DRUG DISCOVERY

Anticancer activities of isoflavones from the *Millettia thonningii* plant and their synthetic derivatives

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ABSTRACT

Three naturally isolated isoflavones (2, 4 and 5), two synthetically modified isoflavones (3 and 6) and four synthesised isoflavones (10, 12, 13 and 15) were tested *in-vitro* to access their activities against Chang liver (normal cell line), JURKAT (leukemia), MCF-17 (breast), HEP-G2 (liver), PC3 (prostate) and LNCap (prostate) cell lines. In the test against liver cancer, the synthetically modified isoflavone 3 was better than the curcumin (CUR) control with IC50 value of 5.14 μM. Synthesised isoflavone 12 showed promising activity against prostate cancer (PC3) (IC50 = 6.11 μM). Whereas intermediate 2,4-dihydroxydeoxybenzoin (10) showed appreciable to moderate activity against breast, liver and prostate (PC3) cancer cell lines (IC50 values of 14.59, 31.79 and 65.20 μM, respectively). By comparison, the synthesised isoflavones showed better activity than the naturally isolated isoflavones against the same cancer cell lines. These results could serve as the basis of a structure activity relationship (SAR) study to identify potential anti-cancer drug candidates, with improved biological properties based on the isoflavone scaffold.

Keywords: Anti-cancer, malignancies, *Milletia thonningii*, isoflavones.

1. INTRODUCTION

Isoflavones are polyphenol compounds, that occur naturally in plants and mostly in soybeans. The scientific interest of isoflavones has increased over the last decade because of their potential health benefits (Dixon and Steele, 1999). Among these benefits are anticancer, antioxidant (due to their ability to transfer free radicals, chelate metal catalysts, and activate antioxidant enzymes), prevention of cardiovascular diseases, and postmenopausal symptoms (Ferrali et al., 1997; Elliott et al., 1992; Cos et al., 1998; Dixon and Steele, 1999). For example, genistein 1 (Figure 1), a simple isoflavonoid compound, is a known protein tyrosine kinase inhibitor (an important factor in many signal transductions pathways, thus influencing cell growth and potentially affecting cancer). In Francophone West Africa, the bark of the *Millettia thonningii* plant (a semi-deciduous tree which

grows in tropical climates all over the world and a good source of isoflavones of type 2) is used as a laxative for children (Wang et al., 2002; Moccia et al., 2003; Asomaning et al., 1995; Asomaning et al., 1999; Harrison et al., 2011). The pulverized root and stem bark decoction is drunk in Nigeria as relief for menstrual pains, whilst the leaf extract is used as a cure for dysentery and diarrhoea (Irvine, 1961).

Additionally, the leaf-juice is reported to be lethal to Bulinus snail, a water snail carrying the microorganisms that causes schistosomiasis (bilharzias), a parasitic disease endemic throughout South America, Africa and the Far east (Irvine, 1961; Abbiw, 1990). Furthermore, 5-hydroxy-3-(4-methoxyphenyl)-8,8-dimethylpyrano[3,2-g]chromen-4(8H)-one 3 (Figure 1) is the first known small molecule in the literature to inhibit hypoxia-inducible factor-1 (HIF-1) activated by simultaneously suppressing mitochondrial respiration and disrupting protein translation *in-vitro* (Mailllard et al., 1993). Despite their potential health benefits, investigations into isoflavones as possible anticancer drug candidates are limited in the literature. Cancer is the leading cause of death in both developed and low middle income countries (LMICs) alike. In 2012, an estimated 14.1 million new cases were diagnosed with 8.2 million deaths worldwide. Liver and breast cancer are the most frequently diagnosed cancers and the leading causes of cancer death in men and women, in most developed countries (Marston and Hostettmann, 1985; Torre et al., 2015). Over the years, the burden has shifted to less developed countries, which currently account for about 57% of cases and 65% of cancer deaths worldwide (Marston and Hostettmann, 1985).

With a rapidly growing and ageing population in most countries, as well as an increasing prevalence of established risk factors, the impact of cancer on human life is increasing substantially (Ferlay et al., 2015). By 2030, it is estimated that 27 million new cases and 17 million deaths are expected globally and LMICs are expected to contribute to 70% of all cancer deaths (Stewart and Kleihues, 2003; Farmer et al., 2010). Cancer remains a dreaded disease and the use of natural products have shown significant therapeutic efficacy against malignancies. Natural products obtained from medicinal plants have played an important role in the treatment of cancer (Lozano et al., 2012). Examples of some of the best-known plant-derived anticancer drugs in clinical use, are the so-called vinca alkaloids, vinblastine and vincristine, isolated from the Madagascar periwinkle plant and taxol from the Pacific yew tree (Prakash et al., 2013). Herein, we report the anticancer activity of isoflavones from three sources (the *Milletia thonningii* plant, derivatives from the natural compound *O*,*O*-dimethylalpinumisoflavone 2 and synthesised isoflavones 10 to 15) against several cancer cell lines *in-vitro*.

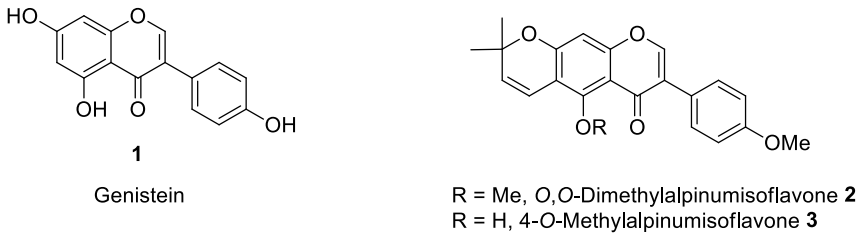


Figure 1 Some naturally occurring isoflavones

2. EXPERIMENTAL PROCEDURE

Isolation and synthesis

General

TLC was performed on precoated TLC plates with silica gel 60 F254 (0.2 mm, Merck, Darmstadt, Germany). 1H and 13C NMR spectra were recorded on a Bruker 500 MHz spectrometer and TMS as internal standard. Column chromatography separation was performed on silica gel 60 (0.063 - 0.200 mm), Fluka, steinheim, Germany. Developed TLC plates were visualised under UV light. All chemicals and solvents were of analar grade. The melting points recorded in this work were uncorrected and were determined in sealed capillary tubes on Stuart melting point apparatus.

Plant Material

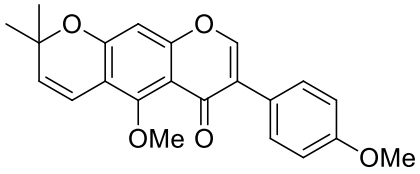
The stem bark of *Millettia thonningii* was collected from the University of Ghana campus on September, 2015, (Legon-Accra, Ghana). A voucher specimen was identified by Abbiw, (1990) and later by Mr YJ Amponsah of blessed memory at the Ghana National Herbarium, Department of Botany of the University of Ghana. The voucher specimen (catalogue NO: 14832) was deposited at the herbarium. The stem bark was shade dried for four weeks and then pulverised.

Extraction and Isolation

The pulverised plant material (1.8 kg) was extracted with ethyl acetate (10.5 L) using a Soxhlet apparatus for 48 h. The extract was filtered and concentrated using a rotary evaporator to give a brown crude (174 g). 96 g of the crude was subjected to column chromatography using 500 g of silica gel as stationary phase. The column was first eluted with 100% petroleum ether (2 L) followed by gradient elution using petroleum ether and ethyl acetate (from 5% ethyl acetate in petroleum ether with increment of 5% until 100% ethyl acetate was reached). In all, 540 eluents were collected and grouped into twelve dominant fractions, F1 - F12, based on their TLC profiles. Out of fraction F6 was obtained white granule solids which was coded 2, (12.8 g); from F11 crushed out white crystals which was further purified through recrystallization and coded 4 (5.2 g); and fraction F12 yielded a light brown solid which was coded 5 (0.2 g). The compounds isolated were characterized by TLC, melting point, mass spectrometry, 1H and 13C-NMR spectra and the results compared to publish data.

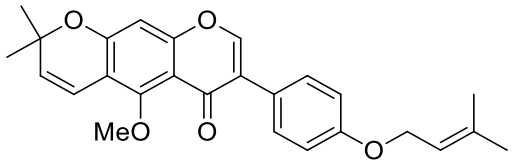
5-Methoxy-3-(4-methoxyphenyl)-8,8-dimethylpyrano[3,2-g]chromen-4(8H)-one (2)

White granules, 12.8 g; m.p. 138 - 140 oC; 1H-NMR (500 MHz, CDCl3) δ: 7.69 (1H, s), 7.38 (2H, d, J = 8 Hz), 6.88 (2H, d, J = 8 Hz), 6.67 (1H, d, J = 10 Hz), 6.53 (1H, s), 5.64 (1H, d, J = 10 Hz), 3.82 (3H, s), 3.76 (3H, s), 1.40 (6H, s) ppm; 13C NMR (500 MHz, CDCl3) δ: 175.0 (C), 159.5 (C), 158.7 (C), 158.0 (C), 155.8 (C), 150.4 (CH), 130.7 (CH), 130.4 (CH), 125.6 (C), 124.3 (C), 116.2 (CH), 114.0 (C), 113.9 (CH), 113.3 (C), 113.2 (C),100.7 (CH), 62.8 (CH3), 55.4 (CH3), 28.3 (CH3) ppm.



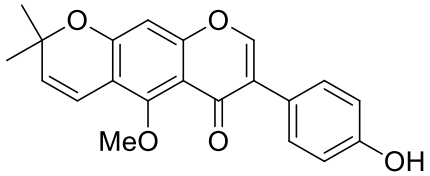
5-Methoxy-8,8-dimethyl-3-(4-((3-methylbut-2-en-1-yl)oxy)phenyl)pyrano[3,2-g]chromen-4(8H)-one (4)

White crystal solids, 5.2 g; m.p. : 108 - 110 oC; 1H-NMR (500 MHz, CDCl3) δ: 7.69 (1H, s), 7.38 (2H, d, J = 8 Hz), 6.88 (2H, d, J = 8 Hz), 6.67 (1H, d, J = 10 Hz), 6.53 (1H, s), 5.64 (1H, d, J = 10 Hz), 5.42 (1H, t, J = 1.25 Hz), 4.5 (2H, d, J = 1.25 Hz), 3.82 (3H, s), 1.73 (3H, s), 1.68 (3H, s), 1.40 (6H, s) ppm; 13C NMR (500 MHz, CDCl3) δ: 175.0 (C), 158.8(C), 158.7 (C), 158.0 (C), 155.8 (C), 150.4 (CH), 138.2 (C), 130.7 (CH), 130.4 (CH), 125.6 (C), 124.2 (C), 119.7 (CH), 116.2 (CH), 114.7 (CH), 113.9 (CH), 113.3 (C), 113.2 (C), 100.7 (CH), 64.8 (CH2), 62.8 (CH3), 28.3 (CH3), 25.8 (CH3), 18.2 (CH3) ppm.



3-(4-Hydroxyphenyl)-5-methoxy-8,8-dimethylpyrano[3,2-g]chromen-4(8H)-one (5)

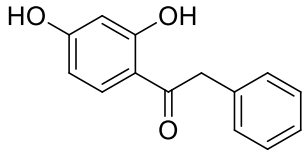
Light brown solids, 0.2 g; m.p. 208 - 210 oC; 1H-NMR (500 MHz, CDCl3) δ: 7.70 (1H, s), 7.26 (2H, d, J = 8 Hz), 6.88 (2H, d, J = 8 Hz), 6.67 (1H, d, J = 10 Hz), 6.54 (1H, s), 5.66 (1H, d, J = 10 Hz), 3.83 (3H, s), 1.4 (6H, s) ppm; 13C NMR (500 MHz, CDCl3) δ: 175.5 (C), 158.8 (C), 158.2 (C), 156.0 (C), 155.8 (C), 150.6 (CH), 130.8 (CH), 130.5 (CH), 125.8 (C), 123.9 (C), 116.1 (CH), 115.7 (CH), 113.4 (C), 113.2 (C),100.7 (CH), 62.9 (CH3), 28.3 (CH3) ppm.



Synthesised compounds

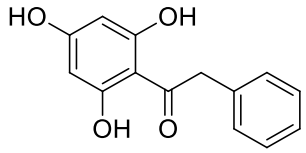
1-(2,4-Dihydroxyphenyl)-2-phenylethanone (10)

To a stirred solution of benzene-1,3-diol 7 (30.00 g, 0.27 mol) was added phenylacetic acid 9 (37.09 g, 0.27 mol) in boron trifluoride diethyl etherate (102.66 mL, 0.82 mol), and heated at 90 0C for 2-3 h. The resulting mixture was extracted with ethyl acetate (60 mL×2) and the combined organic extracts dried with MgSO4, filtered and concentrated *in-vacuo* to give the desired product 1-(2,4-dihydroxyphenyl)-2-phenylethanone 10 in 63% yield (4.9 g) as a white crystal, mp: 130 - 132 oC. 1H-NMR (500 MHz, Acetone) δ: 8.10 (1H, dd, J = 2.0, 8 Hz), 7.24 – 7.45 (5H, m), 6.28 – 6.51 (3H, m), 4.30 (2H, s) ppm; 13C NMR (500 MHz, Acetone) δ: 205.3 (C), 165.8 (C), 164.9 (C), 135.3 (CH), 133.4 (C), 129.5 (CH), 128.4 (CH), 126.7 (CH), 112.7 (C), 108.0 (CH), 102.8 (CH), 44.2 (CH2) ppm.



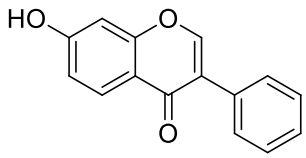
2-Phenyl-1-(2,4,6-trihydroxyphenyl)ethanone (11)

Same procedure as in the synthesis of 1-(2,4-dihydroxyphenyl)-2-phenylethanone 10 was used starting from benzene-1,3,5-triol 8. The product 2-Phenyl-1-(2,4,6-trihydroxyphenyl)ethanone 11 was isolated as a white solid in 49% yield. 1H-NMR (500 MHz, Acetone) δ: 7.22 – 7.44 (5H, m), 5.88 (2H, s), 4.31 (2H, s) ppm; 13C NMR (500 MHz, Acetone) δ: 205.3 (C), 165.8 (C), 164.5 (C), 134.0 (C), 128.9 (CH), 127.8 (CH), 126.7 (CH), 104.3 (C), 103.5 (CH), 43.0 (CH2) ppm.



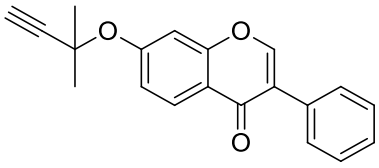
7-Hydroxy-3-phenyl-4H-chromen-4-one (12)

A mixture of 1-(2,4-dihydroxyphenyl)-2-phenylethanone (1.00 g, 4.38 mmol) (10) boron trifluoride diethyl etherate (1.40 mL, 10.95 mmol) and DMF (6.7 mL, 86.79 mmol), was heated on a water-bath. The reaction was cooled to room temperature and resulting solution was concentrated to obtain 7-hydroxy-3-phenyl-4H-chromen-4-one (12), in 65% yield (2.6 g)., mp.: 218 - 220 oC; 1H-NMR (500 MHz, Acetone) δ: 8.28 (1H, s), 8.10 (1H, d), 7.65 (2H, d), 7.44 (2H, t), 7.36 (1H, t), 7.03 (1H, dd), 6.95 (1H, d) ppm. 13C NMR (500 MHz, Acetone) δ: 205.3 (C), 174.5 (C), 162.4 (C), 157.9 (C), 153.2 (CH), 132.6 (C), 129.0 (C), 128.0 (CH), 127.7 (CH), 124.5 (CH), 117.8 (C), 114.9 (CH), 102.4 (CH) ppm.



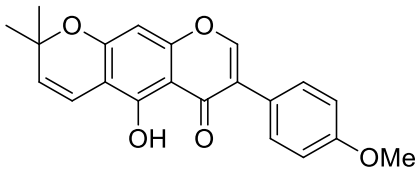
7-((2-Methylbut-3-yn-2-yl)oxy)-3-phenyl-4H-chromen-4-one (14)

A mixture of potassium iodide (0.44 g, 2.63 mmol), copper (I) iodide (2.10 mg), 3-chloro-3-methylbutyne (0.11 g, 1.10 mmol) and 7-hydroxy-3-phenyl-4H-chromen-4-one (12) (0.25 g, 1.05 mmol) in acetone (10 mL) was refluxed for 3 h and then quenched with water (60 mL). The resulting mixture was extracted with ethyl acetate (60 ml ×2) and the combined organic extracts dried with MgSO4, filtered and concentrated *in-vacuo* to give the desired product 7-((2-methylbut-3-yn-2-yl)oxy)-3-phenyl-4H-chromen-4-one (14) in 84% yield (0.27 g) as a pale brown solid that was used in the next step without further purification., mp: 126 - 128 oC; 1H-NMR (500 MHz, CDCl3) δ: 8.15 (1H, dd), 7.89 (1H, s), 7.50 (2H, d), 7.24 - 7.45 (5H, m), 2.63 (1H, s), 1.97 (2H, s), 1.69 (6H, s) ppm; 13C NMR (500 MHz, CDCl3) δ: 205.3 (C), 175.1 (C), 152.7 (C), 132.0 (C), 129.0 (CH), 128.5 (C), 128.1 (CH), 127.3 (CH), 125.3 (C), 118.5 (CH), 106.4 (CH), 84.8 (CH2), 29.5 (CH3) ppm.



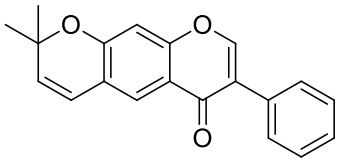
5-Hydroxy-3-(4-methoxyphenyl)-8,8-dimethylpyrano[3,2-g]chromen-4(8H)-one (3)

A solution of 5-methoxy-3-(4-methoxyphenyl)-8,8-dimethylpyrano[3,2-g]chromen-4(8H)-one (2) (1.00 g, 2.74 mmol), formic acid (30 mL, 795.13 mmol) and potassium iodide (5.10 g, 30.73 mmol) in ethyl acetate (30 mL, 0.31 mmol) was refluxed for 1h. After which time water (20 mL) was added to the reaction mixture and set aside to cool to room temperature. The solid precipitate formed was filtered and washed with methanol (2 x 10 mL) to give the product 5-hydroxy-3-(4-methoxyphenyl)-8,8-dimethylpyrano[3,2-g]chromen-4(8H)-one (3) as a pale yellow needle-like crystals in 42 % yield (0.40 g)., mp.: 134 -136 oC; 1H-NMR (500 MHz, CDCl3) δ: 7.74 (1H,s), 7.45 (2H,d, J = 8 Hz), 7.00 (2H,d, J = 8 Hz), 6.75 (1H,d, J = 10 Hz), 6.34 (1H,s), 5.64 (1H,d, J = 10 Hz), 3.87 (3H,s), 1.49 (6H,s) ppm; 13C NMR (500 MHz, CDCl3) δ: 175.0 (C), 159.5 (C), 158.7 (C), 158.0 (C), 155.8 (C), 150.4 (CH), 130.7 (CH), 130.4 (CH), 125.6 (C), 124.3 (C), 116.2 (CH), 114.0 (C), 113.9 (CH), 113.3 (C), 113.2 (C),100.7 (CH), 62.8 (CH3), 28.3 (CH3) ppm



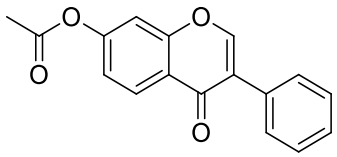
8,8-Dimethyl-3-phenylpyrano[3,2-g]chromen-4(8H)-one (15)

To a solution of 7-((2-methylbut-3-yn-2-yl)oxy)-3-phenyl-4H-chromen-4-one (14) (0.27 g, 0.88 mmol) in o-xylene (18 mL) was added 60% NaH (0.21 g, 5.31 mmol) dispersed in mineral oil. The resulting mixture was heated at 130 oC for 20 h. By which time thin layer chromatography analysis showed that the reaction had gone to completion. The reaction was cooled to room temperature and water (30 ml) was added to the mixture and the pH adjusted to 5 with 1 M HCl. The aqueous layer was extracted with ethyl acetate (30 mL×3), and the combined organic extracts washed with brine, dried (MgSO4) and concentrated *in-vacuo* to give a residue that was filtered and washed with petroleum ether (3 x 10 mL) to afford the product as a pale brown solid in 50% yield (0.13 g)., mp: 172 -174 oC; 1H-NMR (500 MHz, CDCl3) δ: 8.01 (1H, d, J = 8 Hz), 7.99 (1H, s), 7.49 (2H, d, J = 8 Hz), 7.35 (2H, d, J = 8 Hz), 6.77 (1H, s), 5.65 (1H, d, J = 8 Hz), 4.05 (1H, s), 1.97 (3H, s), 1.4 (6H, s) ppm; 13C NMR (500 MHz, CDCl3) δ: 175.7 (C), 171.2 (C), 157.4 (C), 152.3 (C), 132.0 (C), 130.3 (CH), 129.0 (CH), 128.5 (CH), 128.1 (C), 126.8 (C), 125.2 (CH), 118.4 (CH), 115.3 (CH), 114.9 (C), 109.2 (CH), 60.4 (CH3), 32.2 (CH), 28.2 (CH3) ppm.



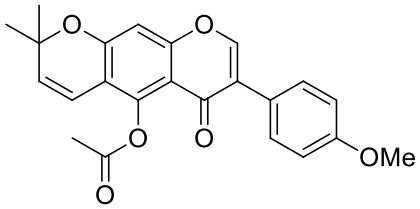
4-Oxo-3-phenyl-4H-chromen-7-yl acetate (13)

A solution of 7-hydroxy-3-phenyl-4H-chromen-4-one (10) in acetic anhydride was heated under reflux for 3 h. The reaction was quenched by adding 2 mL of water leading to the formation of white precipitate. The white precipitate was dissolved in water (40 mL) and the aqueous layer extracted with ethyl acetate. The organic extract was washed with water and brine, dried over MgSO4 and concentrated to give an oily liquid. Addition of water to the oil led to the formation of the product as a white precipitate in 61 % yield (81 mg)., mp: 138 - 140 oC; 1H-NMR (500 MHz, CDCl3) δ: 8.36 (1H, d), 8.03 (1H, s) 7.58 (2H, d), 7.47 (2H, t), 7.42 (1H, t), 7.34 (1H, s), 7.20 (1H, d), 2.39 (3H, s) ppm. 13C NMR (500 MHz, CDCl3) δ: 175.5 (C), 168.5 (C), 156.7 (C), 154.5 (C), 153.1 (CH), 131.6 (C), 129.0 (CH), 128.5 (CH), 128.3 (CH), 128.0 (CH), 125.6 (C), 122.4 (C), 119.5 (CH), 110.9 (CH), 21.2 (CH3) ppm.



7-(4-Methoxyphenyl)-2,2-dimethyl-6-oxo-2,6-dihydropyrano[3,2-g]chromen-5-yl acetate (6)

A solution of 5-hydroxy-3-(4-methoxyphenyl)-8,8-dimethylpyrano[3,2-g]chromen-4(8H)-one (3) (120 mg, 0.33 mmol) in 20 mL of acetic anhydride was refluxed for 3 h. Aqueous sodium bicarbonate (10 g in 100 mL of water) was added and the solid precipitate filtered and purified by chromatography ethyl acetate/petroleum ether in 1:5 ratio as eluent to afford the product 7-(4-methoxyphenyl)-2,2-dimethyl-6-oxo-2,6-dihydropyrano[3,2-g]chromen-5-yl acetate (6) as a white solid in 50 % yield (65 mg)., mp: 204–206 oC; 1H-NMR (500 MHz, CDCl3) δ: 7.77 (1H, s), 7.41 (2H, d, J = 8 Hz), 6.96 (2H, d, J = 8 Hz), 6.73 (1H, s), 6.51 (1H, d, J = 10 Hz), 5.78 (1H, d, J = 10 Hz), 3.85 (3H, s), 2.47 (3H, s), 1.54 (6H, s) ppm; 13C NMR (500 MHz, CDCl3) δ: 174.8 (C), 169.4 (C), 159.6 (C), 158.1 (C), 157.6 (C), 150.9 (CH), 145.0 (C), 132.0 (CH), 130.4 (CH), 125.7 (C), 124.0 (C), 115.2 (CH), 114.0 (CH), 113.0 (C), 111.8 (C), 102.2 (CH), 55.3 (CH3), 28.4 (CH3), and 21.1 (CH3) ppm.



Biological Activity

Materials and Reagents

RPMI culture medium, Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS), Penicillin-streptomycin, Trypsin, 3-(4,5-Dimethylythiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) and Trypan Blue were obtained from Sigma Chemical Company (St. Louis, MO, USA). Fetal bovine serum (FBS) was supplied by GIBCO (Gaithersburg, MD). Dimethyl sulfoxide (DMSO) was obtained from Wako Pure Chem. Ind., Tokyo, Japan. Human liver cancer (HepG2), human breast cancer (MCF-7), normal human liver (Chang Liver), human T-lymphoblast-like leukemia (Jurkat), human prostate cancer (LNCap, PC3), and human vaginal malignant melanoma (HMVII) cell lines were obtained from RIKEN BioResource Center Cell Bank (Japan). All chemicals and reagents used were of analytical grade and obtained from standard suppliers.

Cell Culture

HepG2 and MCF-7 were cultured in DMEM while Chang liver, Jurkat, LNCap, PC3, and HMVII were maintained in RPMI1640 culture medium. All the media were supplemented with 10% FBS and 1% penicillin–streptomycin and then incubated at 37 oC under 5% CO2 under humidified conditions. For the cell treatment, compounds were dissolved in DMSO. DMSO concentrations in the cell culture were 1% (v/v), and the controls were always treated with the similar concentrations of DMSO as used in corresponding experiments.

Cytotoxicity Assay

Cytotoxicity was determined by using tetrazolium based colorimetric (MTT) assay (Zheng et al., 2013). In brief, the monolayer cells were seeded in 96-well plates at a density of 1x104 cells/well. After incubation for 24 h, the cells were treated with the compounds at various concentrations (0 - 100 μg/ml) and incubated as indicated above for 72 h. After incubation, 20 μl of MTT solution (2.5 mg/ml in PBS) was added to each well, and the cells were further incubated for 4 h. Acidified isopropanol containing 1% Triton-X 100 was added to the wells and the plates were kept in the dark at 26 ºC overnight to dissolve any formazan crystals formed. The amount of formazan was measured at 570 nm using a microplate reader (Tecan Infinite M200 Pro, Austria). Cell viability was expressed as a percentage of the control culture.

Statistical Analysis

The percent cell viability was calculated using the formula of (Ayisi et al., 2011). These values were plotted against concentrations of the compounds, and the inhibition concentrations at which 50% activity occurred (IC50) were determined. The selectivity indices (SI) of the compounds were also computed as the ratio of the CC50 values in normal cells to that in cancer cells. All data are expressed as means of three experiments ± SD. Significant differences between the control and test experiments were determined using Student's t-test from Microsoft Excel 2007 version. P values less than 0.05 were considered statistically significant.

3. RESULTS & DISCUSSION

Isolated isoflavones

Ethyl acetate soxhlet extraction of the pulverized of the crude extract of the stem bark of the *Millettia thonningii* plant followed by column chromatography of the crude extract, resulted in the isolation of three isoflavones 2, 3, and 4 (Figure 2). Compound 2 was isolated as a white solid (12.8 g), 4 was a white crystalline solid (5.20 g), and 5 was a light brown solid (200 mg).

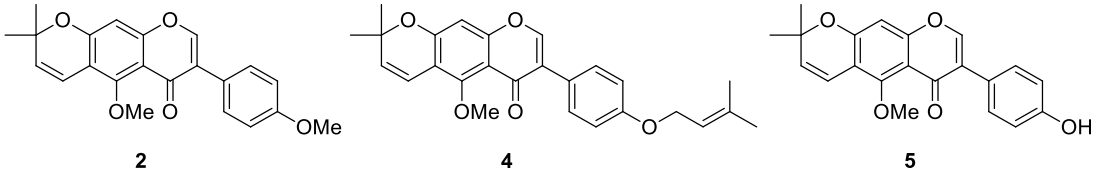
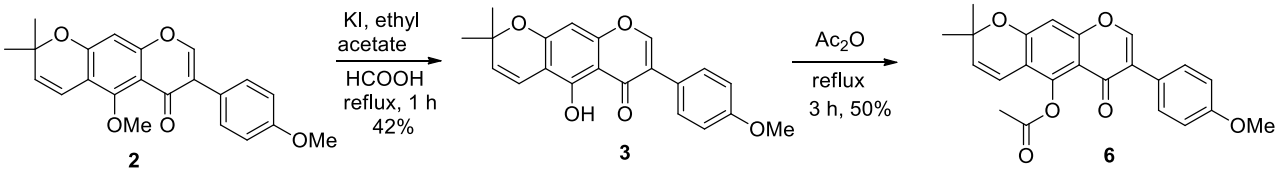


Figure 2 Some isolated isoflavones from the *Millettia Thonningii* plant in Ghana

Synthetic derivatives from isolated isoflavone 2

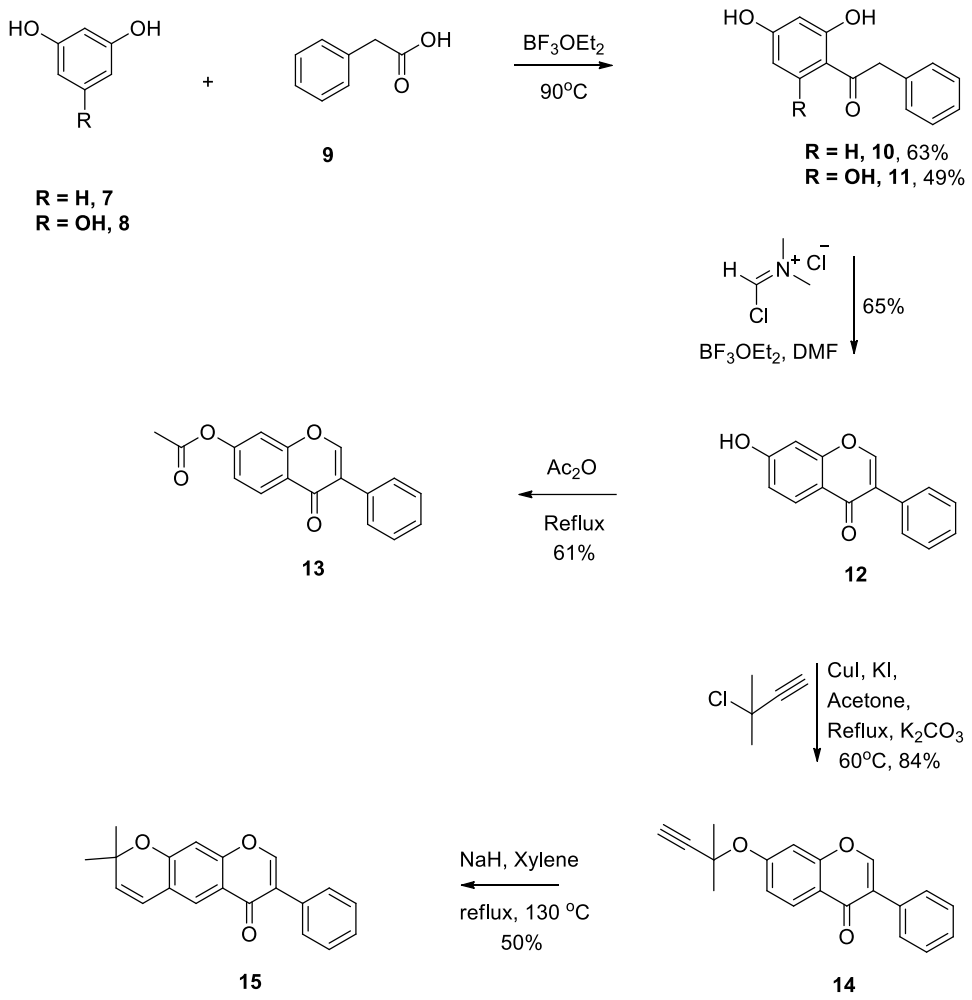
Conversion of naturally isolated isoflavone 2 to 5-hydroxy-3-(4-methoxyphenyl)-8,8-dimethylpyrano[3,2-g]chromen-4(8H)-one 3 was achieved using potassium iodide in the presence of formic acid in refluxing ethyl acetate to afford the desired alcohol as a pale yellow solid in 42 % yield. With compound 3 in hand, O-acylation was effected in refluxing acetic anhydride to give compound 6 as a white solid in 50 % yield (Scheme 1) (Bishayee and Sethi, 2016).



Scheme 1 Synthetic derivatives of 2

Synthesised isoflavones 14 to 17

The synthesised isoflavones began with Friedel – Craft acylation of diol 7 and phenylacetic acid 9 using boron trifluoride diethyl etherate (BF3.Et2O) as a solvent and Lewis acid to yield intermediate 10 as a white solid (Ayine-Tora et al., 2016). Conversion of intermediate 10 to isoflavone 12 was achieved using Vilsmeier reagent (chloromethylene dimethyliminium chloride) in *N,N*-dimethylformamide (DMF) and boron trifluoride diethyl etherate at room temperature. With compound 12 in hand, O-acylation was effected in refluxing acetic anhydride (Ac2O) to give compound 13 as a white solid in 61 % yield (Yadav, 2014). Treatment of compound 12 with 3-chloro-3-methyl-1-butyne in the presence of copper (I) iodide, potassium iodide, and potassium carbonate in refluxing acetone, afforded compound 14 as colourless needle like solid in 84% yield (Yadav, 2014). Compound 14 when refluxed at 130 0C in the presence sodium hydride in *o*-xylene yielded compound 15 a pale brown solid in 50% (Scheme 2) (Yadav, 2014).



Scheme 2 Synthesis of isoflavone compounds 12 to 15

Biological Data Studies

The bioassay experiments carried out have been shown in (Figures 3a – 3f). Table 1 below also contains the values obtained for the 50% inhibition concentration (IC50) and selective indices of isoflavones on different human cancer and normal cell lines after 72 h exposure. Thus, the results obtained from the graphs were statistically analysed. From the table, based on the IC50 values, compounds 2 and 54 exhibited good activity against breast cancer (MCF-7) with IC50 values of 47.12 µg/mL and 6.94 µg/mL respectively. They also showed appreciable inhibition of prostate cancer (PC3) cell line with respective IC50 values of 14.55 µg/mL and 6.87 µg/mL. Compound 5 exhibited very good activity with IC50 values in the range 8.32-20.37 µg/mL against all the cell lines understudied except for Leukemia (JURKAT).

The modified compound 3 exhibited rather strong activity in HMV11 with IC50 value of 8.99 µg/mL and 5.14 µg/mL in liver cancer (HEPG2) cell line. The acetylated derivative 6 also exhibited a strong activity against (MCF-7) with IC50 value of 5.09 µg/mL and 4.59 µg/mL in (PC3). Whereas, synthesised compounds 10, 12, and 13 exhibited good to moderate activity against (MCF-7) with IC50 values of 14.58, 45.31, 16.49 µg/mL, respectively. The same compounds exhibited moderate and good activity against (PC3) with respectively IC50 values of 31.79, 6.11 and 11.37µg/mL. Other activities exhibited by these compounds are shown in (Table 1).

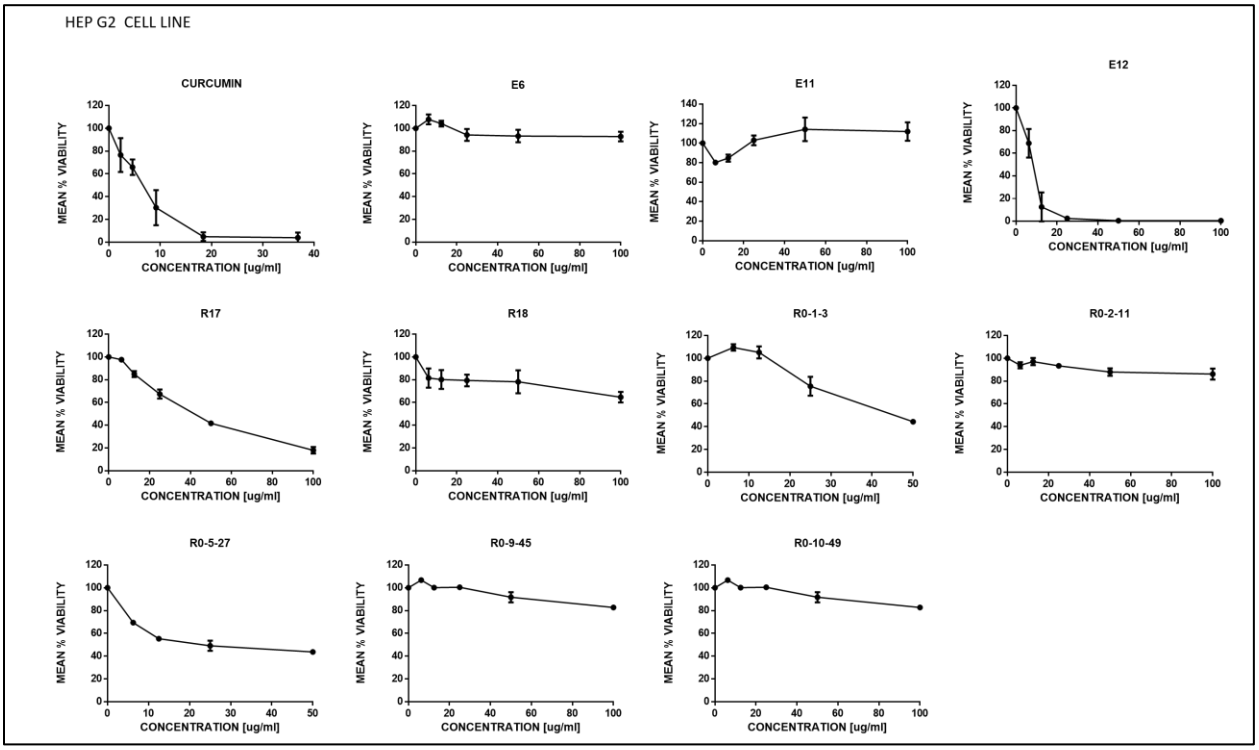


Figure 3a Graphs showing percent cell viability of compounds isolated from *Millettia Thonningii* and their derivatives in (HEP G2) cells after 72 h of treatment

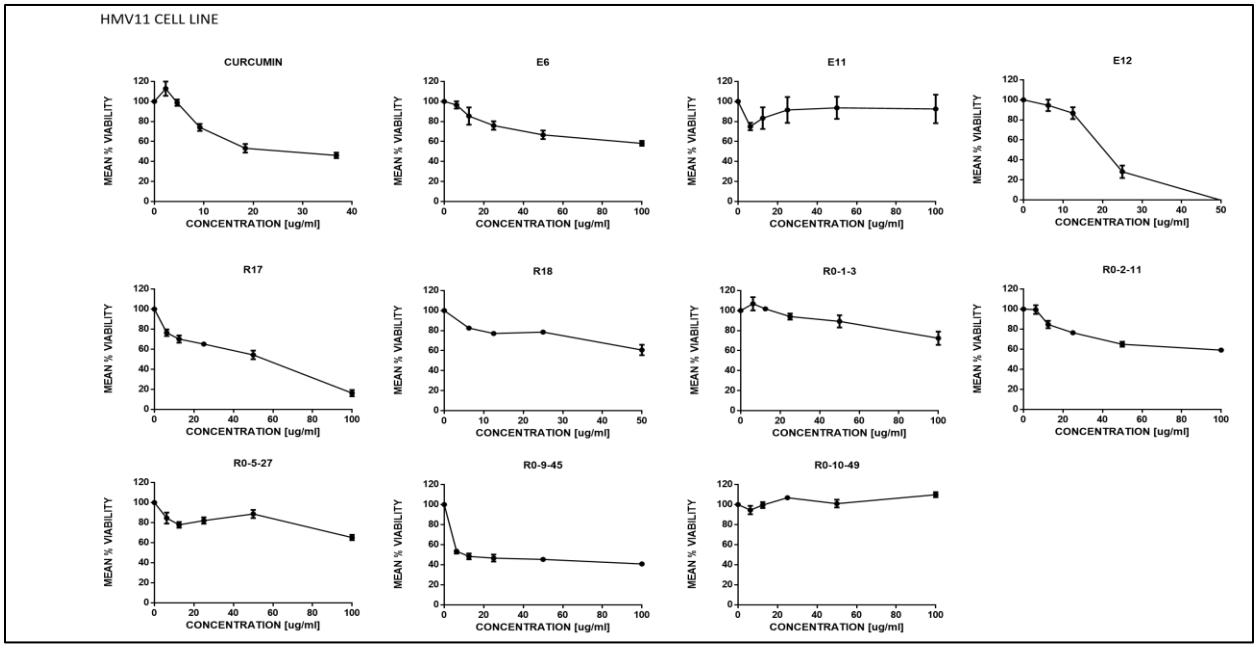


Figure 3b Graphs showing percent cell viability of compounds isolated from *Millettia Thonningii* and their derivatives in (HMV-11) cells after 72 h of treatment

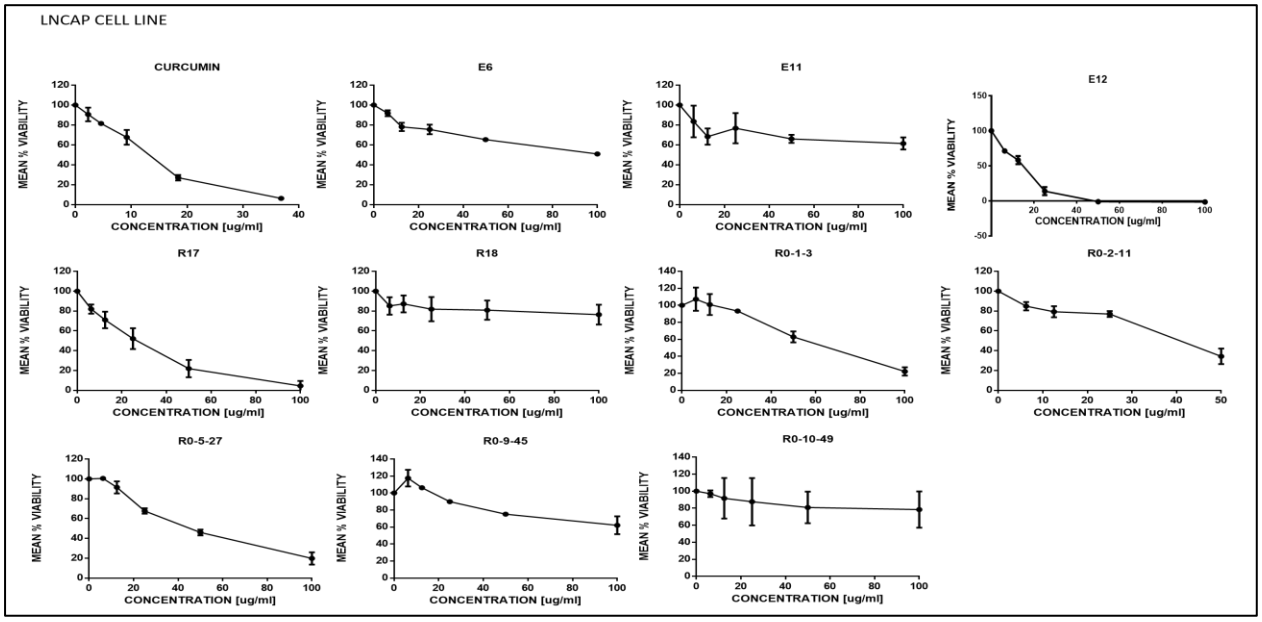


Figure 3c Graphs showing percent cell viability of various compounds isolated from *Millettia Thonningii* and their derivatives in (LNCaP) cells after 72 h of treatment.

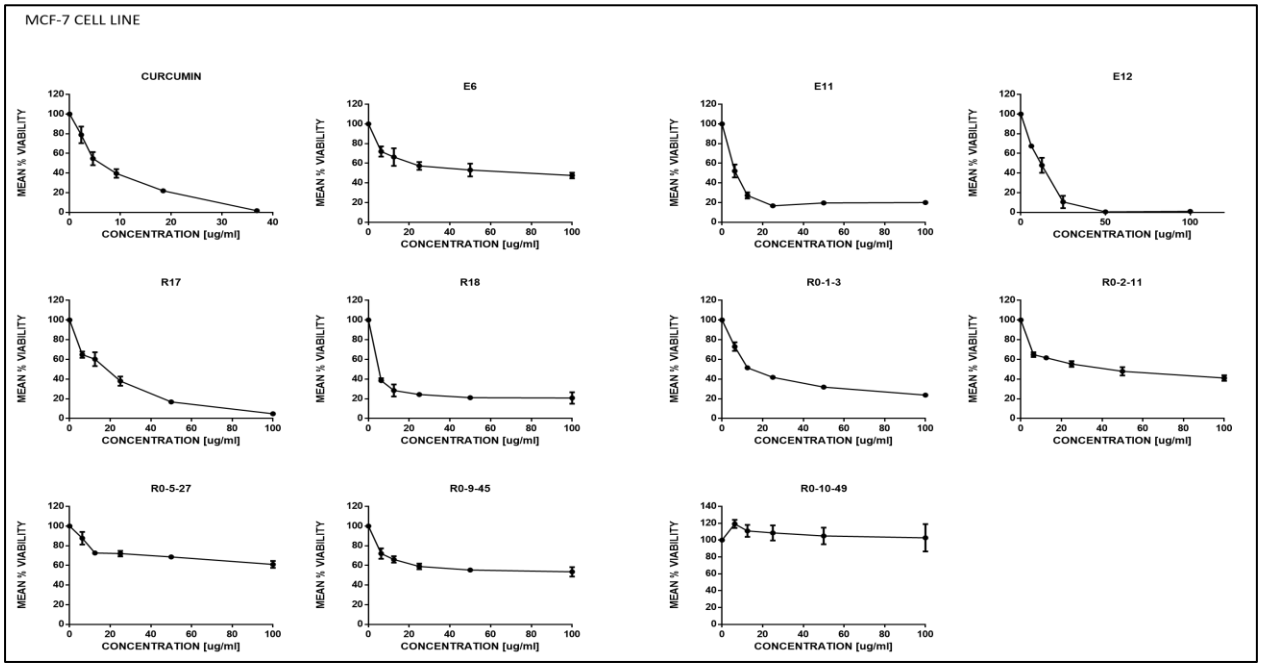


Figure 3d Graphs showing percent cell viability of compounds isolated from *Millettia Thonningii* and their derivatives in (MCF-7) cells after 72 h of treatment

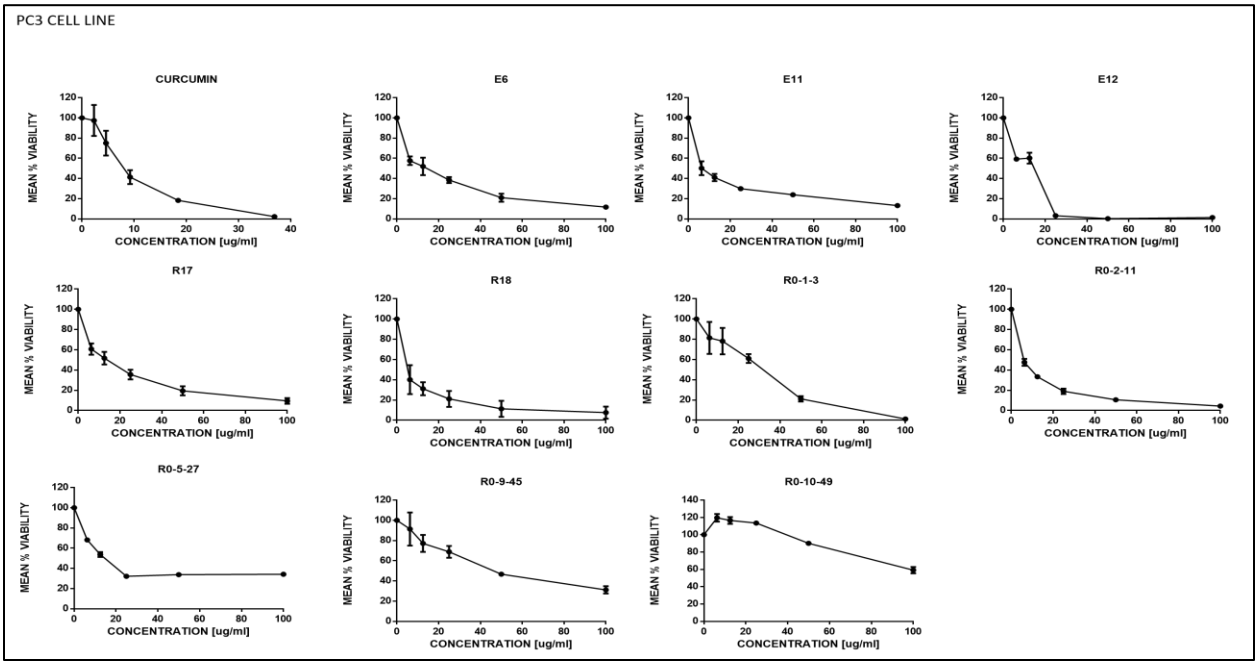


Figure 3e Graphs showing percent cell viability of compounds isolated from *Millettia Thonningii* and their derivatives in (PC3) cells after 72 h of treatment

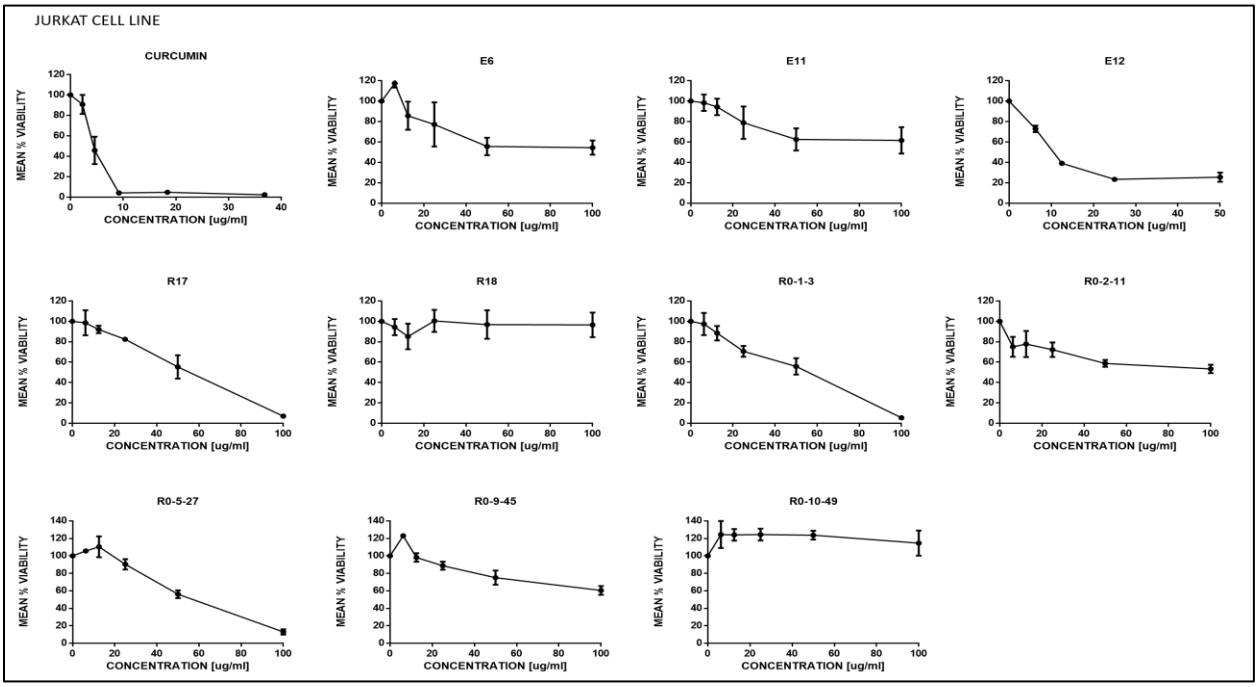


Figure 3f Graphs showing percent cell viability effect of compounds isolated from *Millettia Thonningii* and their derivatives in human Jurkat cells after 72 h of treatment.

Table 1 Fifty percent inhibition concentration (IC50) values and selectivity indices of isolated isoflavones from *Millettia Thonningii* on different human cancer and normal cell lines after 72 h treatment. Results are expressed as mean ± SD of three replicate experiments.

	CELL LINES												
	MCF-7		PC3		LNCAP		JURKAT		HMV11		HEPG2		CHANG LIVER
COMPOUND	IC50	S.I	IC50	S.I	IC50	S.I	IC50	S.I	IC50	S.I	IC50	S.I	CC50
4	47.12±0.29	2.12	14.55±5.43	6.87	>100	1.00	>100	1.00	>100	1.00	>100	1.00	>100
5	6.94±1.15		6.87±1.76		>100		>100		>100		>100		*
6	12.06±2.23	1.53	14.66±0.95	1.26	14.59±1.27	1.27	10.48±0.08	1.76	20.37±1.08	0.91	8.32±1.39	2.22	18.47±0.78
15	16.49±1.52	1.14	13.79±4.20	1.65	27.05±7.76	0.70	54.45±9.20	0.35	55.91±5.31	0.34	41.83±1.07	0.45	18.81±1.52
18	5.09±0.19		5.88±2.25		>100		>100		>100		>100		*
13	87.64±3.29	1.14	>100	1.00	>100	1.00	>100	1.00	>100	1.00	>100	1.00	>100
12	14.26±0.25	7.01	31.79±2.16	3.15	65.20±4.78	1.53	55.23±6.88	1.81	>100	1.00	45.07±2.32	2.22	>100
14	45.31±13.22	2.21	6.11±6.82	16.37	32.66±6.82	3.06	>100	1.00	>100	1.00	>100	1.00	>100
16	>100		14.60±1.28		45.44±3.32		57.09±4.90		>100		25.07±9.91		*
7	>100	*	45.88±2.61	*	>100	*	>100	*	8.99±2.44	*	5.14±0.15	*	*
17	>100	1.00	>100	1.00	>100	1.00	>100	1.00	>100	1.00	>100	1.00	>100
Cur	5.85±1.63	1.53	4.9±1.18	1.83	12.08±2.65	0.74	4.57±0.62	1.96	13.48±0.09	0.66	6.89±1.03	1.30	8.95±0.96

* Not determined.

Docking studies of genistein (1) and compound (12)

In order to better understand the molecular basis for the potent inhibitory activity shown by some of the synthesised compounds, molecular modelling experiments were embarked on. The active molecule 12 was docked against the crystal structure of ERα (PDB ID: 1X7R, resolution 2.0 Å), 22 ERβ (PDB ID: 1X7J, resolution 2.3 Å)22, DAPK1(PDB ID: 5AUZ, resolution 1.6 Å)23 and TTR (PDB ID: 3KGT, resolution 1.95 Å).24 Modelling against the ERα shows the ligand has plausible binding pose similar to genistein for CS, ChemPLP and GS scoring functions. The binding mode shows compound 12 forms hydrogen bond with the side chain imidazole of Histidine (His524) and the backbone carbonyl oxygen of Glycine (Gly521). Modelling against ERβ shows similar binding mode and forms hydrogen bond with only the side chain imidazole of Histidine (His475). The binding modes are shown in (Figure 4).

Modelling against DAPK1 also shows plausible binding mode for all the scoring functions except GS. Compound 12 forms hydrogen bonds with the backbone carbonyl group of glutamic acid (Glu94) and ammonium group of valine (Val96) as shown in (Figure 5). Finally modelling against TTR shows plausible binding modes for all the scoring function except ChemPLP. Compound 12 forms hydrogen bond with the side chain of Serine (Ser117) (Figure 6) H6.

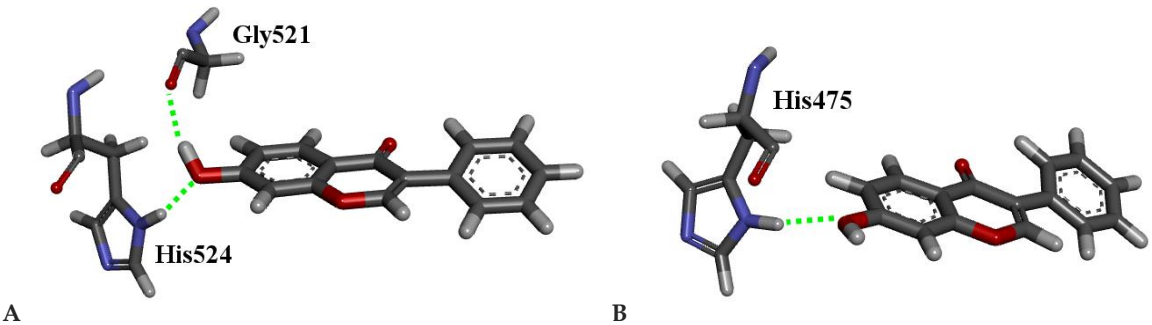


Figure 4 (A) The docked configuration of 12 in the binding site of ERα. Hydrogen bonds are shown as green dotted lines between ligand 12 and the amino acids Gly521 and His524 using ChemPLP. (B) The docked configuration of 12 in the binding site of ERβ. Hydrogen bond is shown as a green dotted line between ligand 12 and the amino acid His475 using ChemPLP.

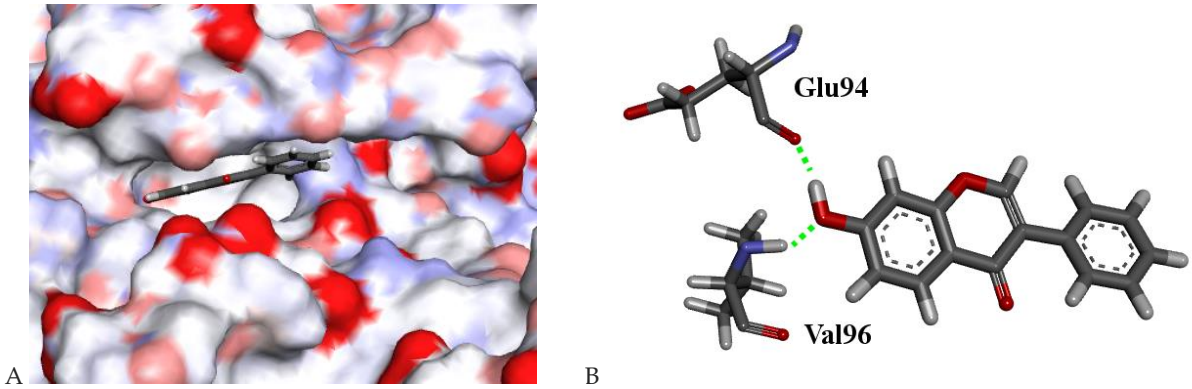


Figure 5 The docked configuration of 12 in the binding site of DAPK1 (A) The protein surface is rendered. The ligand occupies the binding pocket. Red depicts a negative partial charge on the surface, blue depicts positive partial charge and grey shows neutral/lipophilic areas. (B) Hydrogen bonds are shown as green lines between ligand 12 and the amino acids Glu94 and Val96 for ChemScore configuration.

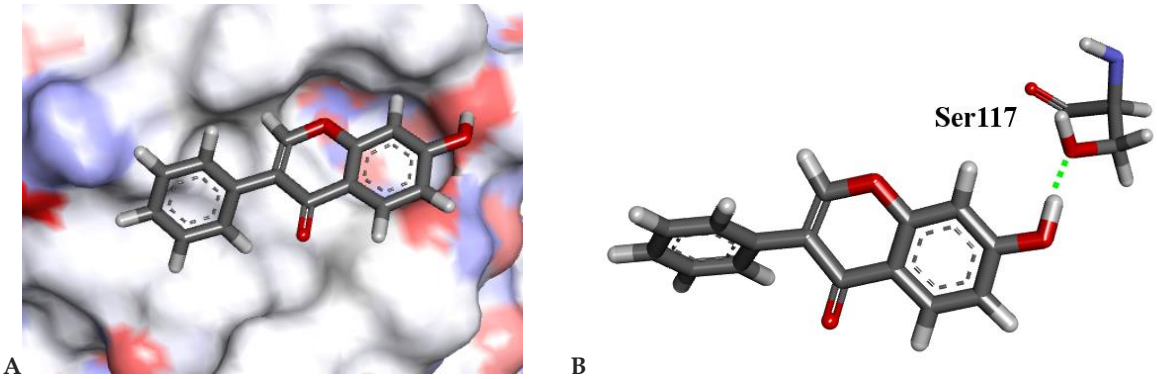


Figure 6 The docked configuration of 12 in the binding site of TTR (A) The protein surface is rendered. The ligand occupies the binding pocket. Red depicts a negative partial charge on the surface, blue depicts positive partial charge and grey shows neutral/lipophilic areas. (B) Hydrogen bonds are shown as a green line between ligand 12 and the amino acid Ser117 for ChemScore configuration.

Both compounds have similar scores however genistein 1 generally has higher scores than 12, which indicates that genistein 1 is likely to be more active than the 12. This is due to the higher number of hydroxyl moieties on genistein 1. Comparing the estrogen receptors, ERβ generally obtained higher scores than its counterparts which indicate the ligands binds more favourably to the ERβ than ERα.

Table 2 Results of the scoring function for the ligands

	ASP		ChemPLP		ChemScore		GoldScore	
Protein	Genistein 1,	12	Genistein 1,	12	Genistein 1,	12	Genistein 1,	12
ERα	33.1	31.2	60.6	62.8	28.0	34.5	46.5	45.4
ERβ	38.5	33.1	70.1	65.7	28.8	32.3	52.3	49.4
DAPK1	28.9	25.2	72.7	57.6	34.3	28.5	49.1	44.4
TTR	17.9	15.3	39.2	35.9	19.1	20.6	38.2	34.5

Chemical Space

The molecular weight (MW) and polar surface area (PSA) are within the lead-like chemical space whereas the Hydrogen bond donors (HD), Hydrogen bond acceptors (HA), Polar surface area (Å2) (PSA), Rotatable bonds (RB) are within the drug-like chemical space as shown in (Table 2). These values indicate the ligand is in favorable chemical space for further development.

4. CONCLUSION

Results from the biological activity test for the synthesized isoflavone derivatives reveal that isoflavone 3 has stronger activity than curcumin control against the liver cancer cell lines. Isoflavone 3 is a product from the *O*-demethylation of the phenolic methyl ether of the benzopyrone ring of 2 as shown in (Scheme 1). The presence of the polar hydroxyl group on the benzopyrone ring of the alpinumisoflavone 3 is necessary and key for the very good activity observed. Synthesized isoflavone 12 also has strong activity against prostate cancer cell lines. The presence of the benzopyrone ring in 2 makes it more rigid (compared to compound 10) which enhances the hydrogen bonding interactions thereby giving compound 12 a better activity than compound 10.

In addition, the presence of the polar hydroxyl group on the ring is responsible for the better activity of compound 12 over compound 14 against the prostate cancer cell lines. Hence the alcohol protecting alkyl group in 14 (Scheme 2) is responsible for the lower activity observed. The benzopyrone ring and the hydroxyl group are very necessary for biological activity against the prostate cancer cell lines. These results, forms the basis of a structure activity relationship (SAR) study currently undergoing at the University of Ghana to identify anti-cancer drug candidates with improved biological properties based on the isoflavone scaffold.

Acknowledgements

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Informed consent

Not applicable.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for experimentation.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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