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Evaluation of synthesized coumarin derivatives on aromatase inhibitory activity

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ABSTRACT

In women across the world, the most common type of cancer is breast cancer. Among medical treatments, endocrine therapy based on aromatase inhibitors (AI) is expected to be effective against not only post-menopausal but also pre-menopausal breast cancer. In this study, we examined the structure–activity relationship between the aromatase inhibitory effects of 7-diethylaminocoumarin derivatives with a substituent at position 3 and coumarin derivatives with a substituent at position 7. Consequently, we found that 7-(pyridin-3-yl)coumarin (IC_{50} values 30.3 nM) and 7,7′-diethylamino-3,3′-biscoumarin (28.7 nM) are the most potent inhibitors of aromatase. These inhibitors were found to be comparable to the existing CYP19 inhibitor exemestane (42.5 nM).

Breast cancer is affecting a growing number of patients all over the world. According to GLOBOCAN 2012, which was published by International Agency for Research on Cancer (AIRC), the type of cancer that affects the most number of women worldwide is breast cancer, both in terms of new cases (1.7 million cases, 25.2% of the total number of cases) and deaths (522,000, 6.4%).[1] Breast cancer is a highly estrogen-dependent tumour, and it has been reported that one-third of all breast cancer cases and two-thirds of all post-menopausal breast cancer cases are estrogen-dependent. In women, estrogen is secreted by the ovaries or synthesized from androgen by CYP19 (aromatase), which is a type of cytochrome P450. The route of estrogen secretion changes before and after menopause, i.e., while the former is the main route in pre-menopausal women, the latter is pre-dominant in post-menopausal women. Pharmaceutical treatment of breast cancer includes chemotherapy, Her2 antibody therapy and endocrine therapy. There are two types of endocrine therapies that can inhibit the action of estrogen: tamoxifen, which inhibits estrogen receptors, and aromatase inhibitors (AI), which inhibits the biosynthesis of estrogen. Of these two, endocrine therapy based on AI has witnessed spectacular advancements since the early 2000 s, and in the last 15 years, its recognition as a drug therapy for breast cancer has significantly increased. Meanwhile, many studies have compared the use of tamoxifen with that of AI in endocrine therapy,[2–6] and a large number of those studies have confirmed that the third-generation AI, e.g., letrozole, are more effective than tamoxifen for pre- and post-operative administration to post-menopausal breast cancer patients.[7] Although endocrine therapy has favoured the use of tamoxifen for post-menopausal breast cancer patients in the past, the use of AI is preferred nowadays. Moreover, the possibilities that AI present are expanding, and combining AI with gonadotropin-releasing hormone (GnRH) agonists has proven effective against pre-menopausal breast cancer.[8] Additionally, it has been found that it is possible to use AI as a therapeutic agent against other highly estrogen-dependent disorders such as endometrial cancer,[9] endometriosis,[10] and uterine leiomyomas.[11] AI has even proven to be effective against infertility as it promote the secretion of follicle-stimulating hormones.[12] Therefore, there is an increasing demand for AI. However, only three types of AI are used currently: steroidal exemestane and the triazole derivatives anastrozole and letrozole. Finding AI that have different chemotypes is desirable from the viewpoint of drug tolerance and side-effect reduction; however, aromatase has a high substrate specificity, and most AI currently being developed are either steroidal or triazole derivatives. Since Chen, Leonetti et al. reported the inhibition of aromatase by coumarin derivatives **1**[13] and **2**[14] in 2004; attention has been focused on developing

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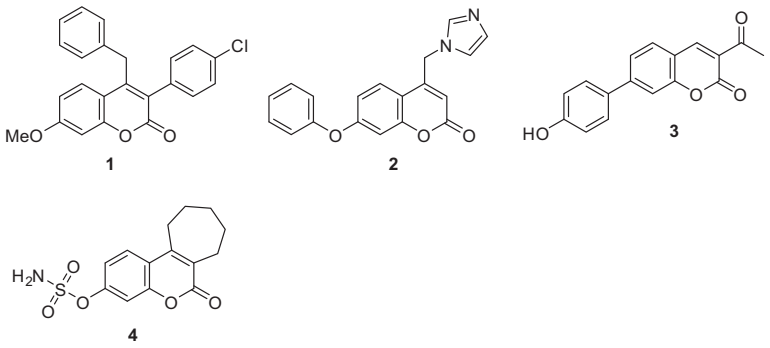

Fig. 1. Structure of coumarin derivatives having aromatase,[13,14] 17β-hydroxysteroid dehydrogenase,[22] or steroid sulfatase[23] inhibitory effect.

coumarin derivatives as a basis of breast cancer treatment drugs (Fig. 1).

Many coumarin derivatives can be found in nature. There are more than 1300 types of those purely derived from plants, and if synthetic products are included, their number is immeasurable.[15] According to the earliest existing records extant, herbs containing Psoralen-type compounds, e.g., methoxsalen, were used in the treatment of vitiligo as long ago as 1400 BCE.[16] Among these is one of the most famous coumarin derivatives, warfarin, which is a blood anti-coagulant, and other derivatives are also known to possess a variety of pharmacological characteristics, such as anti-inflammatory, anti-oxidant, anti-viral, anti-bacterial, anti-hyperlipidemic and anti-tumour characteristics.[17–19] Moreover, the use of their derivatives goes beyond pharmaceutical applications, for they are also used as fluorescent substances. Additionally, furanocoumarin derivatives are used as drug-metabolizing enzyme inhibitors, and their utility in drug metabolism studies or *in vitro* diagnostics is, among other characteristics, a subject of investigation.[20,21] Furthermore, it has been reported that coumarin derivatives **3**[22] and **4**[23] inhibit 17β-hydroxysteroid dehydrogenase and steroid sulphatase (STS) (Fig. 1). In recent years, with progress being made in the research of structure–activity relationships using docking studies,[24] structure–activity relationships concerning the anti-tumour characteristic of coumarin derivatives have also been investigated alongside the design of drugs that utilize coumarin at the active site of enzymes.[25]

We also conducted studies that focus on coumarin derivatives and reported on the fluorescence characteristics of 7-diethylaminocoumarin analogues that have an Ar group at position 3.[26,27] We investigated the structure–activity relationship of coumarin and furanocoumarin derivatives with the inhibitory effects of CYP2A6 and CYP3A4.[28–30]

AI **1**, **2** 17β-hydroxysteroid dehydrogenase inhibitor **3**, and STS inhibitor **4** have a substituent at lactone ring (3 or 4 position) or benzene ring (7 position). Leonetti reported structure-activity relationship study of CYP19 activity and coumarin derivatives having heterocyclic ring at 3–8 position, the optimal position of the hetero ring is 3 or 4 position.[14] We have conducted studies that focus on coumarin derivatives and reported on the fluorescence characteristics of 7-diethylaminocoumarin analogues that have an Ar group at position 3.[26,27] Thus we have technique synthesis of coumarin derivatives and fluorescent 7-diethylaminocoumarin derivatives having hetero ring at 3 position.

In this paper, we focus on the aromatase inhibitory effect of coumarin derivatives and study the structure–activity relationship between coumarin derivatives and their aromatase inhibitory effect.

The coumarin derivatives **5**–21 were prepared (Fig. 2). The coumarin derivatives **5**–**13** used in the present study have previously been synthesized.[26] Furthermore, 7-methoxycoumarin (**14**) is a commercially available item, and **15** and **16** were synthesized according to the method reported by Starcevic et al.[22] The other derivatives **17**–**21** were newly synthesized via a coupling reaction using a Pd catalyst (Schemes 1–3).

CYP19 metabolizes the A-ring of androstenedione onto the aromatised estrone. Because one molecule of formic acid and one molecule of water are released simultaneously, a $^{3}H_2O$ release assay and a $H^{14}COOH$ release assay, using [1β-^{3}H]-androstenedione and [19-^{14}C]-androstenedione as substrates, are used to measure CYP19 activity.[31] Herein, CYP19 activity was measured in a similar way by using the metabolic reaction of [1β-^{3}H]-androstenedione.[32] The percentage of control is indicated for 10 μM of coumarin derivatives **5**–**21** and positive control aminoglutethimide (Fig. 3). The value of IC_{50} was calculated by probit analyses (Table 1). Aminoglutethimide has IC_{50} values 7.0 μM as same as reference value 9.0 μM.[33]

The residual activities of diethylaminocoumarin derivatives **5**–**13** were as follows—diethylaminocoumarin **5**: 86%, 3-thiazolyl coumarin derivatives **6**–**9**: 15%–65%, 3-oxazolyl coumarin derivatives **10**–**13**: 22%–104%. The residual activities of **6**–**9** were as follows: 15% for **6**, which had Me as the 4-substituent of the thiazolyl group; 22% for the COOEt derivative **7**; 55% for the CH_2COOMe derivative **8** and 65% for the phenyl derivative **9**. This means that in comparison to diethylaminocoumarin **5** unsubsituted at position 3, the activity of the derivatives that had a thiazolyl group was higher. Furthermore, with bulky substituents at position 4 of the thiazolyl group, the activity tended to decrease. No correlation, however, was observed between residual activity and the bulkiness of the substituents of the oxazolyl group in **10**–**13**.

Regarding the 7-substituted coumarin monomers and dimers, the residual activities of the monomers that had an aliphatic substituent diethyl amino group or methoxy group were 86% for **5** and 96% for **14**. It seems that they were practically unaffected by the substituent. Conversely, residual activity significantly changed in dimers **20** (77%) and **21** (0% and 30% for 10 and 0.1 μM, respectively). In addition, there was a remarkable change in the activity of the derivatives that also had an aromatic ring at position 7 of the coumarin ring (0–96%). Of these, for the 7-position pyridyl group-substituted compounds, the 2-pyridyl derivative **17** showed 96% (10 μM), the 3-pyridyl derivative **18** showed 0% (10 μM) and 25% (0.1 μM) and the 4-pyridyl derivative **19** showed 14% (10 μM) and 92% (0.1 μM); thus, residual activity changed significantly according to the position of the nitrogen atom.

The IC_{50} values of CYP19 inhibiting **18** and **21** were 30.3 and 28.7 nM, respectively, which is comparable to the IC_{50} value of the existing CYP19 inhibitor exemestane (42.5 nM).[34]

According to the X-ray crystal structure of the CYP19 and androstenedione (ASD) complexes (PDB ID: 3EQM), the 17-keto oxygen makes a hydrogen bond with the backbone amide of Met347 and a weak contact with NH1 of Arg115, located at the rear