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Phosphate tricyclic coumarin analogs as steroid sulfatase inhibitors: synthesis and biological activity†

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In the present work, we report convenient methods for the synthesis and biological evaluation of phosphate tricyclic coumarin derivatives as potential steroid sulfatase inhibitors. The described synthesis includes the straightforward preparation of 7-hydroxy-2,3-dihydro-1*H*-cyclopenta[*c*]chromen-4-one, 3-hydroxy-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-6-one and 3-hydroxy-8,9,10,11-tetrahydro-7*H*-cyclohepta[*c*]chromen-6-one modified with various phosphate moieties. The inhibitory effects of the synthesized compounds were tested on STS isolated from human placenta as well as the MCF-7, MDA-MB-231 and MDA-MB-435S cancer cell lines. Most of the new STS inhibitors possessed IC_{50} values between 21 to 159 μM. In the course of our investigation, the largest inhibitory effects in the STS enzyme assays were observed for the three compounds **9p**, **9r** and **9s**, with IC_{50} values of 36.4, 37.8 and 21.5 μM, respectively (IC_{50} value of 1.0 μM for the 665-COUMATE used as a reference). The compound **9r**, exhibited the highest potency against MCF-7, an estrogen receptor positive (ER+) cell line, with a GI_{50} value of 24.7 μM. The structure-activity relationships of the synthesized coumarin derivatives with the STS enzyme are discussed.

Introduction

Breast cancer is a major cause of mortality, and there is an urgent need for novel treatment strategies. The World Health Organization (WHO) lists estrogens as one of the most important factors inducing the development of breast cancer. In females in industrialized countries, breast cancer is the most frequently diagnosed cancer. Estimates show that in the United States, more than 190 000 new cases of breast cancer were diagnosed and more than 40 000 deaths occurred from this disease in 2009.[1] One strategy for the treatment of hormone-dependent breast cancer (HDBC) involves inhibitors to prevent the synthesis of estrogens in peripheral tissues.[2] In recent years, there has been intensive research toward finding novel effective inhibitors of STS, an enzyme involved in the biosynthesis of estrogen in the mammary gland. Approaches to design effective STS inhibitors include three different categories of compounds: alternative substrates (including competitive reversible inhibitors), reversible inhibitors, and irreversible inhibitors.[3] Initial reports on the synthesis and biological evaluation of STS inhibitors appeared in the 1970s and were focused on natural and synthetic unconjugated steroids.[4] In the 1980s, a series of 2-(hydroxyphenyl)indole sulfates were reported as the first class of STS inhibitors. Among them, compound **1**, with an IC_{50} value of 80 μM, exhibits the highest activity. In the 1990s, intensive research on natural and synthetic steroid derivatives with potent activity against STS has continued. During this time, Evan's research group reported 5-androstene-3β,17β-diol-3-sulfate **2**, which showed potent activity toward STS with an IC_{50} = 2 μM.[5] Because of numerous side effects from the production of estrogenic metabolites that bind to the ER, these compounds have not been utilized in hormone therapy for breast cancer.

There has been a concerted effort by various research groups to find an effective, reversible STS inhibitor that does not show estrogenic action. A series of estrone sulfate (E1S) analogues with different functional groups has been synthesized, and these functional groups include: sulfonates, sodium methylenesulfonate, sulfonamide, sulfonyl halides, methylenesulfonyl groups, phosphates and phosphonates.[6] The most promising compound was estrone-3-*O*-sulfamate **3** (EMATE), which exhibited very high activity in MCF-7 cells, with an IC_{50} value of 65 pM.[7] Unfortunately, due to its estrogenic properties, clinical trials for EMATE have been discontinued. An important class of compounds that exhibits high activity against STS are the coumarin derivatives. In contrast to EMATE, they exhibit fewer

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