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UV Filters with Antagonistic Action at Androgen Receptors in the MDA-kb2 Cell Transcriptional-Activation Assay

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The fact that certain ultraviolet (UV) filters used in cosmetics display estrogenic activity prompted us to study potential actions on androgen receptors (AR) in the human breast carcinoma cell line MDA-kb2, which expresses functional endogenous AR and glucocorticoid receptors (GR) and is stably transfected with a luciferase reporter plasmid. Dihydrotestosterone (DHT), methyltrienolone (R1881), methyltestosterone, danazol, and androstenedione increased luciferase activity, with EC₅₀ values between 0.11 nM (R1881), 0.14 nM (DHT), and 73.5 nM (androstenedione). DHT-induced luciferase gene expression was inhibited by nonsteroidal antiandrogens, hydroxyflutamide, flutamide, bicalutamide, and vinclozolin. In contrast, the steroidal AR agonist/antagonist cyproterone acetate showed agonistic activity in the absence and presence of DHT, which was not blocked by hydroxyflutamide and thus seems not to be mediated by AR. GR-mediated activation of luciferase by dexamethasone was 100 times less potent than DHT and was not antagonized by hydroxyflutamide. The cell line was used for screening of UV filters, benzophenone-3 (Bp-3), benzophenone-4, 3-benzylidene camphor, 4-methylbenzylidene camphor, butyl-methoxy-dibenzoylmethane, homosalate (HMS), octyldimethyl-PABA, and octyl-methoxycinnamate. Two of these, Bp-3 and HMS, antagonized DHT-induced AR activation below cytotoxic concentrations, with IC₅₀ of 5.57 10⁻⁶ M (HMS) and 4.98 10⁻⁶ M (Bp-3). None of the eight UV filters displayed agonistic activity when tested alone, but high concentrations of Bp-3 induced an increase of luciferase activity in the presence of dexamethasone, which was not blocked by hydroxyflutamide or the estrogen antagonist, ICI 182,780. These data indicate that the UV filters Bp-3 and HMS possess antiandrogenic activity *in vitro* in addition to estrogenic activity.

Key Words: MDA-kb2 cells; androgen receptor; androgen; antiandrogen; endocrine disruptor; pesticide; UV filter.

The presence in the environment of increasing amounts of synthetic chemicals is causing concern about unknown long-term toxicities, especially the possibility of interference with endocrine systems (IPCS, 2002; Kelce *et al.*, 1998). We previously identified estrogenic activity in several ultraviolet (UV)

filters *in vitro* and *in vivo* (Schlumpf *et al.*, 2001). This prompted us to initiate a study on their potential interaction with androgen receptors. UV filters are added to sunscreen products in continuously higher amounts, since sun protection factors (SPF) increased from SPF 1–2 in 1950 to SPF 40–60 today (SPF = ratio of UV light dose producing minimal erythema in the protected skin versus the unprotected skin). In addition, UV filters are also included in cosmetics for product protection and stability. These persistent chemicals have been detected in fish (Nagtegaal *et al.*, 1997) and in human milk (Hany and Nagel, 1997). Interactions of xenobiotics with androgens may cause developmental abnormalities, as shown following the exposure to the antiandrogenic pesticides vinclozolin, procymidone, or linuron (Kelce *et al.*, 1994, 1997; Lambright *et al.*, 2000; Ostby *et al.*, 1999). As there is 100% sequence homology between rat and human AR ligand binding domains, analogous effects can be expected to be mediated by human AR (Kelce *et al.*, 1998).

In this study, we validated the human AR expressing cell line MDA-kb2 with known androgens and antiandrogens and used the system to test UV filters for androgenic or antiandrogenic activity *in vitro*. Eight of the most frequently used compounds were chosen among the total of 30 UV filters admitted for use in Switzerland. The human breast carcinoma cell line, MDA-kb2, expresses high levels of functional endogenous androgen receptor (AR) and also glucocorticoid receptor (GR), while estrogen receptor alpha and progesterone receptor are not detectable at the RNA level, and estrogen receptor beta is expressed only at low levels (Hall *et al.*, 1992). The cells are stably transfected with a luciferase transporter plasmid driven by the mouse mammary tumor virus promoter (MMTV) that can be activated through both AR and GR (Wilson *et al.*, 2002). Compounds acting through AR or GR can therefore induce luciferase expression. It was shown earlier that known AR antagonists like hydroxyflutamide, the vinclozolin metabolites M1 and M2, p,p'DDE, and linuron-inhibited dihydrotestosterone (DHT)-induced gene expression in this cell line (Wilson *et al.*, 2002).

MATERIALS AND METHODS

Chemicals. Methyltrienolone (R1881, also called metribolone) (CAS no. 965-93-5) was purchased from NEN[™] (NEN[™] Life Science Products, Inc.,

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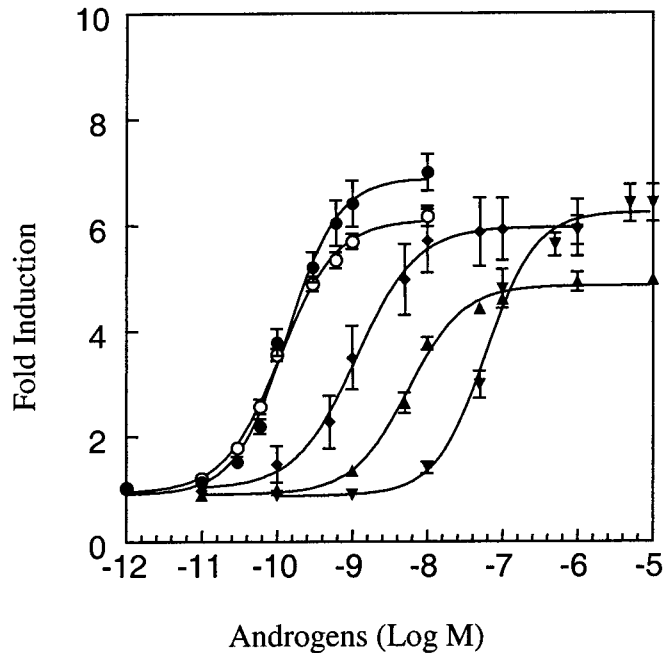


FIG. 1. Androgen receptor (AR)-mediated reporter gene activation induced by androgen agonists in MDA-kb2 cells. Mean \pm SEM of five independent experiments. Ethanol concentrations (v/v): from 1:10¹⁰ to 1:10³. Filled circle, DHT; open circle, R1881; filled diamond, Met-T; filled upward-pointing triangle, danazol; filled downward-pointing triangle, androstenedione.

Boston, MA 02118). 5 α -dihydrotestosterone (DHT) (CAS no. 521-18-6, purity \geq 99.0%), methyltestosterone (Met-T) (CAS no. 58-18-4, purity \geq 97.0%), androstenedione (CAS no. 63-05-8, purity \geq 98.0%), and dexamethasone (DEX) (CAS no. 50-02-2, purity \geq 97.0%) were obtained from Fluka (Fluka Chemie GmbH CH-9471 Buchs, Switzerland), danazol (CAS no. 17230-88-5, purity \geq 98.0%), flutamide (CAS no. 13311-84-7, purity \geq 99%), and cyproterone acetate (CAS no. 427-51-0, purity \geq 98.0%) from Sigma (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany), vinclozolin (CAS no. 50471-44-8, purity \geq 99.4%) from Riedel de Haen (Rdh Laborchemikalien GmbH & Co. KG D-30918 Seelze). Bicalutamide (Casodex, ICI176.334) was a gift from Dr. W. Körner (Bayerisches Landesamt für Umweltschutz, D-86179 Augsburg, MA 02118).

Hydroxyflutamide (OHF) was obtained from Schering-Plough Research Inst., Kenilworth, NJ 07033). The UV screens 3-(4-methylbenzylidene) camphor (4-MBC, Eusolex 6300) (CAS no. 36861-47-9 > 99.7%), 2-ethylhexyl-4-dimethylamino-benzoate (OD-PABA, Eusolex 6007 > 98.5%) (CAS no. 21245-02-3), 4-tert-butyl-4'-methoxy-dibenzoylmethane (B-MDM, Eusolex 9020) (CAS no. 70356-09-1 > 98%), 2-ethylhexyl-4-methoxycinnamate (OMC, Eusolex 2292) (CAS no. 5466-77-3 > 98%), 2-hydroxy-4-methoxybenzophenone (Benzophenone-3, Bp-3, Eusolex 4360) (CAS no. 131-57-7 > 99%), 3,3,5-trimethylcyclohexyl salicylate (Homosalate, HMS, Eusolex HMS) (CAS no. 118-56-9 > 98%), and 3-benzylidencamphor (3-BC) (CAS no. 15087-24-8 > 99.9%) were purchased from Merck (Dietikon, Switzerland), 2-benzoyl-5-methoxy-1-phenol-4-sulfonic acid (Benzophenone 4, Bp-4) (CAS no. 4065-45-6, purity \geq 97.0) from Fluka (Fluka Chemie GmbH, CH-9471 Buchs, Switzerland).

Stock solutions of the compounds were prepared in absolute ethanol at a concentration of 10⁻² M, stored at -20°C, and diluted to desired concentrations in L-15 (LEIBOVITZ) medium (Gibco, Cat No. 11415-049, Lot No. 3041839). The final ethanol concentrations in the medium did not exceed 1% (v/v). This concentration did not affect cell proliferation (Schlumpf *et al.*, 2001).

Cell line and cell culture conditions. The MDA-kb2 cell line was kindly provided by K. Bobseine and L. E. Gray (Endocrinology Branch, U.S. EPA, Research Triangle Park, NC). For routine maintenance, cells were grown in 25-cm² canted neck tissue culture plastic flasks (Falcon, Oxnard, CA) in Leibovitz's L-15 medium at 37°C in a humidified incubator under regular atmospheric conditions (no CO₂). The medium was supplemented with 10% heat-inactivated (56°C, 30 min) fetal bovine serum (FBS, Lot No. 1077868, Cat No. 16000-044, Life & Technologies, GIBCO, Grand Island, NY), and 1% (v/v) (final concentration) antibiotic-antimycotic agent (Gibco, Cat No. 15240-062, Lot No. 1078238).

AR-mediated gene-reporter activation assay in MDA-kb2 cells. Tests were carried out according to the protocol of Wilson *et al.* (2002) with several modifications. MDA-kb2 cells were trypsinized and seeded into 96-well plates (Costar NY, USA) at a density of about 1 \times 10⁴ cells/well with 100 μ l media/well using a multichannel pipettor. After the cells had attached, medium was removed and replaced by dosing medium. For every experiment, wells in column no. 6 were filled with medium as negative control, wells in column no. 7 with 1% ethanol in medium as solvent control, wells in column no. 12 with 10 nM DHT as a positive control (0.1 or 0.5 nM for testing AR antagonists), and wells in column no. 1 with 1 μ M flutamide or bicalutamide alone (for comparison with the test chemical alone). Wells in columns no. 2-5 and 8-11 were filled with different concentrations of the test chemical, rows 1-4

TABLE 1
Potency of Androgen Receptor and Glucocorticoid Receptor Agonists in the MDA-Kb2 Cell Transcriptional Activation Assay

Chemicals	EC ₅₀ (M) ^a			Relative androgenic potency ^{a,b} (%) μ M
	Agonists alone	Agonists + 1 μ M flutamide	Agonists + 1 μ M hydroxyflutamide	
DHT	1.36 \pm 0.17 \times 10 ⁻¹⁰	4.22 \pm 0.32 \times 10 ^{-10*}	5.11 \pm 1.33 \times 10 ^{-9**}	100
R1881	1.11 \pm 0.09 \times 10 ⁻¹⁰	3.89 \pm 0.32 \times 10 ^{-10*}		128.99 \pm 22.53
Met-T	1.25 \pm 0.32 \times 10 ⁻⁹	3.09 \pm 1.20 \times 10 ⁻⁹	1.25 \pm 0.11 \times 10 ^{-8**}	11.28 \pm 1.30
Danazol	5.55 \pm 0.52 \times 10 ⁻⁹	1.66 \pm 0.19 \times 10 ^{-8 *}	7.48 \pm 0.43 \times 10 ^{-8**}	2.41 \pm 0.26
Androstenedione	7.35 \pm 1.33 \times 10 ⁻⁸	1.69 \pm 0.24 \times 10 ^{-7 *}	2.35 \pm 0.63 \times 10 ^{-6**}	0.21 \pm 0.03
Dexamethasone	1.26 \pm 0.14 \times 10 ⁻⁸	1.23 \pm 0.18 \times 10 ⁻⁸	1.29 \pm 0.23 \times 10 ⁻⁸	

^aMean \pm SEM of five independent experiments.

^bRAP = $\frac{EC_{50}(DHT)}{EC_{50}(test\ compound)} \times 100$.

* and **, different from agonists alone for $p < 0.05$ and $p < 0.01$, respectively.

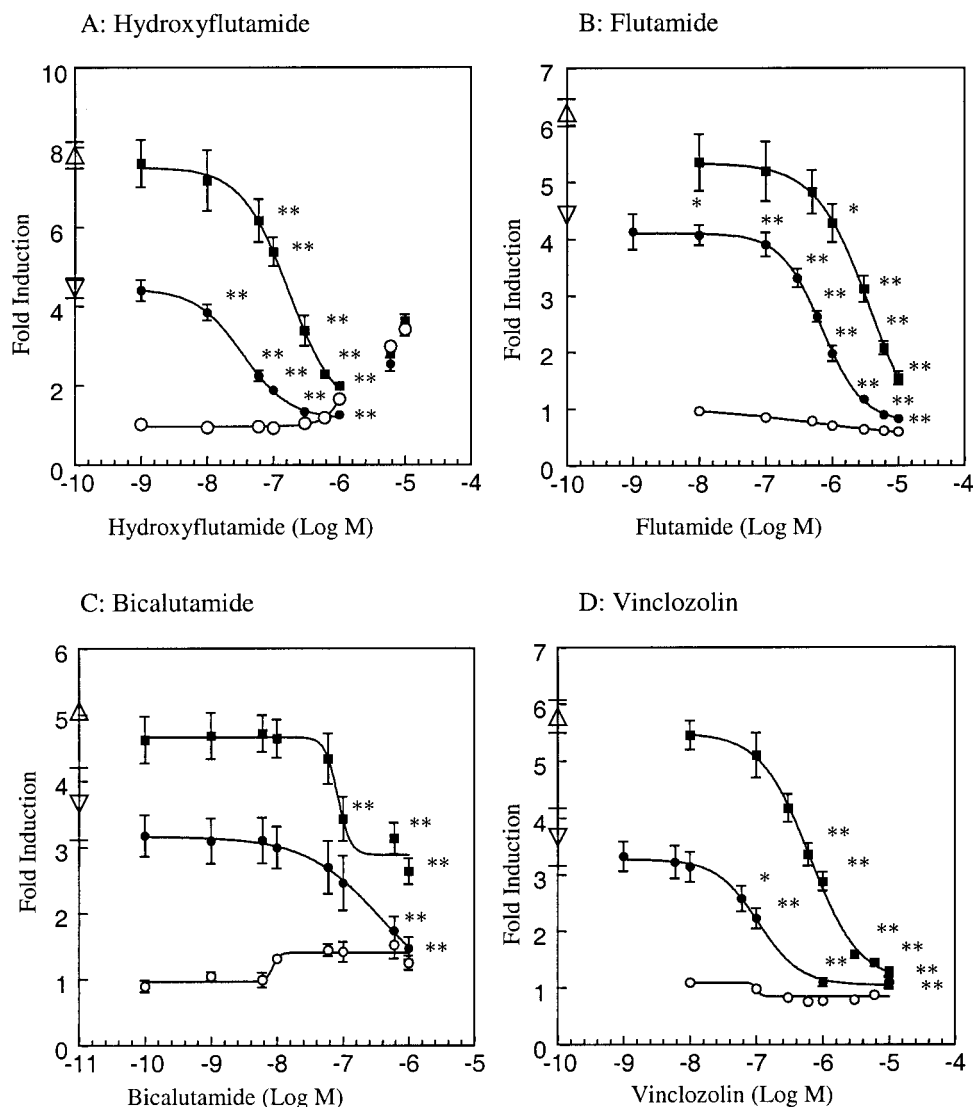


FIG. 2. Effects of androgen receptor antagonists in MDA-kb2 cells. Data are expressed as mean \pm SEM. Open circle, antagonist alone; filled square, antagonist in presence of 0.5 nM DHT; filled circle, antagonist in presence of 0.1 nM DHT; open upward-pointing triangle, 0.5 nM DHT; open downward-pointing triangle, 0.1 nM DHT. * and ** significant difference ($p < 0.05$ and $p < 0.01$) as compared to activation by 0.1 or 0.5 nM DHT, respectively. Ethanol concentrations (v/v): from 1:10⁸ to 1:10³.

TABLE 2
Antiandrogenic Potency of Antiandrogens and UV Filters in MDA-kb2 Cells

Chemicals ^a	IC ₅₀ (in 0.5 nM DHT) (M) ^b	IC ₅₀ (in 0.1 nM DHT) (M) ²
Hydroxyflutamide	2.54 \pm 0.09 \times 10 ⁻⁷ (4)	3.45 \pm 0.20 \times 10 ⁻⁸ (4)
Flutamide	3.62 \pm 0.19 \times 10 ⁻⁶ (4)	7.88 \pm 1.05 \times 10 ⁻⁷ (6)
Bicalutamide	8.30 \pm 0.46 \times 10 ⁻⁸ (4)	3.84 \pm 1.44 \times 10 ⁻⁷ (5)
Vinclozolin	7.92 \pm 2.13 \times 10 ⁻⁷ (5)	1.09 \pm 0.14 \times 10 ⁻⁷ (5)
Bp-3	2.85 \pm 1.18 \times 10 ⁻⁵ (6)	4.98 \pm 0.64 \times 10 ⁻⁶ (6)
HMS	1.31 \pm 0.51 \times 10 ⁻⁵ (6)	5.57 \pm 0.54 \times 10 ⁻⁶ (6)

Note. Inactive UV filters (Fig. 5): Bp-4, 3-BC, 4-MBC, B-MDM, OD-PABA, OMC.

^aChemicals: Bp-3: benzophenone-3; HMS: homosalate; Bp-4: benzophenone 4; 3-BC: 3-benzylidene camphor; 4-MBC: 3-(4-methylbenzylidene) camphor; B-MDM: 4-tert-butyl-4'-methoxy-dibenzoylmethane; OD-PABA: 2-ethylhexyl-4-dimethylamino-benzoate; OMC: 2-ethylhexyl-4-methoxycinnamate.

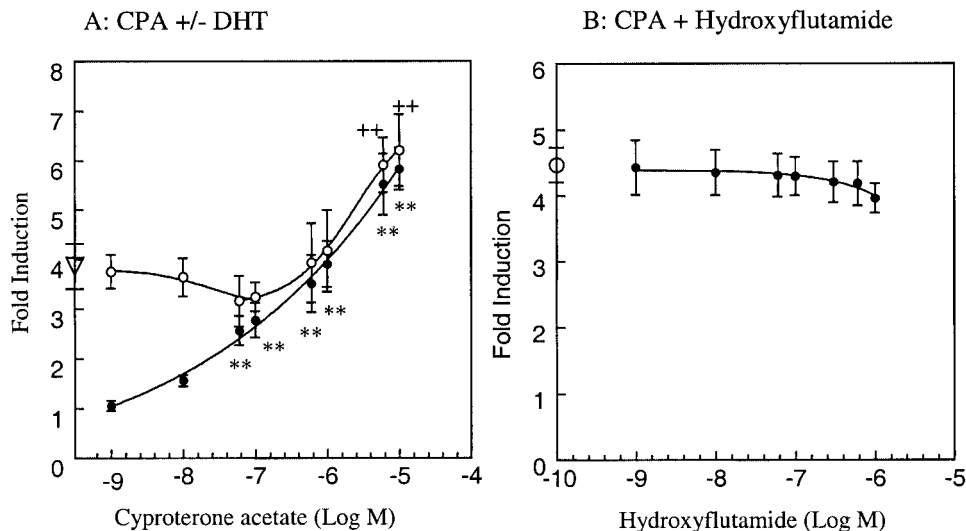
^bMean \pm SEM and number of independent experiments.

together with DHT and rows 5–8 without DHT. Cells were incubated overnight at 37°C.

For measuring luciferase activity, medium was removed. Cells were washed gently two times with Dulbecco's phosphate-buffered saline (PBS) at room temperature. Lysis Buffer (Promega, Cat No. E1351, Lot No. 119684) was added (25 μ l/well), and cells were left at room temperature for 30 min. The contents of the wells (25 μ l/well) were transferred onto a white Dynatech microtiter plate (DYNEX Technologies, Inc., Chantilly, VA). The plate was read in a luminometer ML 1000 [Dynatech Laboratories, Chantilly, VA], with injection set to deliver 25 μ l 1 mM D-luciferin and 25 μ l reaction buffer (25 mM glycylglycine, 15 mM MgCl₂, 5 mM ATP, 0.1 mg/ml BSA, pH 7.8) using a flash-detection program.

MTT reduction assay. Cytotoxicity was estimated with the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye reduction assay as described by Mosmann (1983), with some modifications. Briefly, cells were seeded at 10,000 cells/100 μ l into 96-well flat-bottom culture plates, grown for 24 h, and then treated with the chemicals at the indicated concentrations and time intervals. After incubation with chemicals, 0.1 mg (20 μ l/well of 5 mg/ml in PBS) MTT was added. Plates were further incubated for 4 h at 37°C. The medium was then discarded, 100 μ l of DMSO was added to each well to

FIG. 3. (A) Effect of cyproterone acetate (CPA) on luciferase activity of MDA-kb2 cells in the presence or absence of 0.1 nM DHT. Open circle, CPA in presence of 0.1 nM DHT; filled circle, CPA alone; open downward-pointing triangle, 0.1 nM DHT. Mean \pm SEM of four independent experiments. **Significant difference from negative control ($p < 0.01$). ++Significant difference ($p < 0.01$) as compared to activation by 0.1 nM DHT alone. Ethanol concentrations (v/v): from 1 : 10⁷ to 1 : 10³. (B) Effect of hydroxyflutamide on luciferase activity of MDA-kb2 cells in the presence of 1 μ M CPA. Mean \pm SEM of three independent experiments. Open circle, 1 μ M CPA; filled circle, hydroxyflutamide in presence of 1 μ M CPA. Ethanol concentration (v/v): from 1:10⁷ to 1:10³.



dissolve the formazan crystals formed, and the plate was agitated for 1 min. Absorption at 540 nm (reference filter 620 nm) was quantified with a microplate reader (Anthos labtec reader). MTT-reduction for each treatment was expressed as a percentage of control values.

Data analysis. Results were expressed as mean fold induction compared to negative control (medium) \pm SE of the mean (SEM). Luciferase activities of negative control and solvent control were identical. Data were analysed by two-way ANOVA followed by Bonferroni pairwise comparisons (SYSTAT

5.01 software). Differences between groups were considered statistically significant at $p < 0.05$.

Nonlinear regression and calculation of EC₅₀ and IC₅₀ values were performed with GraphPad Prism 2.01 (GraphPad Software, Inc., San Diego, CA 92121). Mean EC₅₀ or IC₅₀ values of compounds were calculated from the EC₅₀ or IC₅₀ values of individual experiments.

EC₅₀ is the concentration of the agonist producing 50% maximal induction of luciferase activity in MDA-kb2 cells, whereas, IC₅₀ is the concentration of the antagonist producing 50% inhibition of agonist-induced luciferase activity in the cells.

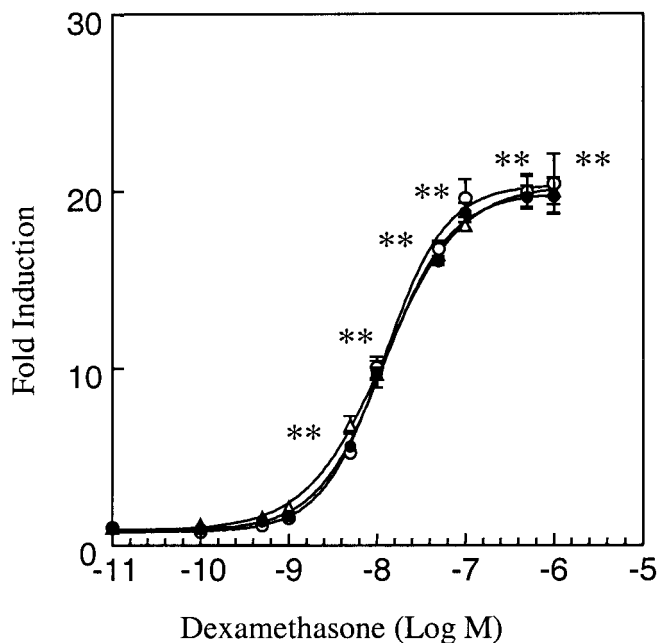


FIG. 4. Effect of dexamethasone on luciferase activity in MDA-kb2 cells in the absence and presence of 1 μ M flutamide and 1 μ M hydroxyflutamide. Mean \pm SEM of five independent experiments. Filled circle, dexamethasone; open circle, dexamethasone in presence of 1 μ M flutamide; open triangle, dexamethasone in presence of 1 μ M hydroxyflutamide. **Dexamethasone without flutamide and hydroxyflutamide, significant difference from negative control ($p < 0.01$). Ethanol concentrations (v/v): from 1:10⁹ to 1:10⁴.

RESULTS

Validation of the MDA-kb2 Cell Assay: Androgen Receptor Agonists

MDA-kb2 cells were exposed to known androgenic chemicals, 5 α -dihydrotestosterone (DHT), methyltrienolone (R1881, metribolone), methyltestosterone (Met-T), danazol, and androstenedione. The five androgens increased luciferase activity of MDA-kb2 cells in a concentration-dependent manner (Fig. 1). DHT and R1881 induced luciferase expression significantly from 0.06 nM and 0.03 nM, respectively (compared with negative control $p < 0.05$). The rank order of EC₅₀ and relative androgenic potency values corresponded to the known potencies of the chemicals (Table 1) (Foster and Cunha, 1999; Wiita *et al.*, 1995).

Effect of Androgen Antagonists on DHT-Induced AR Activation

Nonsteroidal AR antagonists. When administered alone, flutamide, bicalutamide, and the pesticide vinclozolin did not show a detectable effect on luciferase activity in MDA-kb2 cells (Fig. 2). Hydroxyflutamide induced luciferase activity at the highest concentration tested (10 μ M) in the absence or presence of DHT. All four compounds antagonized luciferase activation by 0.1 or 0.5 nM DHT in a concentration-dependent

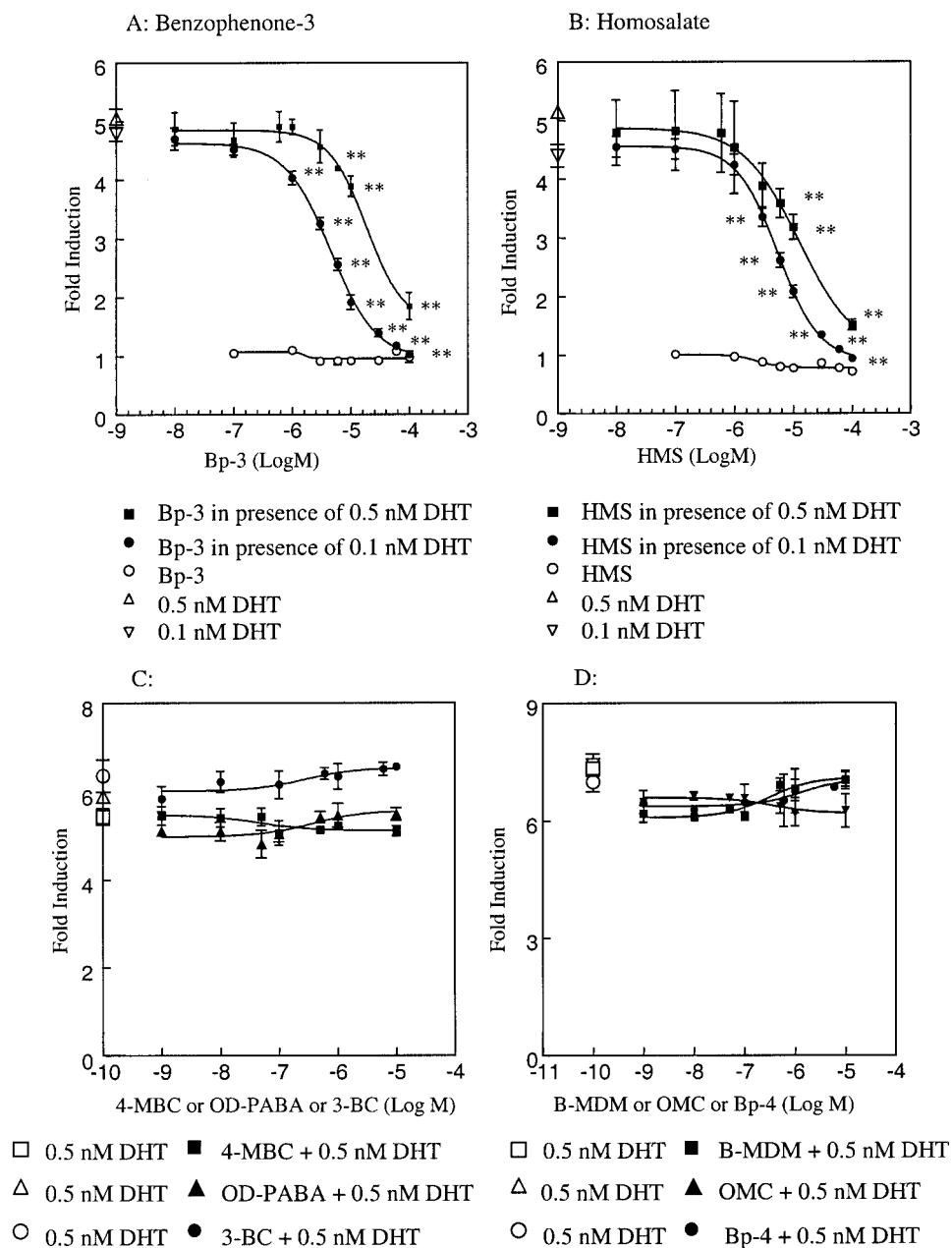


FIG. 5. Antagonism by the UV filters benzophenone-3 (Bp-3) (A) and homosalate (HMS) (B) of DHT (0.1 nM or 0.5 nM)-induced androgen receptor (AR) activation in MDA-kb2 cells and absence of antagonism by other six UV filters (C and D). Mean \pm SEM of six independent experiments. ** significant difference vs. 0.1 nM or 0.5 nM DHT ($p < 0.01$). Ethanol concentrations (v/v): from 1:10⁶ to 1:10².

manner. IC₅₀ values are shown in Table 2. The effect of hydroxyflutamide was significant at and above concentrations of 0.01 μ M or 0.06 μ M (Fig. 2A).

Cyproterone acetate. In contrast to the nonsteroidal AR antagonists, cyproterone acetate (CPA) increased luciferase activity dose-dependently in MDA-kb2 cells when given alone (Fig. 3A). The effect of DHT (0.1 nM) on luciferase activity seemed almost unchanged by low concentrations of CPA; the slight reduction at around 100 nM CPA is statistically not significant. At concentrations above 1 μ M, CPA increased luciferase activity to the same extent as in the absence of DHT

(Fig. 3A). The agonistic effect of 1 μ M CPA was not inhibited by hydroxyflutamide (Fig. 3B).

Glucocorticoid Receptor (GR) Agonist-Induced Luciferase Activation

Since MDA-kb2 cells contain both endogenous AR and GR, compounds that act through either receptor could activate the luciferase reporter. Dexamethasone (DEX) increased luciferase expression in MDA-kb2 cells in a concentration-dependent manner (Fig. 4), with a significant effect at 5 nM and above (compared with negative control $p < 0.01$). The EC₅₀ of

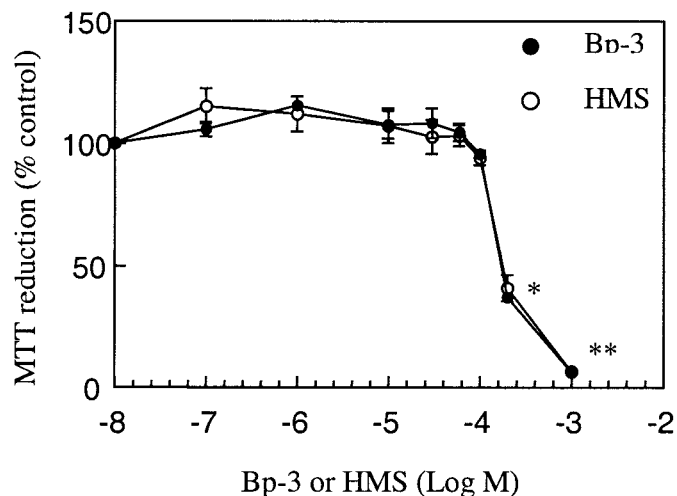


FIG. 6. Concentration-response curves for effects of Bp-3 and HMS on MTT reduction in MDA-kb2 cells. Mean \pm SEM of four independent experiments. * and **, significantly lower than control ($p < 0.05$ and $p < 0.01$).

dexamethasone, 12.6 ± 1.38 nM, was about 100 times higher than that of DHT.

Differentiation between AR and GR agonists. When AR and GR agonists were tested in the presence of $1 \mu\text{M}$ flutamide

or $1 \mu\text{M}$ hydroxyflutamide, both compounds antagonized the increase in luciferase activity induced by androgens, but showed no antagonism against dexamethasone (Table 1, Fig. 4).

Screening of UV Filters for Androgenic or Antiandrogenic Activity

In order to assess possible agonistic or antagonistic actions of UV filters on AR in MDA-kb2 cells, the cells were exposed to eight UV filters in the absence or presence of 0.1 or 0.5 nM DHT. The list of test chemicals included 4-MBC, 3-BC, OD-PABA, B-MDM, OMC, Bp-3, Bp-4, and HMS. None of the compounds displayed agonistic activity (Fig. 5). However, two UV-filters, Bp-3 and HMS, reduced DHT-induced AR activation in MDA-kb2 cells in a concentration-dependent manner (Figs. 5A and 5B; Table 2). As indicated by the MTT reduction assay, this effect was not due to cytotoxicity, which was seen only at and above $200 \mu\text{M}$ (Fig. 6). 4-MBC, 3-BC, OD-PABA, B-MDM, Bp-4, and OMC did not inhibit AR activation by DHT across a wide concentration range (Figs. 5C and 5D). Bp-3 and HMS were also tested with respect to possible interactions with GR activation (Fig. 7). HMS did not change the effect of 50 nM dexamethasone on luciferase activity. High concentrations of Bp-3 induced a small increase in luciferase activity beyond the level produced by dexamethasone alone.

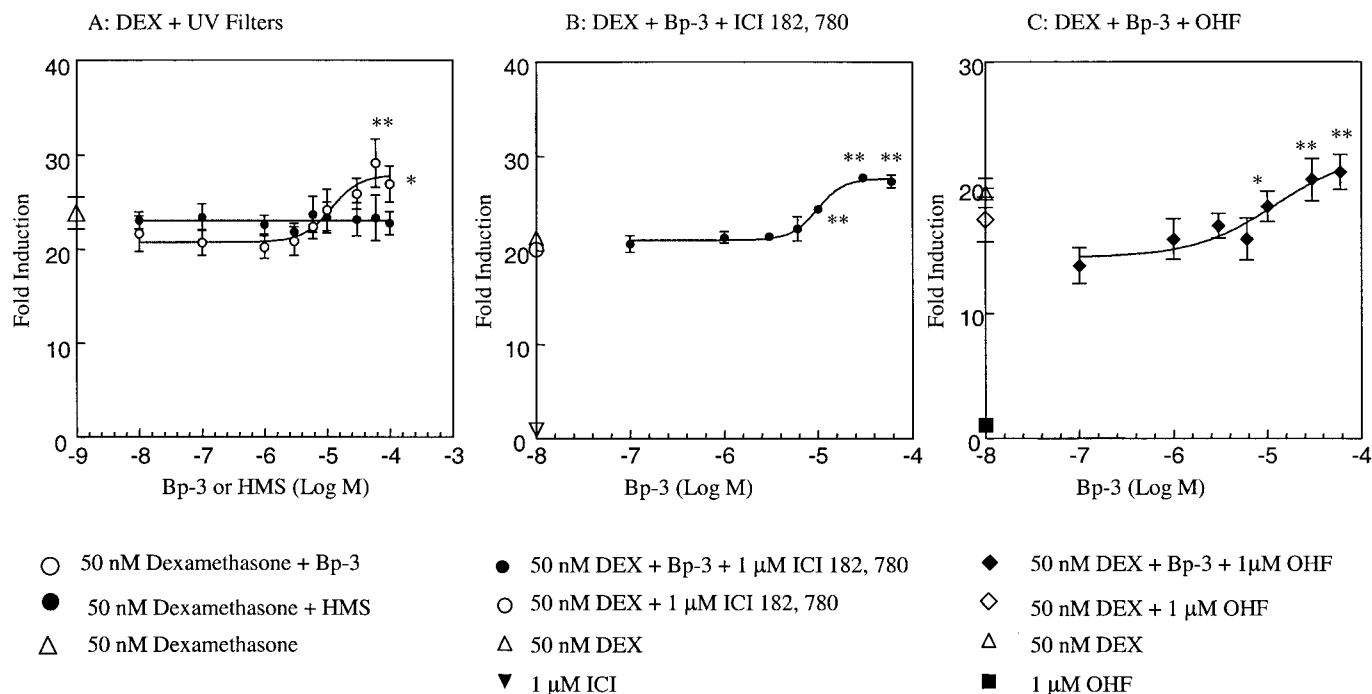


FIG. 7. (A) Effect of Bp-3 and HMS on luciferase activity in MDA-kb2 cells in the presence of 50 nM dexamethasone. Mean \pm SEM of five independent experiments. Ethanol concentrations (v/v): from $1 : 10^6$ to $1 : 10^2$. (B) Effect of Bp-3 on luciferase activity in MDA-kb2 cells in the presence of 50 nM dexamethasone and $1 \mu\text{M}$ ICI 182,780. Mean \pm SEM of five independent experiments. **Significant difference vs. 50 nM dexamethasone and $1 \mu\text{M}$ ICI 182,780 ($p < 0.01$). Ethanol concentrations (v/v): from $1 : 10^6$ to $1 : 10^2$. (C) Effect of Bp-3 on luciferase activity in MDA-kb2 cells in the presence of 50 nM dexamethasone + $1 \mu\text{M}$ hydroxyflutamide. Mean \pm SEM of five independent experiments. * and **: significant difference vs. 50 nM dexamethasone + $1 \mu\text{M}$ hydroxyflutamide ($p < 0.05$ and $p < 0.01$). Ethanol concentrations (v/v): from $1 : 10^6$ to $1 : 10^2$.

This effect was not prevented either by the estrogen antagonist ICI 182,780 nor by hydroxyflutamide (Fig. 7B,C).

DISCUSSION

MDA-kb2 cells represent a cell line that expresses both endogenous human AR and GR and has been stably transfected by Kathy Bobseine with the MMTV-luciferase gene (Wilson *et al.*, 2002). In our validation experiments, AR agonists [5α -dihydrotestosterone (DHT), methyltrienolone (R1881, metribolone), methyltestosterone, androstenedione, and danazol] displayed EC_{50} values and relative androgenic potencies corresponding to the potency range reported in earlier studies (Foster and Cunha, 1999; Wiita *et al.*, 1995). A distinction between AR and GR agonists can be achieved with androgen antagonists such as hydroxyflutamide, which antagonized the effect of DHT on luciferase expression without concomitant antagonism of dexamethasone. These results are comparable to findings of Wilson *et al.* (2002). The data indicate that the cell line represents a sensitive tool for the screening of AR agonists.

The assay likewise proved to be suitable and sensitive for the detection of AR antagonistic effects of nonsteroidal antiandrogens such as hydroxyflutamide, flutamide, bicalutamide, and vinclozolin (Bauer *et al.*, 1998; Gray *et al.*, 1994; Kelce *et al.*, 1994; Waller *et al.*, 1996; Wong *et al.*, 1995). Vinclozolin is converted *in vivo* into two metabolites, M1 and M2, with higher antiandrogenic activity than the parent compound (Kelce *et al.*, 1994). The IC_{50} value of the parent compound observed in the present study is in the range of the activity reported by Wilson and coworkers (2002).

A complex situation was encountered with cyproterone acetate (CPA). CPA was characterized by marked agonistic activity across a wide concentration range when given alone, a slight, nonsignificant tendency of DHT antagonism at low CPA concentrations ($0.1\mu\text{M}$), and an identical dose-response relationship at high CPA concentrations in the presence or absence of DHT. CPA is known to possess significant partial AR agonist activity (Labrie *et al.*, 1987). However, the failure by hydroxyflutamide to reduce its agonistic effect speaks against a major role of AR for the agonistic CPA action in the present cell system. The effect might result from an action on the GR expressed by MDA-kb2 cells; CPA has been reported to exhibit glucocorticoid activity (Lamberts *et al.*, 1988; Poulin *et al.*, 1991). A predominance of agonistic effects of CPA was also observed in MCF7-AR1 cells expressing the human AR (A-SCREEN assay) (Ma, 2002). Thus, antiandrogens with mixed activities may present problems for analysis in both assay systems.

As mentioned in the introduction, the use of UV filters has greatly increased over the last decades. Since they are lipophilic, they may bioaccumulate in the food chain. Evidence for this has been presented for fish (perch and roach in the Meerfelder Maar lake, Germany; Nagtegaal *et al.*, 1997), and

for human milk (Hany and Nagel, 1997). The presence in human milk has recently been confirmed for one UV filter in this laboratory (unpublished observations). When analyzed for potential endocrine activity, 4-MBC, OD-PABA, OMC, Bp-3, and HMS exhibited estrogenic activity *in vitro* on MCF-7 cells (increased proliferation, pS_2 protein induction). 4-MBC, OMC, and Bp-3 also increased uterine weight in immature rats (Schlumpf *et al.*, 2001). Two of these compounds, benzophenone-3 (Bp-3) and homosalate (HMS), also showed significant androgen (DHT) antagonism *in vitro* in MDA-kb2 cells. Both chemicals were completely devoid of agonistic actions when tested alone. The IC_{50} values of the two UV filters were in the low micromolar range, which is the effective range of other environmental antiandrogenic chemicals such as vinclozolin and linuron (Vinggaard *et al.*, 1999; Wilson *et al.*, 2002). While in this cell line, HMS appeared only to antagonize androgen actions and did not interfere with glucocorticoid effects, high concentrations of Bp-3 elicited an unexpected increase of luciferase activity in the presence of dexamethasone, beyond the level induced by the glucocorticoid. Since hydroxyflutamide and the estrogen antagonist ICI 182,780 were unable to block this effect, its nature remains uncertain.

In conclusion, our investigation identified two out of eight UV filters tested, Bp-3 and HMS, as antiandrogens in the *in vitro* MDA-kb2 cell transcription assay. Both compounds also act as estrogen agonists on MCF-7 cells *in vitro*, and Bp-3 is estrogenic in the *in vivo* uterotrophic assay (Schlumpf *et al.*, 2001). *In vitro* estrogenic potencies (ED_{50} $3.73\mu\text{M}$ for Bp-3 and $1.56\mu\text{M}$ for HMS) and antiandrogenic potencies (Table 2) are in a similar range, but such comparisons should be done with caution, considering the different endpoints of the two assays (proliferation versus reporter gene). Our data support the notion that some endocrine disrupting chemicals may interact with endocrine regulation by more than one mechanism (Sohoni *et al.*, 1998). Whether Bp-3 and HMS are also AR antagonists *in vivo* remains to be clarified.

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