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Structure-Activity Relationships Among Antitumorigenic 6-Substituted-1,3,8-trichlorodibenzofurans and in the 7,12-Dimethylbenz[a]anthracene-Induced Rat Mammary Tumor Model

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Abstract

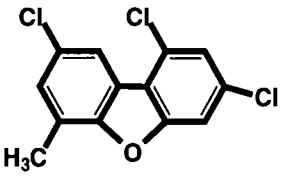
Four 6-alkyl-substituted-polychlorinated dibenzofurans (PCDFs) and 6-amino-1,3,8-trichlorodibenzofuran (ACDF) were tested for their activity as inhibitors of tumor growth in the 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumor model in female virgin Sprague-Dawley rats. After initial development of mammary tumors (150 to 200 mm^3), rats were treated with the test compounds in corn oil or corn oil alone once per week for three weeks and sacrificed one week after the last treatment. All compounds significantly inhibited tumor growth at a dose of 5 mg/kg/week, but varied in their antitumorigenic potency. The most effective compound, 6-isopropyl-1,3,8-trichlorodibenzofuran, was also active at doses of 1.0 and 0.5 mg/kg/week. Hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity was not significantly induced by any treatment.

Introduction

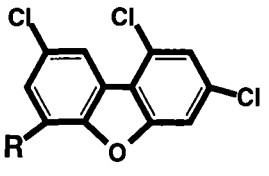
Kociba and co-workers found that dosing female Sprague-Dawley rats for two years with TCDD at 100, 10, and 1 pg/kg/day caused significant inhibition of spontaneous development of uterine and mammary tumors [1]. TCDD inhibits tumor growth in mice [2] and in the DMBA-induced rat mammary tumor model [3], and inhibits 17β-estradiol induced gene expression and proliferation in the female rat uterus and in MCF-7 human breast cancer cells [4]. 6-Methyl-1,3,8-trichlorodibenzofuran (6-MCDF) and its analog 8-MCDF exhibit antitumorigenic activity in the DMBA-induced rat mammary tumor model [5]. 6-MCDF was initially investigated as a partial aryl hydrocarbon (Ah) receptor agonist, and was found to exhibit low toxicity. Moreover, this compound inhibited TCDD-induced toxic responses and induction of CYP1A1 gene expression [6,7]. 6-MCDF is also antiestrogenic, as evidenced in both the female rat uterus [8,9] and in Ah-responsive breast cancer cells [10]. 6-MCDF is approximately 300-700 times less potent than TCDD as an antiestrogen and 10,000-100,000 times less toxic than TCDD for traditional Ah-receptor mediated toxic responses [9]. These studies demonstrated that 6-MCDF and its alkyl-PCDF analogs are relatively nontoxic, antagonized TCDD-induced toxic effects, but exhibited antiestrogenic acitivty both *in vitro* and *in vivo*. 6-MCDF may represent a new class of antiestrogens that act through the Ah-receptor and this class of compounds may have potential for the clinical treatment of breast cancer. This study reports the antitumorigenic effects of 4 alkyl-PCDFs and ACDF in the DMBA-induced rat mammary tumor model.

Experimental Methods

Chemicals DMBA was purchased from Sigma (St. Louis, MO). Five alkyl-substitued-PCDF analogs were synthesized in this laboratory as previously described [9]. The compound names were abbreviated as follows: 6-amino-1,3,8-trichlorodibenzofuran (ACDF), 6-ethyl-1,3,8-trichlorodibenzofuran (ECDF), 6-isopropyl-1,3,8-trichlorodibenzofuran (IPDF), 6-propyl-1,3,8-trichlorodibenzofuran (PCDF), and 6-tertbutyl-1,3,8-trichlorodibenzofuran (TBDF).



6-MCDF



6-Alkyl-PCDF

R =	
CH_3-CH_2-	Ethyl
CH_3-CH_2-CH_2-	Propyl
$(CH_3)_2$-CH-	Isopropyl
$(CH_3)_3$-C-	Tertbutyl
NH_2-	Amino

Animal Treatment. Mammary tumors were induced in virgin 50 ± 1 day old Sprague Dawley rats obtained from Harlan (Houston, TX). They were treated with a single dose of 20 mg DMBA oral gavage per rat as previously described [3,5,11]. After 30-60 days, carcinomas were detected by palpation in the ductal tubules of the mammary glands. After the largest (or only) tumor reached a small predetermined size (150-200 mm^3), rats were dosed with the test compound in corn oil (1.6 ml/kg) once per week for 3 weeks by intraperitoneal injection. In the first experiment, rats were given 5 mg/kg TBDF, IPDF, or ECDF. In the second experiment rats were were dosed with PCDF or ACDF at 5 mg/kg or with lower doses of IPDF (1 and 0.5 mg/kg). Control animals received corn oil alone. Tumor sizes were measured biweekly with calipers and volumes were calculated using the formula (length/2) x (width/2) x (depth/2) x (4/3π). One week after the third injection rats were euthanized and tumors were removed, weighed and sectioned.

EROD Assay. Hepatic microsomal EROD activities were determined by fluorimetric procedures (530/590 excitation/emission) as previously described [12]. This activity is a surrogate of potential AhR-mediated toxicity.

Results and Discussion

Figure 1 summarizes a preliminary structure-activity study on the effects of 3 alkyl-PCDFs: TBDF, ECDF, and IPDF in the DMBA-induced rat mammary tumor model. All three caused highly significant decreases in both tumor mass and tumor volume at a low dose (5 mg/kg). Figure 2 summarizes a second study performed to test two additional analogs, PCDF and ACDF (at 5 mg/kg), and to test IPDF, which was most effective antitumorigenic agent detected in experiment 1, at two lower doses (1.0 and 0.5 mg/kg). No significant changes in rat whole body weight, hepatic EROD activity, liver wet weight, or uterine wet weight were observed for any of the compounds, and these results were consistent with initial studies. These results further confirm that 6-alkyl-substituted PCDFs are potent antiestrogens in the DMBA-induced mammary tumor model and inhibit tumor growth at doses as low as 0.5 mg/kg/week. Jordan [11] previously reported that the dose of tamoxifen required to inhibit mammary tumor growth in this model was 5 mg/kg twice daily, illustrating the relatively high antitumorigenic potency of the alkyl PCDFs. Moreover, the results also show that 6-alkyl-PCDFs did not induce hepatic microsomal EROD activity or cause any changes in liver weight or pathology; thus the antitumorigenic responses in the mammary are not accompanied by any changes in the liver, which is a

major site of action for PCDFs. Ongoing studies are focused on further development of these compounds as a potential new class of Ah receptor-based antiestrogens for treatment of breast cancer. This research was supported by the National Cancer Institute (CA-64081).

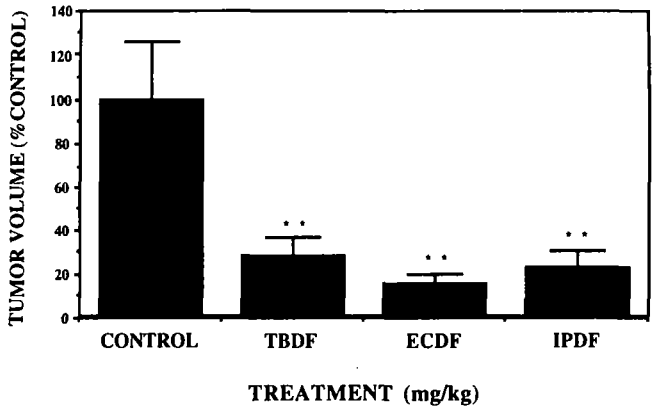


Figure 1. Effects of TBDF, ECDF and IPDF on mammary tumor growth. Fifty-day old female Sprague-Dawley rats were treated orally with DMBA (20 mg/kg). After the initial tumor was detected (150-200 mm^3), rats were dosed with 5 mg/kg TBDF, ECDF or IPDF once per week for three weeks and euthanized one week after the third injection. Tumor volumes reported are those at the time of euthanasia.

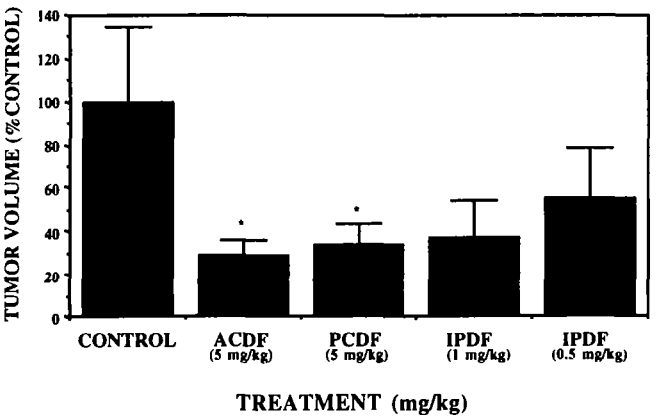


Figure 2. Effects of ACDF, PCDF and low doses of IPDF on mammary tumor growth. fifty-day old female Sprague-Dawley rats were treated orally with DMBA (20 mg/kg). After the initial tumor was detected (150-200 mm^3), rats were dosed with 5 mg/kg TBDF, ECDF or 1 and 0.5 mg/kg IPDF once per week for three weeks and euthanized one week after the third injection. Tumor volumes reported are those at the time of euthanasia.

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