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Design and synthesis of silicon-containing steroid sulfatase inhibitors possessing pro-estrogen antagonistic character

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ABSTRACT

Steroid sulfatase (STS) is a potential target for treatment of postmenopausal hormone-dependent breast cancer. Several steroidal STS inhibitors have been reported, but steroidal compounds are difficult to optimize and may interact with other targets. On the other hand, we have shown that diphenylmethane (DPM) derivatives act as estrogen receptor (ER) agonists and antagonists. Here, we aimed to design and synthesize non-steroidal DPM-type STS inhibitors that would also serve as pro-estrogen antagonists, releasing a metabolite with ERα-antagonistic activity upon hydrolysis by STS. We synthesized a series of compounds and evaluated their biological activities by means of STS-inhibitory activity assay and ER reporter gene assay. Among them, silicon-containing compound **16a** showed strong STS-inhibitory activity (IC_{50} = 0.17 μM). Further, its putative metabolite (**12a**) exhibited potent ERα-antagonistic activity (IC_{50} = 29.7 nM).

1. Introduction

Breast cancer is one of the most common types of cancer in women; 70–80% of these cancers are hormone-dependent[1] and more than 70% express high levels of estrogen receptor alpha (ERα).[2] Endocrine therapies are standard treatments for hormone-dependent breast cancer, based on the use of an anti-estrogen or estrogen antagonist to block production or utilization of estrogens or to inhibit their functional effects.[3,4] In premenopausal women, estrogens promoting breast tumor growth are mainly synthesized in the ovaries, and therefore, a luteinizing hormone-releasing hormone (LH-RH) agonist[5] that suppresses the function of pituitary hormone to promote estrogen synthesis in the ovaries may be particularly useful. On the other hand, in postmenopausal women, whose ovarian function is lowered, estrogens are mainly synthesized from androgen secreted from the adrenal glands. Since the conversion of androgen to estrogen is performed by aromatase, aromatase inhibitors are often used to treat postmenopausal hormone-dependent breast cancer. However, the therapeutic effect of aromatase inhibitors is not always sufficient, and drugs with another mechanism of action may also be required.

In addition to aromatase, steroid sulfatase (STS) is involved in estrogen synthesis in breast cancer. STS catalyzes the hydrolysis of steroid-3-sulfates to 3-hydroxysteroids. Since the systemic circulating precursors for estrogens and androgens are predominantly sulfates such as dehydroepiandrosterone sulfate (DHEAS) and estrone sulfate (E1S), STS plays a crucial role in production of androgens and estrogens [estrone (E1) and estradiol (E2)].[6] Further, the activity of STS in cancer tissue is several hundred times higher than that of aromatase.[7] Since STS produces estrogens through a different route from aromatase, it is expected that simultaneous administration of STS inhibitors and aromatase inhibitors might show an additive or synergistic anti-tumor effect. Therefore, STS is considered to be a promising target for anticancer drug development.[8] Several STS inhibitors have been reported (Fig. 1). Among them, estrone 3-*O*-sulfamate (EMATE) was evaluated in a clinical trial. However, it was found that metabolites of EMATE exhibit potent estrogenic activity[9], and so might promote tumor growth. Therefore, the development of EMATE was discontinued. Since steroidal compounds are difficult to optimize, and they or their metabolites might also act on other targets, non-steroidal STS inhibitors have attracted attention. For example, STX64, which has a coumarin skeleton, shows potent STS-inhibitory activity and a clinical trial has been carried out.[10]

The non-steroidal diphenylmethane (DPM) derivative **1** has also been reported as an STS inhibitor (Fig. 2).[11] However, its putative metabolite **1m** has ER-agonistic activity.[12] Therefore, compound

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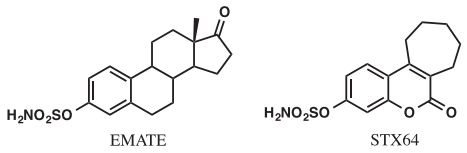

Figure 1. Structures of EMATE (estrone 3-*O*-sulfamate) and STX64.

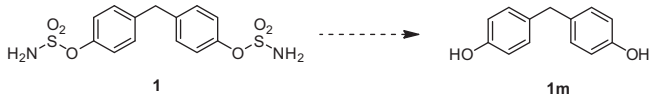

Figure 2. Structures of diphenylmethane (DPM) derivative **1** and its putative metabolite **1m**.

1 was considered unsuitable for use as a therapeutic agent for the same reason as EMATE.

Recently, we found that although some DPM derivatives have ER-agonistic activity, others have ER-antagonistic activity.[12] Therefore, we set out to design novel non-steroidal DPM-type STS inhibitors that would release ER antagonist metabolites upon hydrolysis by STS (Fig. 3). A similar approach has already been reported for steroid analogs.[13]

In recent years, various silicon-containing bioactive compounds have been reported.[14,15] The introduction of silicon into compounds can improve various biological properties, including selectivity, potency, pharmacokinetics, pharmacodynamics and cell penetration. Indeed, several silicon-containing agents have advanced to clinical trials.[16,17] Also, we have developed silicon-containing tubulin polymerization inhibitors.[18] It has already been reported that replacement of quaternary carbon in DPM with sulfur increased the STS-inhibitory activity. Since the silicon atom is larger than carbon, like sulfur, we speculated that introduction of silicon could also enhance STS-inhibitory activity.

In this paper, we describe the design and synthesis of non-steroidal, silicon-containing STS inhibitors that release an ER antagonist upon hydrolysis by STS. The biological activities of these inhibitors were examined by means of STS-inhibitory activity assay and ER reporter gene assay.

2. Results and discussion

2.1. Molecular design and computational studies

It has been reported that the STS-inhibitory activity of sulfur derivative **2** was stronger than that of carbon derivative **1**.[11] Generally, bonds involving sulfur are longer than those involving carbon. The single-bond covalent radii of carbon and sulfur are 75 pm and 103 pm, respectively, whereas that of silicon is 116 pm.[19] Therefore, we hypothesized that replacement of quaternary carbon in the DPM skeleton with silicon might also result in enhancement of STS-inhibitory activity. Based on this hypothesis, we designed silicon-containing compound **3a**. We next calculated the distances between the two benzene ring (*d*) in compounds **1**, **2** and **3a**. Structure optimizations were performed at the MP2/6-31+G* level of theory with the Gaussian 09 program package.[20,21] The results are illustrated in Figure 4. As expected, distance (*d*) in silicon compound **3a** was longer than those in carbon compound **1** and sulfur compound **2**.

2.2. Chemistry

Based on our hypothesis, we planned to prepare silicon-containing compound **3a** and its derivatives **8**, **10** and **15a**–**18a**. These compounds were prepared by usual organic synthetic methods as illustrated in Scheme 1. Compounds **9a**, **11a**, **12a** and **14a** were prepared according to the reported method.[22] Briefly, bromophenols (**5a**–**b**) were reacted with dichlorodialkylsilane in the presence of *n*-butyllithium (*n*-BuLi) in tetrahydrofuran (THF). Compounds **3a**, **15a**, **16a** and **18a** were synthesized by reaction of **9a**, **11a**, **12a** and **14a** with excess sulfamoyl chloride.[23] Compound **17a** was prepared in an analogous manner, starting from dichlorodipropylisilane **4d** and bromophenol **5c**. Mono sulfamate

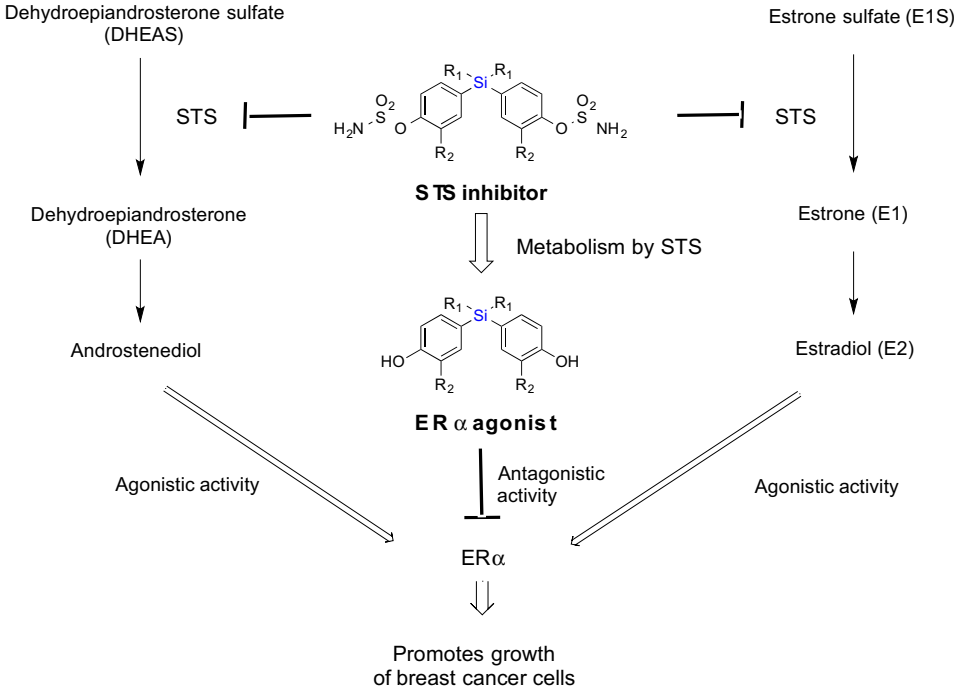

Figure 3. Schematic illustration of the expected action mechanisms of our DPM-type STS inhibitors with pro-estrogen antagonistic character.