Methyl-Substituted Diindolymethanes as AhR-Based Antitumorigenic/Antiestrogenic Compounds

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INTRODUCTION

Indole-3-carbinol (I3C) is a secondary metabolite of 3-indolylmethyl glucosinolate, a naturally occurring indole in cruciferous vegetables. Several studies have reported the anticarcinogenic activity of I3C, which enhances the metabolic deactivation of carcinogens (reviewed in 1). Diindolylmethane (DIM) is the dimerization product of I3C that forms as an acid condensation product in the stomach (2). Both DIM and I3C bind to the aryl hydrocarbon receptor (AhR) (3) and exhibit AhR agonist and partial antagonist activity *in vitro* (4). Recently, DIM was shown to inhibit the growth of established tumors in the 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary carcinoma model (5). In the current study, methyl- and methoxy-substitued analogs of DIM were tested for their ability to inhibit mammary tumor growth. 5,5'-Dimethoxydiindolylmethane (5,5'-MeODIM) and 1,1'-dimethyldiindolylmethane (1,1'-DDIM) were tested at 5.0 mg/kg/every other day in the DMBA-induced rat mammary carcinoma model. Subsequently, 1,1'-DDIM was retested concurrent with 2,2'-dimethyldiindolylmethane (2,2'-DDIM) and 1,1',2,2'-tetramethyldiindolylmethane (1,1',2,2'-TDIM) at 1.0 mg/kg/every other day. Results indicate that both 5,5'-MeODIM and 1,1'-DDIM are effective antitumorigenic agents in the rat mammary gland at 5 mg/kg, and that 2,2-DDIM and 1,1',2,2'-TDIM retain activity at 1.0 mg/kg. DIM and its structural analogs represent a new class of nontoxic antiestrogens that work through the AhR and may have potential in the clinical treatment of human breast cancer.

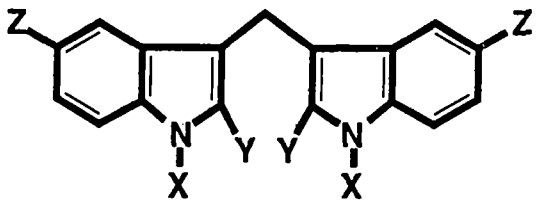
MATERIALS and METHODS

Chemicals. 7,12-DMBA was purchased from Sigma (St. Louis, MO). 5,5'-MeODIM, 1,1'-DDIM, 2,2'-DDIM and 1,1',2,2'-TDIM were synthesized in this laboratory.

Animals. At 50 days of age, virgin female Sprague-Dawley rats were treated with 20 mg/rat of DMBA. After 30-60 days, mammary carcinomas were palpable in the ductual tubules, as previously described (6). When the largest tumor reached a small size (10 mm at its longest axis), each animal was treated every other day for 3 weeks by gavage. In cases were two tumors of close size were present on the same animal, the tumor volumes were averaged. In the first experiment, animals were treated with 5,5'-MeODIM or 1,1'-

DDIM at 5.0 mg/kg, or with vehicle alone (1% ethanol in water). In the second experiment, rats were treated with 1,1'-DDIM, 2,2'-DDIM, or 1,1',2,2'-TDIM at 1.0 mg/kg/every other day, or with vehicle. Tumor sizes were measured with calipers and volumes were calculated using the formula: (length/2) x (width/2) x (depth/2) x (4π/3). Tumors were excised at the time of euthanasia; organ wet weights were measured for the liver, uterus, heart, spleen and kidneys, and the hepatic EROD activity was determined.

Ethoxyresorufin-O-deethylase (EROD) Assay. Hepatic microsomal EROD activities were determined by fluorimetric procedure (530/590 excitation/emission) as previously described (7). This activity is a sensitive surrogate of potential AhR-mediated toxicity.



	X	Y	Z
1,1'-DDIM	CH_3	H	H
2,2'-DDIM	H	CH_3	H
1,1',2,2'-TDIM	CH_3	CH_3	H
5,5'-MeODIM	H	H	CH_3O

Figure 1: Structure of Methyl- and Methoxy-substituted DIM analogs.

RESULTS and DISCUSSION

1. Figure 2 summarizes an initial study testing the effects of 5,5'-MeODIM and 1,1'-DDIM on mammary tumor growth. Both compounds caused highly significant decreases in both tumor volume and tumor mass at time of euthanasia at a dose of 5 mg/kg/every other day.

2. Figure 2 summarizes the antitumorigenic effects of 1,1'-DDIM, 2,2'-DDIM, and 1,1',2,2'-TDIM at a dose of 1.0 mg/kg/every other day. 2,2'-DDIM significantly inhibited tumor growth ($p < 0.05$) using ANOVA with Duncan's New Multiple Range Test. The effects of 1,1',2,2'-TDIM on the inhibition of tumor growth were significant ($p < 0.05$) when compared against controls using student's t test, although not by ANOVA.

3. No significant changes were observed for hepatic EROD activity, a surrogate for AhR mediated toxicity. These results are consistent with in vitro studies showing that high doses of DIM were required to induce EROD activity (4) and in vivo sudies in which EROD induction was not observed at doses which completely inhibited tumor growth (5).
4. None of the structural analogs affected whole body weight, or organ wet weights for the liver, uterus, heart, spleen, and kidneys. Histological examination of the liver revealed no differences between animals tested with DIM analogs and vehicle treated animals.
5. DIM and its structural analogs represent a new class of nontoxic antiestrogens that work through the AhR and may have potential in the clinical treatment of human breast cancer.

ACKNOWLEDGEMENTS

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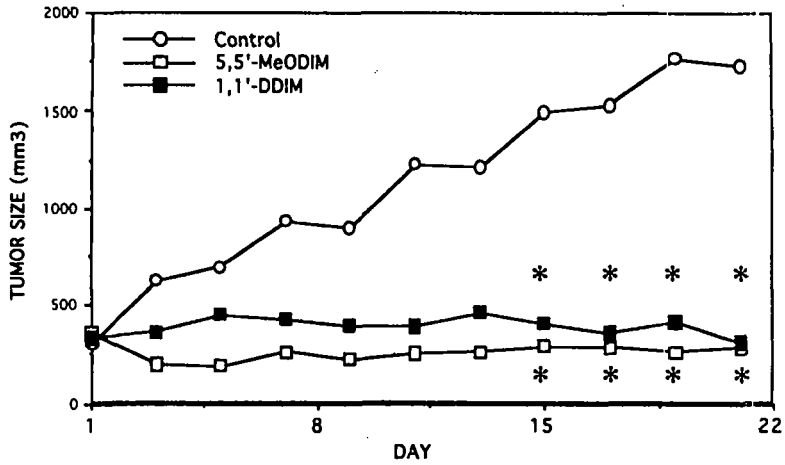

Figure 2: Effects of 5,5'-MeODIM and 1,1'-dimethyl-DIM (5.0 mg/kg/every other day) on mammary tumor growth in the DMBA-induced tumor model. Both 5,5'-MeODIM and 1,1'-DDIM significantly inhibited tumor growth compared to control tumors.

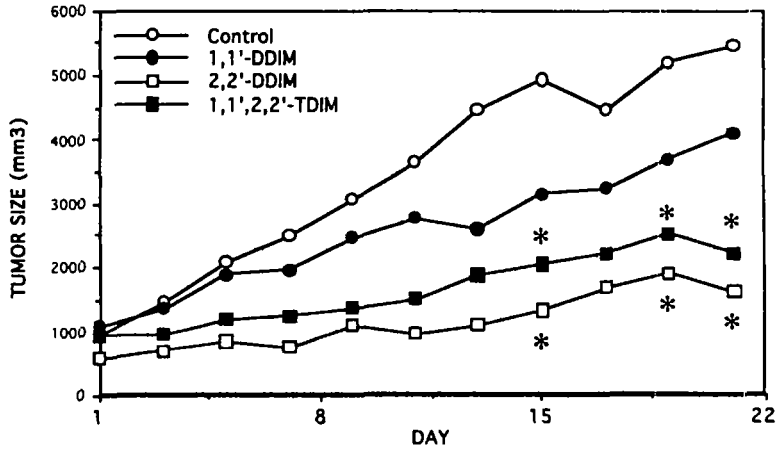

Figure 3: Effects of 1,1'-DDIM, 2,2'-DDIM and 1,1',2,2'-TDIM (1.0 mg/kg/every other day) on mammary tumor growth in the DMBA-induced tumor model. 2,2'-DDIM significantly inhibited tumor growth ($p < 0.05$) using ANOVA with Duncan's New Multiple Range Test. The effects of 1,1',2,2'-TDIM on the inhibition of tumor growth were significant ($p < 0.05$) when compared against controls using student's t test, although not by ANOVA.