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Antitumor Activity of Antiestrogenic Phenylindoles on Experimental Prostate Tumors*

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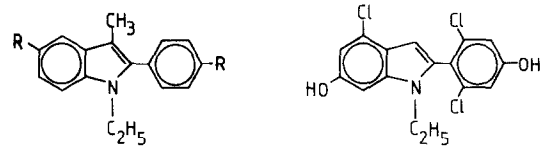
Abstract—*Two antiestrogenic phenylindoles (D* 16726 *and D* 15413) *were tested for their prostatic tumor-inhibiting activity. Both compounds exerted a strong inhibitory effect on prostate and seminal vesicle weight of intact rats and mice comparable to that of diethylstilbestrol. Their estrogenic properties, however, are much lower than those of DES. Therefore, there is no direct correlation between estrogenic potency and inhibition of accessory sex organ weights. The tumor-inhibiting activity of D* 16726 *and D* 15413 *on the androgen-dependent R* 3327 *Dunning prostatic carcinoma and the human prostatic tumor PC* 82 *implanted in nude mice equals that of castration or of diethylstilbestrol. Both* 2*-phenylindoles had good affinities for estrogen receptors from calf uterine and R* 3327 *tumor cytosol, but no affinities for androgen and progesterone receptors. As these* 2*-phenylindoles have much lower estrogenic properties than diethylstilbestrol, they may also have low side-effects, and can therefore be of interest for the therapy of the prostatic carcinoma.*

INTRODUCTION

THE THERAPY usually applied for the disseminated prostatic carcinoma is androgen ablation [1–3]. This can be achieved by various treatment modalities like castration or the administration of estrogens, antiandrogens or LHRH analogs [1–5]. Diethylstilbestrol (DES), a non-steroidal estrogen, is still very often used to treat prostatic cancer [3]. However, this therapy is associated with severe side-effects like gynecomastia and cardiovascular complications [1].

In the prostatic carcinoma as well as in the normal prostate receptors for androgens and for estrogens are present [1, 2]. The occurrence of estrogen receptors (ER) in prostate tumors was one of the reasons for studying the effect of the antiestrogen tamoxifen against this disease [1, 6]. In several studies on human and experimental prostatic tumors, tamoxifen exerted certain tumor-inhibiting effects [1, 6]. However, there are controversial results about its effects on gonadotropin and testosterone levels [7, 8].

In view of the severe side-effects of DES caused by its estrogenic properties, the use of suitable antiestrogens for the treatment of the prostatic carcinoma which might have similar antitumor effects, but lower side-effects than DES, might offer a more acceptable alternative to conventional hormone therapy. As we have developed many new antiestrogens of different chemical structures during the last few years [9], we have evaluated the potential prostatic tumor inhibiting properties of two 2-phenylindoles (D 16726 and D 15413). These compounds (Fig. 1) have reasonable affinities to the ER, low estrogenicity and partial antiestrogenic activity, and exerted strong tumor-inhibiting effects on several hormone-dependent experimental mammary tumors [10–14].



R = OCOCH3 : D 16726
= OH : D 15414

D 15413

Fig. 1. *Structures of D* 16726, *D* 15414 *and D* 15413.

In this paper, we have tested D 16726 and D 15413 for their growth inhibitory effects on prostate and seminal vesicle weights of intact mature rats and mice and for their antitumor activity on the hormone-dependent R 3327 Dunning prostatic carcinoma as well as on the androgen-dependent human prostatic tumors PC 82 and PCEW

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Dedicated to Prof. Dr. Dr. N. Brook on his 75th birthday. ?

implanted in athymic nude mice. To get more insight into the pharmacology of these compounds, their potential antiandrogenic properties in castrated rats and mice and their affinities for androgen receptors (AR), ER and progesterone receptors (PR) in various cytosol preparations were determined.

MATERIALS AND METHODS

Chemicals

D 16726 (MG 351.4), D 15414 (MG 267.3) and D 15413 (MG 256.6) were synthesized in the authors' laboratory as previously described [10, 12, 13]. DES (MG 268.3), testosterone, testosterone propionate, progesterone and triamcinoline acetonide were purchased from Sigma, Steinheim, F.R.G. CPA was generously provided by Schering AG, Berlin, F.R.G. [2,4,6,7-^{3}H]Estradiol (90–115 Ci/mmol), [17α-methyl-^{3}H]methyltrienolone (R 1881, 70–87 Ci/mmol), and [17α-methyl-^{3}H]promegestone (R 5020, 70–87 Ci/mmol) were purchased from New England Nuclear, Dreieich, F.R.G.

Growth inhibitory effect on accessory sex organ weights

Mature male SD rats (180–200 g at the start of the test, age: 9–10 weeks) and mature male NMRI mice (23–25 g, age: 8–9 weeks), both from Ivanovas, Kissleg, F.R.G., were used [15–18]. In the experiments with castrated rats or mice, animals were castrated via the scrotal route under ether anesthesia. The dose of testosterone propionate (TP) to restore accessory sex organ weights in castrated animals to intact levels was determined in previous experiments. According to these results, the dose was 0.1 mg TP for rats in 0.2 ml and 0.3 mg TP for mice in 0.1 ml of olive oil. Intact and castrated rats and castrated mice were injected for 9 consecutive days, intact mice for 4 days. Twenty-four hours after the last injection, blood was drawn (intact animals) by cardiac puncture under ether anesthesia. Prostates and seminal vesicles were removed, dissected free from adhering fat and tissue, blotted dry (prostates) or dried overnight at 100° C (seminal vesicles) and weighed. The plasma of five rats (one sample/group) and of three mice (two samples/group) was pooled and assayed for testosterone level in duplicate.

Testosterone radioimmunoassay

Testosterone was assayed in plasma after centrifugation using a direct double antibody radioimmunoassay kit purchased from DRG, Marburg, F.R.G.

Estrogenic activity

The uterotrophic activity was determined in the immature mouse uterine weight test [10, 19, 20].

Androgen-dependent R 3327 prostate carcinoma

Male Copenhagen × Fisher F_1 rats bearing the androgen-dependent R 3327 Dunning rat adenocarcinoma were kindly provided by Dr. N. Altman, Papanicolaou Cancer Research Institute, Miami, U.S.A. [6, 21]. Seventy to 73 days after tumor implantation, they were randomly distributed into groups of seven to eight rats (average tumor area/rat = 51.8 ± 6.7 mm^2). Rats were injected with the test compounds dissolved in olive oil three times a week s.c., castration was performed on day 1 of treatment. Tumor area was determined weekly by calculating the product of caliper measurements made in two perpendicular diameters. At the end of therapy, blood was drawn by cardiac puncture under ether anesthesia. Tumors, seminal vesicles and prostates were removed and dissected free from adhering tissue and fat. Plasma of each animal was assayed individually for testosterone level in duplicate.

PC 82 and PC EW human prostatic carcinoma in nude mice

Male nude mice of BALB/c origin were transplanted with one 1 × 1 × 2 mm particle of PC 82 or PC EW tumor at the age of 4–5 weeks. At the start of treatment, mice were randomly distributed into groups of six to eight animals (one tumor/mouse with a diameter of 4–6 mm) and injected with the test compound dissolved in olive oil s.c. daily for 20 days. Tumor diameter was determined by calculating the average caliper measurements made in two perpendicular diameters.

Histology

Paraffin-embedded sections of R 3327 tumors were stained with hematoxylin and eosin and treated by the periodic acid Schiff staining method for histologic examination.

Binding affinities for steroid hormone receptors

ER-affinities were determined with calf uterine cytosol and with cytosol of R 3327 prostatic tumor [6, 10, 11, 19, 20]. The prostate cytosol of 24 hr castrated rats, R 3327 tumor cytosol and calf uterine cytosol were used as the source of AR [6, 22–26]. PR-affinities were determined with calf uterine cytosol [27]. Tissues were rinsed in ice-cold 0.9% NaCl solution, cleaned, minced and homogenized in 1 vol. of the relevant buffer (10 mM Tris, 1.5 mM EDTA, 3 mM NaN_3, pG 7.4 for ER, 10 mM Tris, 1.5 mM EDTA, 20 mM Na_2MoO_4, glycerol 10%, pH 7.4, for AR, 10 mM Tris, 1.5 mM EDTA, 0.25 M sucrose, pH 7.4, for PR). The homogenate was centrifuged at 9000 ***g*** for 10 min, and the supernatant was centrifuged at 105,000 ***g*** for 90 min. Relative binding affinities (RBA) were determined by the dextran-coated charcoal (DCC)

method: 100 µl aliquots of the cytosols were incubated with 100 µl (1 nM) of [^{3}H]estradiol (ER), [^{3}H]R 1881 (AR) or [^{3}H]R 5020 and different concentrations of the test compounds 0–4° C for 16 hr (ER) or 2 hr (AR, PR). Non-specific radioligand binding was determined by a parallel incubation containing 20 µM of estradiol (ER), testosterone (AR) or progesterone (PR). After incubation, dextran-coated charcoal suspension (0.625% dextran 80,000, 1.25% Norit A in the relevant buffer) was added for 90 min (ER) or 10 min (AR, PR) at 0–4° C. After centrifugation for 10 min at 800 ***g***, the radioactivity of a 200 µl supernatant aliquot was counted. The percentage of bound radioligand was plotted against the concentration of unlabeled test compounds. A standard curve for unlabeled estradiol, testosterone or progesterone was included in each assay. Four to six concentrations of each competitor were tested. They were chosen to provide a linear portion on a semilog plot crossing the point of 50% competition. From this plot, the molar concentrations of unlabeled standards and of test compounds reducing radioligand binding by 50% were determined. As the preparation of a sufficient amount of prostate cytosol requires too many animals, we have developed an assay with calf uterine cytosol. The RBA values of dihydrotestosterone, testosterone and progesterone were very similar in calf uterine and in rat prostate cytosol (data not shown).

Statistics

The significance of the difference between two means was evaluated on the basis of the *U*-test according to Wilcoxon, Mann and Whitney.

RESULTS

Growth inhibitory effect on accessory sex organ weight

D 16726 and D 15413, administered in equimolar doses to DES, had a strong and highly significant effect on prostate and seminal vesicle weight of the intact mature rat which was identical to that of DES and only slightly lower than that of castration at all doses used (Table 1). In the 100 µg dose, the testosterone levels of the D 16726 and DES treated animals were below the detection limit of the applied RIA.

The 2-phenylindoles as well as DES also exerted a strong, dose-dependent and significant inhibitory activity on the seminal vesicle weight and on testosterone levels of the intact, mature mouse (Table 2). In the 100 µg dose, D 16726 and D 15413 were only slightly less inhibiting than DES. Lowering the dose to 10 µg led to a superiority of DES compared to D 16726. However, the effect of 13.3 µg of D 16726 is comparable to a 1 µg dose of DES.

Estrogenic activity

The inhibitory activity of compounds like DES on accessory sex organs of intact animals is thought to be mainly exerted indirectly via pituitary by suppressing the LH-release due to their estrogenic potencies [2, 3]. Therefore, we have determined the estrogenic activity of DES, D 16726 and D 15413 in the immature mouse uterine weight test [10, 19, 20]. Compared to the strong estrogen DES, D 16726 and D 15413 have only very low uterotrophic properties (Table 3). D 16726 and D 15413 reach the estrogenic effect exerted by DES at the 0.1 µg dose only at a dose of 625 or 25 µg, respectively. Thus, the uterotrophic potency of D 16726 is more than 5000 times and that of D 15413 is about 250 times lower than that of DES. As the inhibitory potency on accessory sex organs of D 15413 is nearly identical and that of D 16726 is about one tenth lower than that of DES (Table 2), there is no direct correlation between the inhibitory activity on prostate and seminal vesicle growth of these 2-phenylindoles and their estrogenic potency.

Prostatic tumor inhibiting effects

The tumor inhibiting effect was tested on the hormone-dependent R 3327 Dunning prostatic tumor of the rat [6, 21]. All treatment modalities led to a strong and highly significant decrease in tumor growth (Table 4). At the 16 mg/kg dose, D 16726 exerted the best tumor inhibiting activity of all groups. The antitumor effect of 16 mg/kg of D 15413 was comparable to that of 2 mg/kg of DES (Table 4). The marked body weight loss under therapy with DES and the phenylindoles was surprising, as we have not found any significant weight loss in mammary tumor experiments with female rats and mice. That is why this weight loss is not due to toxic, but to certain endocrine effects. Therefore, in a further experiment (data not shown), the dose of D 16726 was lowered to 3 × 4 mg/kg/week. The inhibiting effect of D 16726 on tumor area (%T/C = 4) was still at least as pronounced as in the castration group (%T/C = 10), whereas the body weight loss compared to the control was lower (-16%) after 8 weeks of therapy. The %T/C values determined by the tumor weight at the end of therapy correspond very well to those of the final tumor area. In all treatment groups, prostate and seminal vesicle weights were strongly reduced. The greatest decrease was determined in the castration group (Table 4). The testosterone levels were also diminished below the detection level.

The tumor-inhibiting effect of D 16726 was further tested in the human prostatic carcinomas PC 82 and PC EW implanted in nude mice [28–30]. At a dose of 6 × 4 mg/kg/week, D 16726 led to a strong decrease in tumor growth after 20 days of therapy in the PC 82 model (Fig. 2). The effect of

*Table 1. Inhibitory effect of D 16726, D 15413 and DES on the seminal vesicle and prostate weight and on the testosterone plasma level of the intact mature rat**

Group	Dose† (mg)	Mean prostate (mg)	Relative prostate weight‡	Mean seminal vesicle (mg)	Mean relative seminal vesicle weight‡	Testosterone plasma level (ng/ml)	Final body weight (g)
Control	—	156	56.0 ± 10.8	101	36.2 ± 6.9		279 ± 6
Castrated§	—	31‖	10.9 ± 1.6‖	27‖	9.4 ± 1.7‖		282 ± 15
D 16726	6.5	42‖	20.3 ± 4.4‖	28‖	13.6 ± 2.1‖	ND	207 ± 6
	1.3	58‖	24.8 ± 4.5‖	35‖	15.0 ± 2.5‖		236 ± 21
D 15413	6.5	41‖	17.0 ± 2.4‖	30‖	12.6 ± 1.9‖		240 ± 16
	1.3	47‖	19.5 ± 4.4‖	35‖	14.5 ± 1.6‖	238 ± 16	
DES	1.0	63‖	27.2 ± 2.9‖	34‖	14.6 ± 9.0‖		234 ± 17
Control	—	159	65.8 ± 3.0	75	31.3 ± 6.0	1.6	242 ± 12
D 16726	0.13	48‖	21.2 ± 16.0‖	17‖	7.3 ± 7.0‖	< 0.1	230 ± 6
D 15413	0.13	64‖	26.3 ± 13.6‖	32‖	12.9 ± 12.2‖	0.4	245 ± 7
DES	0.10	40‖	21.7 ± 8.4‖	21‖	11.1 ± 3.0‖	< 0.1	188 ± 6
CPA	1.0	107	47.7 ± 15.8	55	24.5 ± 9.6	1.2	225 ± 5
Control	—	199	71.6 ± 12.4	81	29.3 ± 1.9	1.7	277 ± 9
Castrated§	—	32	12.3 ± 2.4‖	16‖	6.1 ± 1.5‖	< 0.1	260 ± 10
CPA	5.0	81	331 ± 0.6‖	28‖	11.4 ± 2.5‖	0.6	244 ± 17
	10.0	77	35.4 ± 7.8‖	18‖	8.1 ± 1.4‖	0.6	219 ± 9

*Compounds were administered daily for 9 days s.c. (five rats/group). Organs were removed at day 10.
†Dose per animal and day; doses of D 16726 and D 15413 are equimilar to DES.
‡Relative organ weight = organ wt (mg)/body wt (g) × 100. Mean ± S.D.
§Castrated on day 1 of treatment.
‖Significant ($P < 0.01$).
ND = not determined.

*Table 2. Inhibitory effect of D 16726, D 15413 and DES on the seminal vesicle weight and on the testosterone plasma level of the intact mature mouse**

Group	Dose† (μg)	Mean seminal vesicle weight (mg)	Relative seminal vesicle weight‡	Testosterone plasma level (ng/ml)	Final body weight (g)
Control	—	33	114.0 ± 12.7	4.3	28.6 ± 1.4
D 16726	1310	10§	36.4 ± 10.1§	0.3	27.5 ± 1.5
	131	15§	53.4 ± 4.9§	0.6	28.6 ± 1.5
D 15413	1330	10§	35.6 ± 6.1§	0.1	27.4 ± 1.3
	133	17§	59.2 ± 7.0§	0.4	29.3 ± 1.1
DES	100	11§	38.4 ± 8.8§	0.6	27.8 ± 1.4
Control	—	26	90.7 ± 9.7	4.0	29.3 ± 3.5
D 16726	13.1	15§	55.4 ± 14.1§	1.6	27.7 ± 2.9
D 15413	13.3	12§	43.0 ± 6.2§	0.9	28.3 ± 1.5
DES	10.0	9§	30.3 ± 7.7§	1.0	31.7 ± 3.7
	1.0	15§	49.2 ± 6.0§	2.2	29.7 ± 3.9

*Compounds were administered daily for 4 days s.c. (six to seven mice/group). Organs were removed at day 5.
†Dose per animal and day; doses of D 16726 and D 15413 are equimilar to DES.
‡Relative seminal vesicle weight = organ wt (mg)/body wt (g) × 100. Mean ± S.D.
§Significant ($P < 0.01$).

D 16726 was even slightly better than that of castration. In the PC EW model, the antitumor activity of D 16726 was similar to that in the PC 82 tumor and comparable to that of castration (data not shown).

*Table 3. Estrogenic activity of D 16726, D 1543 and DES in the immature mouse uterine weight test**

Group	Dose† (μg)	Estrogenic activity‡
D 16726	1	16.4 ± 3.6
	5	19.6 ± 1.7
	25	21.0 ± 1.7
	125	30.5 ± 6.5
	625	41.6 ± 7.9
Solvent	—	16.3 ± 3.9
D 15413	1	21.5 ± 3.9
	5	22.2 ± 2.4
	25	45.8 ± 7.6
	125	40.6 ± 1.5
Solvent	—	19.3 ± 3.4
DES	0.01	15.9 ± 3.5
	0.04	29.5 ± 5.2
	0.1	44.9 ± 5.4
	0.4	49.8 ± 7.0
	1.0	55.9 ± 8.2
Solvent	—	12.5 ± 3.5

*Compounds were administered on 3 consecutive days s.c. (seven mice/group). Uteri were removed at day 4.
†Dose per animal and day.
‡Estrogenic activity = uterine dry wt (mg)/body wt (g) × 100. Mean ± S.D.

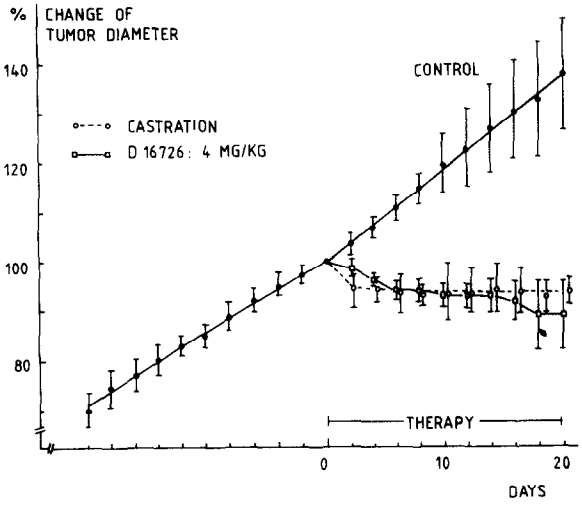

Fig. 2. Effect of D 16726 (4 mg/kg) and castration on growth of the human, androgen-dependent prostatic carcinoma PC 82 implanted in nude mice. Compounds were administered daily s.c. Tumors were termed 100% at a diameter of 4–6 mm.

Histology of the R 3327 prostatic tumor

All tumors of the control animals showed a consistently similar histological structure, which was identical to that described [21]. Castration led to significant changes in tumor morphology. A considerable increase in stroma, but especially in the amount of collagen fibers, was seen (Fig. 3). The size of glandular acini was generally reduced. The epithelial height and secretory activity of the acini were diminished. Application of DES caused a complete change in tumor structure. Acini of various sizes and shapes were separated by broad strands of edematous stroma. The epithelial height of the acini was significantly reduced and quite frequently a squamous metaplasia of the epithelium was observed. In many acini distinct vacuolation of the supranuclear cytoplasm was also seen as a result of lipid storage. Treatment with D 16726 resulted in severe damage of the tumor tissue. Glandular acini showed a wide variation in size and shape. Many were filled by a homogeneous eosinophilic mass probably due to degenerating epithelial cells. The glandular epithelium was highly vacuolated and showed a pronounced degeneration. The chromatin of stromal nuclei also appeared highly consensed and signs of necrosis could be frequently observed in the stromal cells.

Table 4. Effect of D 16726, D 15413, DES and castration on growth of the R 3327 Dunning prostate carcinoma on prostate, seminal vesicle and body weight, and on testosterone level

	Dose (mg/kg)	Tumor wt* (g) median (range)	%T/C*	Tumor area* (mm^2)	%T/C	Seminal vesicle weight** (mg)	Prostate weight** (mg)	Testosterone level* (ng/ml)	Final body weight§ (g)
Control	—	4.35 (1.61–13.80)	100	647 ± 100	100	400 ± 83	468 ± 81	2.6 ± 1.2	350 ± 24
D 16726	8.0	0.74 (0.11–3.03)	17‖	110 ± 59	17‖	71 ± 6‖	33 ± 8‖	<0.1‖	265 ± 20‖
	16.0	0.47 (0.14–4.68)	11‖	83 ± 89	13‖	69 ± 12‖	20 ± 10‖	<0.1‖	250 ± 31‖
D 15413	16.0	0.88 (0.05–4.14)	20‖	138 ± 84	21‖	79 ± 18‖	33 ± 10‖	<0.1‖	280 ± 17‖
DES	2.0	0.68 (0.00–5.78)	16‖ 16‖	112 ± 73	17‖	63 ± 8‖	17 ± 11‖	<0.1‖	258 ± 29‖
Castration	—	0.69 (0.06–3.1)	1	84 ± 59	13‖	50 ± 10‖	12 ± 5‖	<0.1‖	335 ± 15‖

Compounds were administered three times weekly for 6 weeks s.c. (eight to nine rats/group; two tumors/rat).
*Determined after 6 weeks of therapy. %TC = value of treatment group/control group × 100.
†Dry wt.
‡Wet wt.
§Body wt minus tumor wt.
‖Significant ($P < 0.01$).

*Table 5. Antiandrogenic effect of D 16726, D 15413, DES and and CPA on the seminal vesicle and prostate weight of the 5 day castrated, TP-stimulated mature rat**

Group	TP† (mg)	Dose‡ (mg)	Mean prostate weight (mg)	Relative prostate weight§	Mean seminal vesicle weight (mg)	Relative seminal vesicle weight§	Final body weight (g)
Standard	0.1	—	106	41.0 ± 1.4	58	22.5 ± 1.6	261 ± 4
Castrated	—	—	17	7.1 ± 0.5	15	6.4 ± 0.6	240 ± 9
D 16726	0.1	1.0	101	45.2 ± 2.4	63	28.4 ± 2.8	223 ± 4
D 15413	0.1	1.0	101	43.5 ± 0.5	48	20.7 ± 1.6	233 ± 8
Intact control	—	—	119	44.3 ± 4.8	76	28.1 ± 4.0	270 ± 7
Standard	0.1	—	146	44.5 ± 10.0	132	40.0 ± 8.5	330 ± 17
Castrated	—	—	27	9.1 ± 0.5	24	7.8 ± 1.2	301 ± 14
DES	0.1	1.0	116	49.3 ± 5.6	101	42.6 ± 1.7	238 ± 19
CPA	0.1	1.0	58‖	17.1 ± 3.8‖	38‖	12.8 ± 2.7‖	292 ± 15

*Compounds were administered daily for 9 days s.c. (five rats/group). Organs were removed at day 10.
†TP = testosterone propionate (dose: 0.1 mg/animal/day s.c.).
‡Dose per animal and day.
§Relative = organ wt (mg)/body wt (g) × 100. Mean ± S.D.
‖Significant ($P < 0.01$).

Antiandrogenic effect

The determination of the antiandrogenic effect in castrated, testosterone-substituted animals offers the possibility of detecting a direct antagonistic activity at the target level. In our tests, cyproterone acetate (CPA) exerted a strong inhibiting effect on prostate and seminal vesicle weight, whereas the estrogen DES and the 2-phenylindoles D 16726 and D 15413 had no effect (Table 5).

As differences in antiandrogenic activity of estrogens between rats and mice have been described [31], we tested the phenyindoles also in castrated, mature, testosterone-substituted mice. Similar to the assay in rats, CPA exerted a strong inhibiting effect on seminal vesicle growth. Unexpectedly, DES as well as D 16726 and D 15413 exhibited a certain, but still significant antiandrogenic effect (Table 6). However, this effect was less pronounced than that of CPA and almost disappeared at low doses which were comparable to the effective doses used in intact mice.

Affinities for steroid hormone receptors

D 16726, its hydroxy-substituted analog D 15414, D 15413 (Fig. 1) as well as DES did not show any affinity for AR from castrated rat prostate, calf uterine or R 3327 prostatic tumor cytosol, whereas CPA had affinities in all three cytosol preparations (Table 7).

The 2-phenylindoles exerted good affinities for ER from calf uterine as well as R 3327 tumor cytosol. The hydroxy-substituted compound D 15414 had a higher RBA value than its acetoxy-substituted analog D 16726 and the chlorinated compound D 15413 (Table 7). In contrast to CPA, the 2-phenylindoles had only low (D 153413) or nearly no affinity (D 16726, D 15414) to PR (Table 7). Therefore, a progestomimetic activity of these compounds is unlikely.

DISCUSSION

The results shown above clearly demonstrate that compounds like D 16726 and D 15413 with only low estrogenic properties can exert strong inhibitory effects on the growth of the accessory sex organs of rats and mice to nearly the same extent as did the potent estrogen DES (Table 1, 2). Therefore, partial antiestrogens can exert androgen depriving effects similar to estrogens. This offers the possibility of using such antiestrogens, which may have lower side effects than DES, for the treatment of the prostatic carcinoma. In the R 3327 prostatic tumor model, D 16726 and D 15413 inhibited the tumor growth in a 4, 8 or 16 mg/kg dose to the same extent as did a 2 mg/kg dose of DES or castration (Table 4). These results were confirmed for D 16726 in two human, hormone-dependent prostatic tumors implanted in nude mice (Fig. 2).

The mechanism of the growth inhibiting effect on accessory sex glands and experimental prostate tumors of these 2-phenylindoles is not yet exactly known. D 16726 and D 15413 reduced the testosterone levels in intact rats and mice as much as did DES (Table 1, 2). Therefore, a major part of their

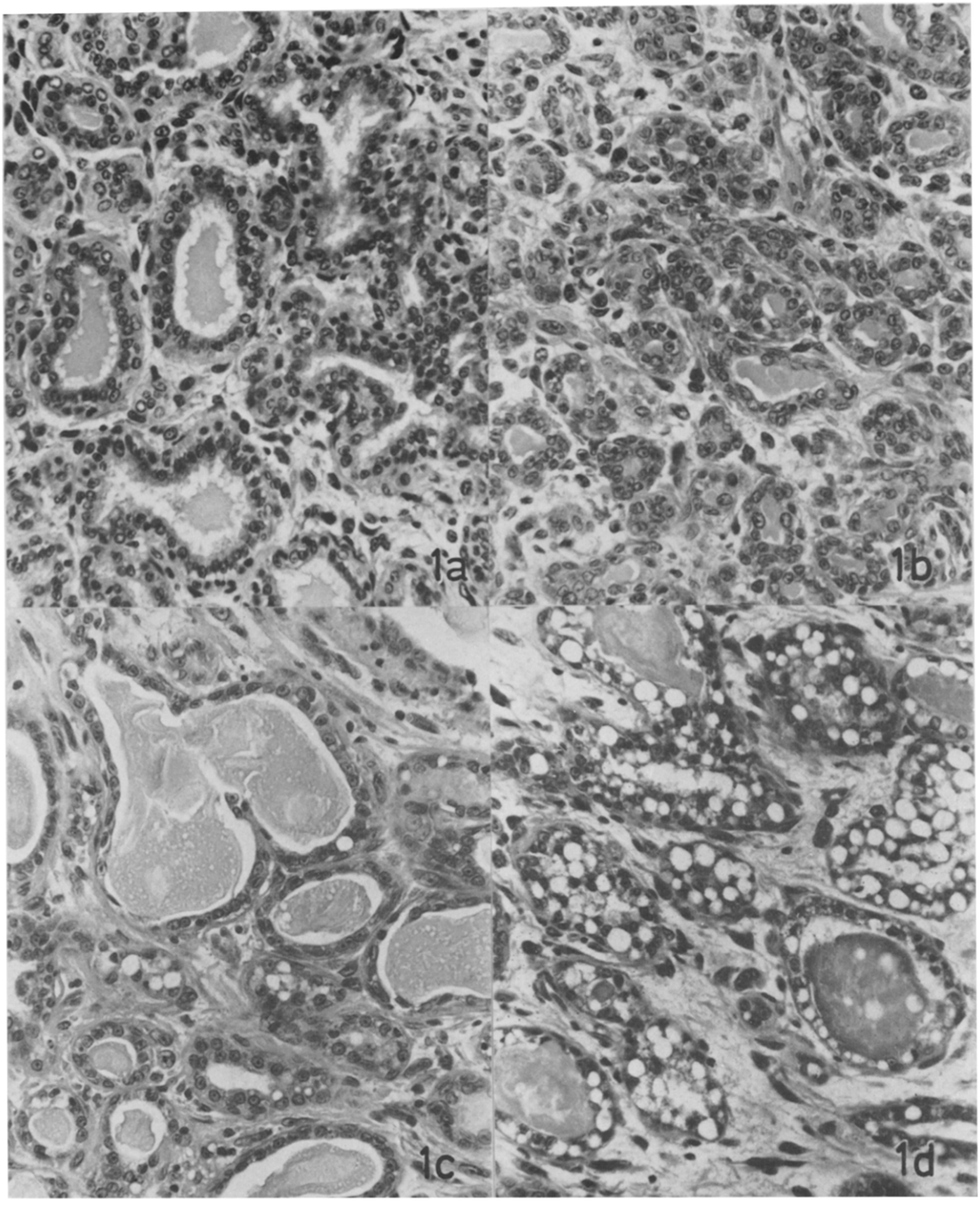

Fig. 3. Histology of the R 3327 Dunning tumor. Hemotoxylin–eosin staining. Magnification: 256×. *a, Control. b, Castration. c. DES,* 2 mg/kg. *d, D* 16726, 16 mg/kg. *Experimental details are identical with those in Table* 4. *Tumors were taken after* 6 *weeks of treatment.*

*Table 6. Antiandrogenic effect of D 16726, D 15413, DES and CPA on the seminal vesicle weight of the 5 day castrated, TP-stimulated mature mouse**

Group	TP† (μg)	Dose† (μg)	Mean seminal vesicle weight (mg)	Relative seminal vesicle weight‡	Final body weight (g)
Standard	300	—	47	31.1 ± 1.8	35.8 ± 2.3
Castrated	—	—	4	11.1 ± 0.6	34.6 ± 3.3
D 16726	300	1310	32§	100.8 ± 6.0§	32.0 ± 3.9
D 15413	300	1330	34§	102.3 ± 2.4§	33.6 ± 5.1
DES	300	1000	25§	85.6 ± 5.7§	29.1 ± 3.6
CPA	300	1000	18§	56.0 ± 1.2§	32.4 ± 1.7
Intact control	—	—	47	136.7 ± 3.5	34.3 ± 3.0
Standard	300	—	31	86.8 ± 5.1	35.4 ± 1.6
Castrated	—	—	2	5.1 ± 0.8	33.3 ± 1.7
D 16726	300	665	25§	70.1 ± 7.6§	35.9 ± 3.5
	300	2620	24§	69.4 ± 1.0§	34.1 ± 2.2
D 15413	300	665	25§	70.9 ± 0.5§	34.6 ± 1.5
	300	2660	20§	60.6 ± 5.3§	33.5 ± 1.9
DES	300	500	17§	55.4 ± 1.7§	29.8 ± 1.3
	300	2000	21§	71.5 ± 11.7§	29.2 ± 4.4
Standard	300	—	30	91.0 ± 2.3	33.0 ± 1.9
D 16726	300	13.1	28	80.9 ± 8.2	34.3 ± 1.4
	300	131	30	91.8 ± 4.8	32.8 ± 1.5
D 15413	300	13.3	28	79.0 ± 10.2	34.9 ± 2.1
	300	133	25§	75.5 ± 10.4§	33.4 ± 1.0
DES	300	10.0	32	99.1 ± 10.9	31.9 ± 1.9
	300	100	25§	80.5 ± 2.1§	30.9 ± 1.7
Intact control	—	—	31	88.7 ± 3.5	35.1 ± 2.7

*Compounds were administered daily for 9 days (six to seven mice/group). Organs were removed at day 10. All groups except intact control were castrated 5 days prior to therapy.
†Dose per animal and day; doses of D 16726 and D 15413 are equimolar to DES.
‡Relative seminal vesicle weight = organ wt (mg)/body wt (g) × 100. Mean ± S.D.
§Significant ($P < 0.01$).

Table 7. Relative binding affinities (RBA) of D 16726, D 15413, D 15414, DES and CPA for the estrogen (ER), androgen (AR) and progesterone (PR) receptor

	ER		AR			PR
	Calf uterine cytosol	R 3327 cytosol	Rat prostate cytosol	Calf uterine cytosol	R 3327 cytosol	Calf uterine cytosol
D 16726	1.7	1.6	< 0.05	< 0.05	< 0.05	<0.01
D 15414	9.5	17	< 0.05	< 0.05	< 0.05	0.02
D 15413	1.9	6.7	< 0.05	< 0.05	< 0.05	0.15
DES	60	80	< 0.05	< 0.05	< 0.05	0.03
CPA	ND	ND	4.9	11.2	19.2	42

ND = Not determined.

action should be due to their ability to suppress LH release. However, as this ability does not correlate with their estrogenic potency, it will be of interest if antiestrogens of different chemical structure exert this biological profile, too. D 16726 and D 15413 did not show an affinity for the AR. So it was not surprising that they had no direct antiandrogenic effect in rats, either. It is unlikely that the slight, but still significant, antiandrogenic effect in castrated mice contributes to their tumor-inhibiting properties.

Human as well as experimental prostatic carcinomas contain a considerable amount of ER [1,6]. The functionality of the ER was proved in the R 3327 tumor by the induction of PR [6]. As D 16726, D 15413, DES (Table 7) as well as tamoxifen [6] have affinities for ER from prostatic tumor, the antitumor effect of these compounds can

be partially mediated through ER [6]. However, there can be also other direct effects not involving the ER of these compounds on prostate tumors. In a clonogenic assay with the R 3327 tumor, estrogens had a dose-dependent effect on colony formation *in vitro* [32]. The difference in tumor histology at the end of the R 3327 experiment (Fig. 3) between castration and the administration of DES or D 16726 is a further hint that the action of estrogens or antiestrogens on tumor reduction is not only caused by a deprivation of androgens. Moreover, the histological picture after DES treatment differs from that after D 16726 treatment suggesting certain differences in the mode of the action of these compounds.

In conclusion, it can be stated that antiestrogens with only low estrogenic side-effects might offer a suitable alternative to conventional hormonal treatment of the prostatic carcinoma.

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REFERENCES

1. Stoll BA. Prostatic cancer: methods and results of endocrine therapy. In: Stoll BA, ed. *Hormonal Management of Endocrine-Related Cancer*. London, Lloyd-Luke, 1981, 148–157.
2. Griffiths K, Peeling WB, Harper ME, Davies P, Pierrepoint CG. Prostatic cancer: rationale for hormonal therapy. In: Stoll BA, ed. *Hormonal Management of Endocrine-Related Cancer*. London, Lloyd-Luke, 1981, 131–147.
3. Hodges CV. Hormonal therapy of prostatic cancer. In: Rose DP, ed. *Endocrinology of Cancer*. Boca Raton, CRC Press, 1979, Vol. II, 57–67.
4. Neumann F, Jacobi GH. Antiandrogens in tumor therapy. *Clin Oncol* 1982, **1**, 41–64.
5. Labrie F, Dupont A, Belanger A *et al.* New approach in the treatment of prostate cancer: complete instead of partial withdrawal of androgens. *Prostate* 1983, **4**, 579–594.
6. Ip MM, Milholland RJ, Rosen F. Functionality of estrogen receptor and tamoxifen treatment of R 3327 Dunning rat prostate adenocarcinoma. *Cancer Res* 1980, **40**, 2188–2193.
7. Leinonen P, Bolton NJ, Kontturi M, Vikko R. Rapid endocrine effects of tamoxifen and testolactone in prostatic carcinoma patients. *Prostate* 1982, **3**, 589–597.
8. Bartke A, Mason M, Dalterio S, Bex F. Effects of tamoxifen on plasma concentrations of testosterone and gonadotrophins in the male rat. *J Endocrinol* 1978, **79**, 239–240.
9. Paridaens RJ, Leclercq G, Heuson JC. Steroid hormones, analogs and antagonists. In: Chabner BA, Pinedo HM, eds. *The Cancer Pharmacology Annual* 2. Amsterdam, Elsevier, 1984, 171–192.
10. von Angerer E, Prekajac J, Strohmeier J. 2-Phenylindoles. Relationship between structure, estrogen receptor affinity, and mammary tumor inhibiting activity in the rat. *J Med Chem* 1984, **27**, 1439–1447.
11. von Angerer E, Prekajac J, Berger M. The inhibitory effect of 5-acetoxy-2-(4-acetoxyphenyl)-1-ethyl-3-methylindole (D 16726) on estrogen-dependent mammary tumors. *Eur J Cancer Clin Oncol* 1985, **21**, 531–537.
12. von Angerer E, Engel J, Schneider MR, Sheldrick WS. D 16726. *Drugs Future* 1985, **10**, 281–285.
13. von Angerer E, Prekajac J. 2-(Hydroxyphenyl)indoles: a new class of mammary tumor inhibiting compounds. *J Med Chem* 1983, **26**, 113–116.
14. von Angerer E, Prekajac J, Schneider MR, Berger MR. The inhibitory effect of 4-chloro-2-(2,6-dichloro-4-hydroxyphenyl)-1-ethyl-6-hydroxyindole (D 15413) on estrogen-dependent mammary tumors. *J Cancer Res Clin Oncol* 1985, **110**, 216–220.
15. Sufrin G, Coffey DS. Flutamide. Mechanism of action of a new nonsteroidal antiandrogen. *Invest Urol* 1976, **13**, 429–434.
16. Dekanski JB. Anti-prostatic activity of bifluranol, a fluorinated bibenzyl. *Br J Pharmacol* 1980, **71**, 11–16.
17. Keenan EJ, Klase PA, Thomas JA. Effects of prolactin on DNA synthesis and growth of the accessory sex organs in male mice. *Endocrinology* 1981, **109**, 170–175.
18. Stárka L, Hampl R, Kasal A, Kohout L. Androgen receptor binding and antiandrogenic activity of some 4,5-secoandrostanes and ring B cyclopropanoandrostanes. *J Steroid Biochem* 1982, **17**, 331–334.
19. Kranzfelder G, Schneider M, von Angerer E, Schönenberger H. Entwicklung neurer Antiöstrogene vom Typ des 3,3′-Dihydroxy-α,β-diethylstilbens und ihre Prüfung am DMBA-induzierten, hormonabhängigen Mammacarcinom der SD-Ratte. *J Cancer Res Clin Oncol* 1980, **97**,167–186.
20. Schneider MR, Schönenberger H, Michel RT, Fortmeyer HP. Mammary tumor inhibiting effect of 3,3′-diacetoxy-α,β-dialkylstilbenes and of related stilbene oxides. *J Med Chem* 1982, **25**, 141–145.
21. Smolev JK, Heston WDW, Scott WW, Coffey DS. Characterization of the Dunning R 3327 H prostatic adenocarcinoma: an appropriate animal model for prostatic cancer. *Cancer Treat Rep* 1977, **61**, 273–287.

22. Raynaud JP, Ojasoo T, Bouton MM, Philibert D. Receptor binding as a tool in the development of new bioactive steroids. In: Ariens EJ, ed. *Drug Design.* New York, Academic Press, 1979, Vol. VIII, 169–214.
23. Rowley DR, Thompson SA, Lubaroff DM, Heidger PM Jr. Androgen receptor binding characteristics in the cytosol of the rat dorsolateral prostate gland and the Dunning R 3327 prostatic adenocarcinoma. *Prostate* 1984, **5**, 101–111.
24. Wagner RK, Görlich L, Jungblut PW. Multiple steroid hormone receptor in calf uterus. Binding specificities and distribution. *Hoppe Seyler's Z Physiol Chem* 1972, **353**, 1654–1656.
25. Carroll SL, Rowley DR, Chang CH, Tindall DJ. Exchange assay for androgen receptors in the presence of molybdate. *J Steroid Biochem* 1984, **21**, 353–359.
26. Wakeling AE, Furr BJA, Glen AT, Hughes LR. Receptor binding and biological activity of steroidal and on steroidal antiandrogens. *J Steroid Biochem* 1981, **15**, 353–359.
27. Theofan G, Notides AC. Characterization of the calf uterine progesterone receptor and its stabilization by nucleic acids. *Endocrinology* 1984, **114**, 1173–1179.
28. Hoehn W, Schroeder FH, Riemann JF, Joebsis AC, Hermanek P. Human prostatic adenocarcinoma: some characteristics of a serially transplantable line in nude mice (PC 82). *Prostate* 1980, **1**, 95–104.
29. Hoehn W, Walther R, Hermanek P. Human prostatic adenocarcinoma: comparative experimental treatment of the tumor line PC 82 in nude mice. *Prostate* 1982, **3**, 193–201.
30. Hoehn W, Wagner M, Riemann JF *et al.* Prostatic adenocarcinoma PCEW, a new human tumor line transplantable in nude mice. *Prostate* 1984, **5**, 445–452.
31. Mawhinney MG, Belis JA. Androgens and estrogens in prostatic neoplasia. *Adv Sex Horm Res* 1976, **2**, 141–209.
32. Dibner JJ, Nakeff A. R 3327 adenocarcinoma clonogenic cells: epithelial properties and hormone response. *JNCI* 1983, **70**, 1057–1066.