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Examination of the in vitro (anti)estrogenic, (anti)androgenic and (anti)dioxin-like activities of tetralin, indane and isochroman derivatives using receptor-specific bioassays

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Abstract

Molecules derived from tetralin, indane and isochroman are often used in the synthesis of fragrance materials. The two polycyclic musk fragrances AHTN (6-acetyl-1,1,2,4,4,7-hexamethyltetralin), HHCB (1,2,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran) and ADBI (4-acetyl-1,1-dimethyl-6-*tert*-butylindane) are derived from tetralin, isochroman and indane, respectively. In previous studies, AHTN and HHCB have been shown to antagonize estrogen receptors (ERs), both in vitro and in vivo. Here, we used two newly developed reporter gene assays, to examine the agonistic and antagonistic properties of several indane, tetralin and isochroman derivatives towards the human androgen receptor (AR) and aryl hydrocarbon receptor (AhR). Additionally, we also assessed (anti)estrogenicity of these compounds.

A number of compounds showed weak estrogenic activity towards the human ERα. Several compounds showed (anti)estrogenic effects, starting at a concentration of 0.1 μM. Surprisingly, almost all compounds were found to be AR antagonists, starting at 0.1 μM.

None of the compounds tested, showed either agonism or antagonism towards the AhR. Non-specific effects via crosstalk of the AhR and the ER or AR can therefore be ruled out. As far as we are aware, molecules derived from indane, tetralin and isochroman showing direct interaction with the ER and AR have not been reported previously.

Keywords: Estrogen receptor; Androgen receptor; Dioxin receptor; Indane; Tetralin; Isochroman

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1. Introduction

It has been suggested that substances present in the environment are adversely affecting wildlife and human health by disrupting endocrine function (reviewed in Tyler et al. (1998); Golden et al. (1998)). These so-called endocrine active compounds (EACs) exert their effects via several mechanisms, such as estrogen receptor (ER) or androgen receptor (AR)-mediated processes. Due to worldwide concern about EACs, a large number of assays have been developed to screen for estrogenic and androgenic activity of chemicals. In vitro assays include receptor competitive binding assays, for the measurement of the binding affinity of a chemical for the receptor. Reporter gene assays, such as the Chemically Activated LUciferase eXpression assay (CALUX®) (Legler et al., 1999) or the Yeast Estrogen Screen assay (YES) (Routledge and Sumpter, 1996) assess the receptor binding-dependent transcriptional activity of genes that can easily be measured. Cell proliferation assays, such as the E-SCREEN assay (Soto et al., 1995) measure the increase in cell number of an estrogen-sensitive cell line, e.g. MCF-7 or T47D cells. A major drawback of competitive binding assays is that these do not distinguish between receptor agonists and antagonists, whilst reporter gene assays can. In vivo estrogenic activity can be screened using the immature or ovariectomized rat uterotrophic assay or the recently developed transgenic zebrafish (Legler et al., 2000) and mouse (Ciana et al., 2001) assays, while androgenic activity can be screened by means of the Hershberger assay (Hershberger et al., 1953).

In past decades a large number of EACs have been tested in either one of these assays. Examples of chemicals that have been shown to mimic the natural estrogen 17β-estradiol (E2), are bisphenol-A, *o,p′*-DDT, methoxychlor, several alkylphenols (Routledge and Sumpter, 1997), some phthalate esters (Zacharewski et al., 1998), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (Meerts et al., 2001). Many of these xenoestrogens are only weakly estrogenic at high concentrations. However, diethylstilbestrol (DES) and ethinylestradiol (EE2) are as potent as the endogenous ligand E2. Well-known (anti)estrogens are tamoxifen, raloxifen, ICI 164,384 and ICI 182,780. Androgens and in particular (anti)androgens also seem to play a role in endocrine disruption (Kelce and Wilson, 1997), however, they have so far not been studied extensively. EACs that mimic the endogenous androgens testosterone or dihydrotestosterone (DHT) have not been identified. Currently, known (anti)androgenic EACs are the fungicide vinclozolin (Kelce et al., 1994), the methoxychlor metabolite 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) (Gaido et al., 2000) and several pesticides including *p,p′*-DDE and dieldrin (Kelce et al., 1995; Andersen et al., 2002). Recently, the UV filters benzophenone-3 and homosalate have been shown to have (anti)androgenic activity (Ma et al., 2003).

In the present study, we investigated the (anti)estrogenic and (anti)androgenic activity of several tetralin, indane and isochroman derivatives, which are structurally related to polycyclic musks which are used as fragrance compounds in perfumes, cosmetics and laundry detergents. Also, the dioxin-like properties of the compounds were assessed. The widely used polycyclic musk fragrances AHTN (Tonalide), ADBI (Celestolide), and HHCB (Galaxolide) are derived from tetralin, indane and isochroman, respectively. After use in private households and industrial applications, these materials reach the aquatic environment via sewage treatment plants. Due to their lipophilic nature, they have been shown to bioaccumulate in fish and other aquatic organisms (Balk and Ford, 1999). AHTN and HHCB have previously been shown to act as so-called "selective estrogen receptor modulators" (SERMs) (Schreurs et al., 2002b). Depending on the cell type, the promoter context and the ER subtype (ERα or ERβ) targeted, these materials may act both like agonists and antagonists.

In order to study (anti)estrogenicity, we used the human embryonal kidney 293 cell-line, which was stably transfected with a reporter construct, consisting of three estrogen response elements (EREs) upstream from a TATA box in front of luciferase cDNA, and a hERα or hERβ expression plasmid (Lemmen et al., 2002). (Anti)androgenicity was studied using a human U2-OS osteoblastic bone cell line, consisting of three androgen response elements (AREs) coupled to a TATA box in front of the luciferase gene and a hAR expression plasmid (Sonneveld et al., 2005). A rat-hepatoma H4IIE cell-line stably transfected with a 4× DRE-TATA-Luc-reporter construct was used to assess (anti)dioxin-like properties (Sonneveld et al., 2002). Usually dioxins are