridin-4-ylboronic acid (15) in 40% yield as a coral pink solid: mp. 157-158$^\circ$; $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 8.77 (s, 1H), 8.55 (d, $J$ = 4.2 Hz, 1H), 8.47 (s, 1H), 8.23 (d, $J$ = 8.1 Hz, 1H), 8.10 (d, $J$ = 8.1 Hz, 1H), 7.82 (t, $J$ = 7.5 Hz, 1H), 7.64 (d, $J$ = 8.7 Hz, 1H), 7.52 (t, $J$ = 6.6 Hz, 2H). $^{13}$C NMR (75 MHz, CD$_3$OD) $\delta$ 177.6, 157.9, 156.6, 149.9, 149.4, 138.9, 135.7, 130.2, 127.0, 126.8, 125.3, 125.0, 122.9, 119.6; IR (KBr) 3038, 3037, 1634, 1588, 1566, 1470 cm$^{-1}$; HRMS (ESI): $m/z$ calc for C$_{14}$H$_{10}$NO$_2$ [M + H]$^+$: 224.0712. Found 224.0714.
General procedure for the synthesis of pyrazole analogues of genistein (6-11)

3-Iodochromone (12, 90 mg, 0.3 mmol) and the appropriately substituted 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (16a-f, 0.5 mmol) were dissolved in THF (30 mL), and then aqueous Na$_2$CO$_3$ (3.0 mL of 2 M) followed by tetrakis(triphenylphosphine)palladium (12 mg, 0.01 mmol) were added. The reaction was allowed to proceed at reflux overnight, cooled to 25 °C, water was added (50 mL), and the mixture was extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were washed with water (30 mL × 3), the organic fraction was dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo to afford the crude product. Purification of the product by preparative TLC using 30% ethyl acetate in hexanes as eluent furnished the respective title compounds (6-11) (see Appendix).

3-(1-Methyl-1H-pyrazol-4-yl)-4H-chromen-4-one (6)

This compound was prepared from the Suzuki coupling of 3-iodochromone (12) and 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (16a) in 31.07% yield as a light yellow solid: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.28 (dd, $J$ = 8.1, 1.2 Hz, 1H), 8.22 (s-overlap, 2H), 7.75 (s, 1H), 7.69 – 7.63 (m, 2H), 7.48 - 7.38 (m, 3H), 3.94 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 175.8, 155.9, 150.7, 135.9, 133.5, 132.2, 132.0, 130.4, 128.6, 128.4, 126.2, 125.1, 124.0, 118.1, 117.5, 112.2, 39.1; IR (KBr) = 2936, 1634, 1612, 1544, 1470, 1438 cm$^{-1}$; HRMS (ESI) calculated for C$_{13}$H$_{10}$N$_2$O$_2$ [M + H]$^+$: 227.0820. Found 227.0820.

3-(1-Isopropyl-1H-pyrazol-4-yl)-4H-chromen-4-one (7)

This compound was prepared from the Suzuki coupling of 3-iodochromone (12) and 1-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole
(16b) in 42% yield as a colorless yellow solid: mp. 104-105 °C; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.32 (s, 1H), 8.30 (dd, $J$ = 8.2, 1.0 Hz, 1H), 8.25 (s, 1H), 7.79 (s, 1H), 7.67 (dt, $J$ = 7.5, 1.8 Hz, 1H), 7.48 (d, $J$ = 8.4 Hz, 1H), 7.43 (t, $J$ = 7.2 Hz, 1H), 4.57 (hept, $J$ = 6.7 Hz, 1H), 1.56 (d, $J$ = 6.6 Hz, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 175.8, 155.9, 150.7, 134.9, 133.5, 127.1, 126.1, 125.2, 124.0, 118.1, 117.5, 111.7, 54.2, 22.9; IR (KBr) 3069, 2929, 2978, 1634, 1609, 1540, 1510, 1489, 1469 cm$^{-1}$; HRMS (ESI) m/z: calcd for C$_{15}$H$_{15}$N$_2$O$_2$ [M + H]$^+$: 255.1134. Found 255.1135.

3-(1-(sec-Butyl)-1H-pyrazol-4-yl)-4H-chromen-4-one (8)

This compound was prepared from the Suzuki coupling of 3-iodochromone (12) and 1-sec-butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (16c) in 39% yield as a colorless yellow wax: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.31 (s, 1H) 8.31-8.26 (overlapped, 1H), 8.26 (s, 1H), 7.79 (s, 1H), 7.67 (t, $J$ = 7.5 Hz, 1H), 7.48 (d, $J$ = 8.4 Hz, 1H), 7.42 (t, $J$ = 7.5 Hz, 1H), 4.33-4.22 (m, 1H), 1.98-1.79 (m, 2H), 1.53 (d, $J$ = 6.9 Hz, 3H), 0.85 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 173.6, 153.6, 148.3, 132.8, 131.1, 125.6, 123.8, 122.9, 121.7, 115.8, 115.3, 109.2, 57.9, 27.9, 18.6, 8.4; IR (KBr) 2973, 1644, 1609, 1541 1511, 1490, 1465 cm$^{-1}$; HRMS (ESI) m/z: calculated for C$_{16}$H$_{17}$N$_2$O$_2$ [M + H]$^+$: 269.1290. Found 269.1294.

3-(1-Isobutyl-1H-pyrazol-4-yl)-4H-chromen-4-one (9)

This compound was prepared from the Suzuki coupling of 3-iodochromone (12) and 1-isobutyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (16d) in 42% yield as a white solid: mp. 96-97 °C; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.29 (d, $J$ = 9.3 Hz, 1H), 8.26 (s, 1H), 8.25 (s, 1H), 7.77 (s, 1H), 7.66 (dt, $J$ = 7.5 Hz,
1H), 7.47 (d, J = 8.4 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 3.96 (d, J = 7.2 Hz, 2H),
2.31-2.18 (m, 1H), 0.93 (d, J = 6.6 Hz, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 175.5,
155.7, 150.4, 135.3, 133.2, 129.7, 125.9, 124.9, 123.8, 117.8, 117.3, 111.5, 59.6,
29.4, 19.7; IR (KBr) 3053, 2961, 2928, 2871, 1640, 1611, 1542, 1465 cm$^{-1}$;
HRMS (ESI) m/z: calcd for C$_{16}$H$_{17}$N$_2$O$_2$ [M + H]$^+$: 269.1290. Found 269.1296.

3-(1-(Pentan-2-yl)-1H-pyrazol-4-yl)-4H-chromen-4-one (10)

This compound was prepared from the Suzuki coupling of 3-iodochromone (12)
and 1-(pentan-2-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole
(16e) in 29% yield as a light yellow wax: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.31 (s,
1H), 8.30-8.26 (overlapped, 1H), 8.26 (s, 1H), 7.78 (s, 1H), 7.67 (t, J = 7.5 Hz,
1H), 7.48 (d, J = 8.4 Hz, 1H), 7.42 (t, J = 7.5 Hz, 1H), 4.43-4.32 (m, 1H), 1.99-
1.88 (m, 1H), 1.79-1.67 (m, 1H), 1.53 (d, J = 6.6 Hz, 1H), 1.30-1.18 (m, 2H), 0.90
(t, J = 7.2 Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 173.9, 154.0, 148.7, 133.2,
131.5, 125.8, 124.2, 123.2, 122.1, 116.2, 115.8, 109.6, 56.5, 37.3, 19.4, 17.5, 11.8;
IR (KBr) 3068, 2958, 2930, 2872, 1636, 1609, 1578, 1541, 1463 cm$^{-1}$; HRMS
(ESI) m/z: calcd for C$_{17}$H$_{19}$N$_2$O$_2$ [M + H]$^+$: 283.1447. Found 283.1448.

3-(1-(Pentan-3-yl)-1H-pyrazol-4-yl)-4H-chromen-4-one (11)

This compound was prepared from the Suzuki coupling of 3-iodochromone (12)
and 1-(pentan-3-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole
(16f) in 37% yield as a yellow wax: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.30 (s, 1H),
8.30-8.26 (overlapped, 1H), 8.26 (s, 1H), 7.80 (s, 1H) 7.66 (t, J = 7.6 Hz, 1H),
7.47 (d, J = 8.4 Hz, 1H), 7.41 (t, J = 7.6 Hz, 1H), 3.99-3.94 (m, 1H), 1.95-1.82 (m,
4H), 0.81 (t, J = 7.2 Hz, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 176.0, 156.1, 150.8,
135.3, 133.6, 129.1, 126.3, 125.3, 124.2, 118.3, 117.8, 111.5, 67.0, 28.6, 10.9; IR
(KBr) 2966, 2931, 2876, 1641, 1610, 1541, 1465 cm$^{-1}$; HRMS (ESI) $m/z$: calculated for C$_{17}$H$_{19}$N$_2$O$_2$ [M + H]$^+$: 283.1447. Found 283.1452.

**Cell culture**

All cell lines were initially purchased from American Type Culture Collection (ATCC$^{\text{TM}}$). The PC-3 prostate cancer cells, the LNCaP prostate cancer cells, and the HeLa cervical cancer cells were routinely cultured in RPMI-1640 medium supplemented with 10% FBS and 10% penicillin/streptomycin. Cultures were maintained in 5% carbon dioxide at a temperature of 37°C. The DU-145 prostate cancer cells were routinely cultured in Eagle’s Minimum Essential Medium (EMEM) medium supplemented with 10% FBS and 10% penicillin/streptomycin.

**Effect of Genistein and Analogues on Cell Viability**

PC-3 or DU-145 or LNCaP or HeLa cells were plated in 24-well plates at a density of 20,000 each well in the appropriate 10% FBS medium. The cells were then treated with genistein, or synthesized genistein analogues separately at 50 µM and 100 µM (for PC-3, DU-14, and LNCaP cells) or at 1 µM and 10 µM (for HeLa cells) for 3 days, while equal treatment volumes of DMSO were used as vehicle control. Cell numbers were counted with a cell viability analyzer (Beckman-Coulter). The ratio of drug treated viable cell numbers to vehicle treated viable cell numbers was defined as percentage viability and variation between replicate experiments is not greater than 5%. IC$_{50}$ values were obtained from dose-response curves for each genistein analogs.

**WST-1 cell proliferation assay**

PC-3 or DU-145 or LNCaP or HeLa cells were plated in 96-well plates at a density of 3,200 per well in 200 µL of culture medium. The cells were then
treated with genistein, or synthesized genistein analogues separately at different doses for 3 days, while equal treatment volumes of DMSO were used as vehicle control. The cells were cultured in a CO₂ incubator at 37 °C for 3 days. 10μL of the premixed WST-1 cell proliferation reagent (Clontech) was added to each well. After mixing gently for one minute on an orbital shaker to ensure homogeneous distribution of color, the cells were incubated for additional 3 h at 37 °C. The absorbance of each well was measured using a microplate-reader (Synergy HT, BioTek) at a wavelength of 430nm. The IC_{50} value is the concentration of each compound that inhibits cell proliferation by 50% under the experimental conditions and is the average from at least triplicate determinations that were reproducible and statistically significant. For calculating the IC_{50} values, a linear proliferative inhibition was made based on at least five dosages for each compound.

Figures 10-25 in the next section show the proton and carbon NMR spectra of the genistein analogues.
4. SELECTED NMR SPECTRA

Figure 10. $^1$H NMR spectrum of compound 3

Figure 11. $^{13}$C NMR spectrum of compound 3
Figure 12. $^1$H NMR spectrum of compound 4

Figure 13. $^{13}$C NMR spectrum of compound 4
Figure 14. $^1$H NMR spectrum of compound 5

Figure 15. $^{13}$C NMR spectrum of compound 5
Figure 16. $^1$H NMR spectrum of compound 7

Figure 17. $^{13}$Carbon NMR spectrum of compound 7
Figure 18. $^1$H NMR spectrum of compound 8

Figure 19. $^{13}$C NMR spectrum of compound 8
Figure 20. $^1$H NMR spectrum of compound 9

Figure 21. $^{13}$C NMR spectrum of compound 9
Figure 22. $^1$H NMR spectrum of compound 10

Figure 23. $^{13}$C NMR spectrum of compound 10
Figure 24. $^1$H NMR spectrum of compound 11

Figure 25. $^{13}$C NMR spectrum of compound 11
REFERENCES


APPENDIX: TABLES
Chemical Analysis and NMR Data of Genistein analogues and Intermediate Compounds

### NMR Data of Intermediate Compounds

<table>
<thead>
<tr>
<th>Compound #</th>
<th>Formula, MW</th>
<th>Chemical Structure</th>
<th>NMR Data: $^1$H NMR and $^{13}$C NMR</th>
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<td>12</td>
<td>C$_6$H$_5$IO$_2$ 272.0415 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.29 (s, 1H), 8.26 – 8.23 (m, 1H), 7.73 – 7.68 (m, 1H), 7.48 – 7.43 (m, 2H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 173.3, 157.7, 156.1, 134.1, 126.6, 126.0, 121.8, 118.0, 86.9.</td>
</tr>
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<td>20a</td>
<td>C$_4$H$_3$IN$_2$ 208.0025 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.49 (s, 1H), 7.40 (s, 1H), 3.92 (s, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 144.2, 134.2, 55.9, 39.2.</td>
</tr>
<tr>
<td>20b</td>
<td>C$_6$H$_4$IN$_2$ 236.0565 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.50 (s, 1H), 7.45 (s, 1H), 4.56 - 4.43 (m, 1H), 1.49 (d, $J$ = 6.6 Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 143.7, 131.0, 55.4, 54.4, 22.9.</td>
</tr>
<tr>
<td>20c</td>
<td>C$<em>7$H$</em>{11}$IN$_2$ 250.0835 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.50 (s, 1H), 7.43 (s, 1H), 4.28 - 4.17 (m, 1H), 1.95 – 1.73 (m, 2H), 1.47 (d, $J$ = 6.9 Hz, 3H), 0.81 (t, $J$ = 7.5 Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 143.8, 131.9, 60.6, 55.4, 30.2, 20.9, 10.7.</td>
</tr>
<tr>
<td>20d</td>
<td>C$<em>7$H$</em>{11}$IN$_2$ 250.0835 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.48 (s, 1H), 7.37 (s, 1H), 3.88 (d, $J$ = 7.2 Hz, 2H), 2.20 - 2.07 (m, 1H), 0.86 (d, $J$ = 6.6 Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 144.1, 133.9, 60.1, 55.6, 29.7, 19.9.</td>
</tr>
<tr>
<td>20e</td>
<td>C$<em>8$H$</em>{13}$IN$_2$ 264.1105 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.48 (s, 1H), 7.41 (s, 1H), 4.34 - 4.26 (m, 1H), 1.90 – 1.83 (m, 1H), 1.71 – 1.64 (m, 1H), 1.45 (d, $J$ = 6.8 Hz, 3H), 1.25 – 1.13 (m, 2H), 0.87 (t, $J$ = 7.6 Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 143.7, 131.7, 58.8, 55.3, 39.2, 21.3, 19.3, 13.7.</td>
</tr>
<tr>
<td>20f</td>
<td>C$<em>8$H$</em>{13}$IN$_2$ 264.1105 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.46 (s, 1H), 7.36 (s, 1H), 3.90 – 3.80 (m, 1H), 1.86 – 1.68 (m, 4H), 0.71 (t, $J$ = 7.5 Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 143.9, 132.7, 67.1, 55.2, 28.4, 10.7.</td>
</tr>
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<td>Cmpds #, Formula, and MW</td>
<td>Chemical Structure</td>
<td>NMR Data: $\text{^1H NMR}$ and $\text{^{13}C NMR}$</td>
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</tr>
<tr>
<td>-------------------------</td>
<td>--------------------</td>
<td>-----------------------------------------------</td>
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</tr>
<tr>
<td><strong>3</strong> (phenol) $C_{13}H_{10}O_3$ 238.0630 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$\text{^1H NMR}$ (400 MHz, CD$_2$COCD$_3$) $\delta$ 8.44 (s, 1H), 8.28 – 8.27 (m, 1H), 8.22 – 8.20 (m, 1H), 7.82 – 7.77 (m, 1H), 7.61 – 7.58 (m, 1H), 7.51 – 7.49 (m, 3H), 6.92 – 6.89 (m, 2H). $\text{^{13}C NMR}$ (75 MHz, CD$_2$COCD$_3$) $\delta$ 204.7, 174.9, 156.8, 155.6, 152.4, 133.1, 129.6, 125.2, 124.5, 124.1, 123.9, 122.7, 117.6, 114.4.</td>
<td></td>
</tr>
<tr>
<td><strong>4</strong> (phenyl) $C_{13}H_{10}O_2$ 222.0681 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$\text{^1H NMR}$ (400 MHz, CD$_2$COCD$_3$) $\delta$ 8.22 (s, 1H), 8.11 – 8.08 (m, 1H), 7.71 – 7.66 (m, 1H), 7.53 – 7.47 (m, 3H), 7.40 – 7.36 (m, 3H), 7.32 – 7.25 (m, 3H). $\text{^{13}C NMR}$ (75 MHz, CD$_2$COCD$_3$) $\delta$ 204.9, 174.8, 155.8, 153.5, 146.9, 133.5, 132.0, 128.6, 127.7, 127.5, 125.4, 124.9, 124.4, 124.2, 117.8.</td>
<td></td>
</tr>
<tr>
<td><strong>5</strong> (R: 3-pyridinyl) $C_{14}H_{12}NO_2$ 223.0633 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$\text{^1H NMR}$ (300 MHz, CD$_2$OD) $\delta$ 8.77 (s, 1H), 8.55 (d, $J = 4.2$ Hz, 1H), 8.47 (s, 1H), 8.23 (d, $J = 8.1$ Hz, 1H), 8.10 (d, $J = 8.1$ Hz, 1H), 7.82 (t, $J = 7.5$ Hz, 1H), 7.65 (d, $J = 8.7$ Hz, 1H), 7.52 (t, $J = 6.6$ Hz, 2H). $\text{^{13}C NMR}$ (75 MHz, CD$_2$OD) $\delta$ 177.6, 157.9, 156.6, 149.9, 149.4, 138.9, 135.7, 130.2, 127.0, 126.8, 125.3, 125.0, 122.9, 119.6.</td>
<td></td>
</tr>
<tr>
<td><strong>6</strong> (R: Methyl) $C_{13}H_{10}N_2O_2$ 226.0742 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$\text{^1H NMR}$ (300 MHz, CDCl$_3$) $\delta$ 8.28 (dd, $J = 8.1$, 1.2 Hz, 1H), 8.22 (s, overlap, 2H), 7.75 (s, 1H), 7.69 – 7.63 (m, 2H), 7.48 – 7.38 (m, 3H), 3.94 (s, 3H). $\text{^{13}C NMR}$ (75 MHz, CDCl$_3$) $\delta$ 175.8, 155.9, 150.7, 135.9, 133.5, 132.2, 132.0, 130.4, 128.6, 128.4, 126.2, 125.1, 124.0, 118.1, 117.5, 112.2, 39.1.</td>
<td></td>
</tr>
<tr>
<td><strong>7</strong> (R: Isopropyl) $C_{13}H_{14}N_2O_2$ 254.1055 amu</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$\text{^1H NMR}$ (300 MHz, CDCl$_3$) $\delta$ 8.32 (s, 1H), 8.30 (dd, $J = 8.2$, 1.0 Hz, 1H), 8.25 (s, 1H), 7.79 (s, 1H), 7.70 – 7.66 (m, 1H), 7.50 – 7.40 (m, 2H), 4.57 (hept, $J = 6.7$ Hz, 1H), 1.36 (d, $J = 6.6$ Hz, 6H). $\text{^{13}C NMR}$ (75 MHz, CDCl$_3$) $\delta$ 175.8, 155.9, 150.7, 134.9, 133.5, 127.1, 126.1, 125.2, 124.0, 118.1, 117.5, 111.7, 54.2, 22.9.</td>
<td></td>
</tr>
</tbody>
</table>
# NMR Data of Genistein Analogue

<table>
<thead>
<tr>
<th>Cmpds #, Formula, and MW</th>
<th>Chemical Structure</th>
<th>NMR Data: $^1$H NMR and $^{13}$C NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (R: sec-butyl)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (300 MHz, CDCl₃) $\delta$ 8.31 (s, 1H) 8.31 – 8.26 (Overlap, 1H), 8.26 (s, 1H), 7.79 (s, 1H), 7.50 – 7.40 (m, 2H), 4.33 – 4.22 (m, 1H), 1.98 – 1.79 (m, 2H), 1.53 (d, $J = 6.9$ Hz, 3H), 0.85 (t, $J = 7.5$ Hz, 3H). $^{13}$C NMR (75 MHz, CDCl₃) $\delta$ 173.6, 153.6, 148.3, 132.8, 131.1, 125.6, 123.8, 122.9, 121.7, 115.8, 115.3, 109.2, 57.9, 27.9, 18.6, 8.4.</td>
</tr>
<tr>
<td>9 (R: isobutyl)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (300 MHz, CDCl₃) $\delta$ 8.29 (d, $J = 9.3$ Hz, 1H), 8.26 (s, 1H), 8.25 (s, 1H), 7.77 (s, 1H), 7.69 – 7.63 (m, 1H), 7.48 – 7.39 (m, 2H), 3.96 (d, $J = 7.2$ Hz, 2H), 2.31– 2.18 (m, 1H), 0.93 (d, $J = 6.6$ Hz, 6H). $^{13}$C NMR (75 MHz, CDCl₃) $\delta$ 175.5, 155.7, 150.4, 135.3, 133.2, 129.7, 125.9, 124.9, 123.8, 117.8, 117.3, 111.5, 59.6, 29.4, 19.7.</td>
</tr>
<tr>
<td>10 (R: 2-Pentyl)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (300 MHz, CDCl₃) $\delta$ 8.31 (s, 1H), 8.31– 8.26 (overlap, 1H), 8.26 (s, 1H), 7.78 (s, 1H), 7.70 – 7.64 (m, 1H), 7.49 – 7.40 (m, 2H), 4.43 – 4.32 (m, 1H), 1.99 - 1.88 (m, 1H), 1.79 – 1.67 (m, 1H), 1.53 (d, $J = 6.6$ Hz, 1H), 1.30– 1.18 (m, 2H), 0.90 (t, $J = 7.2$ Hz, 3H). $^{13}$C NMR (75 MHz, CDCl₃) $\delta$ 173.9, 154.0, 148.7, 133.2, 131.5, 125.8, 124.2, 123.2, 122.1, 116.2, 115.8, 109.6, 56.5, 37.3, 19.4, 17.5, 11.8.</td>
</tr>
<tr>
<td>11 (R: 3-Pentyl)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>$^1$H NMR (400 MHz, CDCl₃) $\delta$ 8.30 (s, 1H), 8.30 – 8.26 (overlap, 1H), 8.26 (s, 1H), 7.80 (s, 1H), 7.66 (t, $J = 7.6$ Hz, 1H), 7.48 – 7.393 (m, 2H), 3.99 – 3.94 (m, 1H), 1.95 – 1.82 (m, 4H), 0.81 (t, $J = 7.2$ Hz, 6H). $^{13}$C NMR (75 MHz, CDCl₃) $\delta$ 176.0, 174.8, 156.1, 150.84, 150.82, 135.3, 133.6, 129.1, 126.3, 125.3, 124.2, 118.3, 117.8, 111.5, 67.0, 29.9, 28.6, 21.0, 10.9.</td>
</tr>
</tbody>
</table>
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**Pahoua Xiong**

Type full name as it appears on submission

**June 26, 2015**

Date