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Anti-oestrogenic diarylheptanoids from *Aframomum melegueta* with *in silico* oestrogen receptor alpha binding conformation similar to enterodiol and enterolactone

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ABSTRACT

Aframomum melegueta is a spice widely used in African folk medicine. The chloroform soluble fraction of *A. melegueta* seeds yielded four new diarylheptanoids named gingerenone D (**1**), dihydrogingerenone A (**2**), dihydrogingerenone B (**3**), and dihydrogingerenone C (**4**), in addition to six known diarylheptanoids and hydroxyphenylalkanones. The most potent oestrogen receptor binding ability in an oestrogen receptor alpha (ERα) competitive-binding assay was for compounds **1**, **2** and **5** with IC_{50} values of 50, 79 and 39 μM, respectively, compared with 18 nM for the natural steroid 17β-oestradiol (E2). In addition, the diarylheptanoids **1**, **2** and **5** showed anti-oestrogenic activity in a receptor cofactor assay system for ERα, while the hydroxyphenylalkanone, [6]dehydrogingerdione, (**7**) exhibited an agonistic action. Results were interpreted via virtual docking of the active compounds to an ERα crystal structure, in comparison with the known oestrogenic compounds: enterodiol (END), enterolactone (ENL), genistein and E2. The anti-oestrogenic compounds **1**, **2** and **5** showed a binding similarity to that of END and ENL while compound **7** was similar in binding to genistein and E2 interpreting its agonistic effect.

1. Introduction

Phytoestrogens are plant derived compounds that structurally or functionally mimic mammalian oestrogens. Many women turn to phytoestrogens as an alternative to hormone replacement therapy (HRT) and its undesirable side effects (Cornwell, Cohick, & Raskin, 2004).

In the molecular model of oestrogen action, binding of a phytoestrogen to the oestrogen receptor (ER) through its ligand binding domain (LBD) results in dimerisation of the receptor. The receptor dimer takes a unique conformation for every ligand and based on this conformation it binds to a cofactor protein. If the dimer binds to a co-activator protein the ligand exerts an agonistic effect and if to a co-repressor protein it will be an antagonist (Riggs & Hartmann, 2003). Based on the above mechanism, phytoestrogens could act as agonists, antagonists or selective oestrogen receptor modulators (SERM).

SERMs are non-steroidal chemicals which are unique in that they can function as agonist or antagonists depending on the tissue, ER subtype and the concentration of the endogenous oestrogens (Riggs & Hartmann, 2003). The mechanism of tissue selectivity of SERMs could be explained according to one of the following hypotheses: (a) a preferential action on one of the two ER subtypes (ERα and ERβ) and therefore exerting different tissue-selective effects due to the differences in body distribution of the two subtypes and different responses upon their activation (Hall & McDonnell, 1999; Riggs & Hartmann, 2003), or (b) the difference in the tissue distribution of co-regulator proteins (co-activators and co-repressors), where more than 20 co-regulator proteins have been discovered. This results in binding of a molecule to a co-activator in a certain tissue and a repressor in another, based on its receptor dimer unique conformation (McKenna, Lanz, & O'Malley, 1999).

Several classes of phytoestrogens have been reported, such as isoflavonoids (genistein and daidzein), coumestans (coumesterols) and stilbenes (resveratrol) (Ososki & Kennelly, 2003). An additional class of dietary phytoestrogens is lignans, which could provide health benefits by decreasing the risk of hormone-sensitive cancers (Adolphe, Whiting, Juurlink, Thorpe, & Alcorn, 2010). The oestrogenic action of dietary lignans is mainly due to their metabolites named enterolactone (ENT) and enterodiol (END) (Wang, 2002). Enterolactone was found to induce tissue-specific oestrogen response and inhibit the E2-induced proliferation of MCF-7 breast cancer cells (Penttinen et al., 2007; Wang, 2002). Both END and

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ENL protect against breast cancer in women and prostate cancer in men. Various *in vitro* experiments suggested that END and ENL significantly inhibited the growth of human colon tumour cells (Wang, 2002).

Aframomum melegueta K. Schum (Zingiberaceae) is a perennial herb widely cultivated for its valuable seeds in the tropical regions of Africa (Umukoro & Ashorobi, 2007). The seeds of this plant are generally called 'grains of paradise'. Melegueta pepper is the only major spice that is native to Africa and viewed as an African panacea (Duke, 2002). The seed of *A. melegueta* is used as a herbal remedy against diarrhoea, stomachache, treating painful inflammatory conditions and in the control of postpartum haemorrhage (Akendengue & Louis, 1994; Rafatullah, Galal, Al-Yahya, & Al-Said, 1995). The anti-ulcer, cytoprotective, antimicrobial (Galal, 1996; Rafatullah, Galal, Al-Yahya, & Al-Said, 1995) anti-nociceptive (Umukoro & Ashorobi, 2007) and sexual performance enhancing (Kamtchouing et al., 2002) effects of the aqueous seed extract of this plant have been reported. In spite of the vast pharmacological investigations of the plant, its phytochemical content is not well studied until now.

In our continuous search for phytoestrogens (El-Halawany, Chung, Abdallah, Nishihara, & Hattori, 2010; El-Halawany et al., 2007; El-Halawany, El Dine, Chung, Nishihara, & Hattori, 2011; Hegazy et al., 2011), the alcoholic extract of *A. melegueta* showed a potent anti-oestrogenic effect (El-Halawany, El Dine, Chung, Nishihara, & Hattori, 2011). Its possible diarylheptanoid content, similar structure to END and ENL (Fig. 1), and its reported anti-oestrogenic effect encouraged the authors to study the possible oestrogen receptor agonist/antagonist effect of the plant constituents.

In addition, investigation of the mechanism of these compounds on a molecular level, compared to 17β-oestradiol (E2), ENL and END, was carried out using a computational docking study of the isolated compounds to ERα.

2. Materials and methods

2.1. Reagents and kits

E2 was purchased from Calbiochem Co. (Darmstadt, Germany), tamoxifen and ERα competitor screening kit were purchased from Wako Chem. Co. (Osaka, Japan). EnBio receptor cofactor assay system (RCAS) for ERα kits were purchased from Fujikura Kasei Co. Ltd., Ibaraki, Japan. Genistein was isolated from *Sophora japonica* as previously reported by the authors (El-Halawany, Chung, Abdallah, Nishihara, & Hattori, 2010). ENL and END were kind gifts from Dr. Jong-Sik Jin, Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center.

2.2. General experimental procedures

Ultraviolet (UV) spectra were measured with a UV-2200 UV–Vis recording spectrophotometer (Shimadzu Co., Kyoto, Japan). Infrared (IR) spectra were measured using Jasco FT/IR-230 infrared spectrometer (Jasco, Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra, including correlation spectroscopy (COSY), heteronuclear multiple-bond correlation (HMBC), and heteronuclear single-quantum coherence (HSQC) experiments, were recorded on a JHA-LAA 400 WB-FT (^{1}H, 400 MHz; ^{13}C, 100 MHz; Jeol Co., Tokyo, Japan) spectrometer, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. High-resolution electron impact mass spectroscopy (HR-EIMS) was performed by using a JMX-AX 505 HAD mass spectrometer (JEOL) with anionisation voltage of 70 eV. TLC was carried out on pre-coated silica gel 60 F_{254} (0.25 mm, Merck; Darmstadt, Germany) and RP-18 $F_{254}S$ (0.25 mm, Merck Co.). Column chromatography (CC) was carried out on a BW-820MH silica gel, Wakosil C-300 silica gel (40–63 μm) (Wako Chem. Co.). Medium pressure liquid

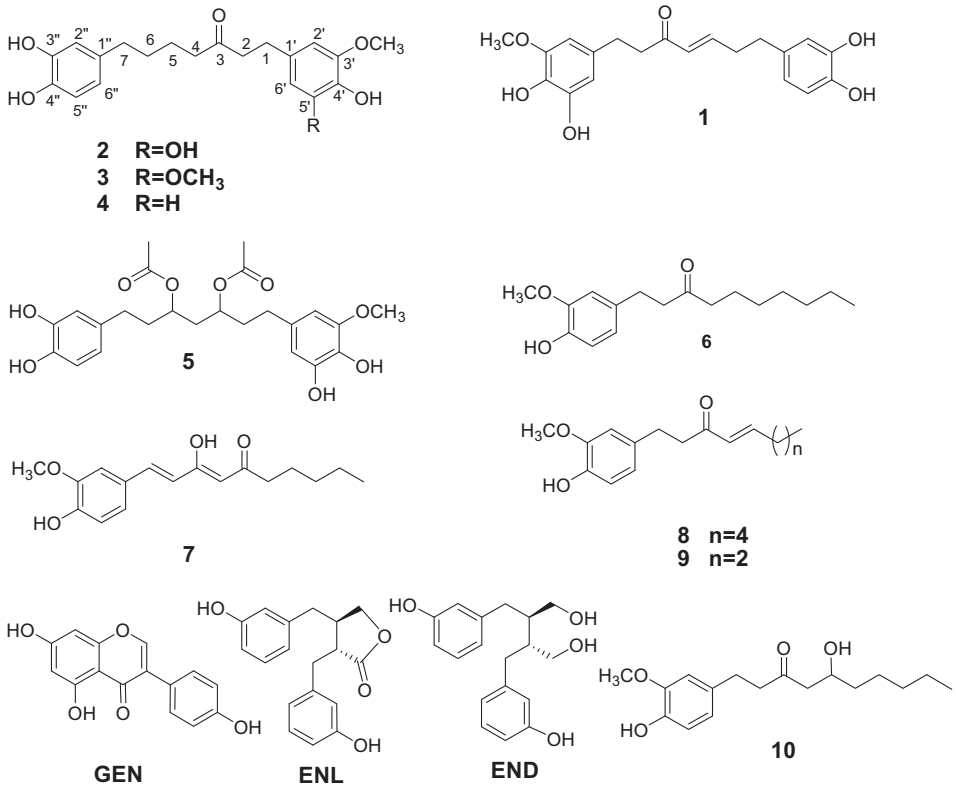

Fig. 1. Compounds isolated form *A. melegueta* (**1**–**10**), genistein (GEN), enterodiol (END) and enterolactone (ENL).