at test beginning; 10 mice/group) were injected sc daily for three consecutive days with solutions of the test compounds in olive oil (0.1 mL/mouse). The uteri were removed 24 h after the last injection, fixed with Bouin's solution, dried, and weighed.

Hormone-Dependent Human MCF-7 Breast Cancer Cells. The applied method was identical with that previously described by us.[5]

Hormone-Dependent, Transplantable MXT Mammary Tumor of the BDF_1 Mouse.[15,16] The applied method was identical with that previously described by us.[5] The MXT tumor used in these studies was the MXT line 3.2 kindly provided by Dr. Bogden, Laboratory of Experimental Oncology, EG&G Bogden Laboratories, Worcester, MA. The tumor was transplanted in pieces of about 2 mm^3 (one tumor piece–animal) subcutaneously in female, 8-weeks-old BDF_1 mice (body weight, 20 ± 1.6 g; Charles River Wiga, West Germany). After transplantation, the animals were randomly distributed into groups of 10. Starting with the first day after transplantation, the test compounds were injected sc 3 times a week (Monday, Wednesday, Friday) as olive oil solutions (0.1 mL/mouse). The duration of treatment was 6 weeks. At the end of treatment, the animals were killed by cervical dislocation and weighed. The tumors were removed, washed in 0.9% sodium chloride solution, blotted dry, and weighed, and the average tumor weight was calculated. The uteri were also removed and prepared as described in ref 14 to serve as an indicator of the estrogenic side effects of the compounds.

Acknowledgment. We thank Dr. H. Schönenberger for helpful discussions, Dr. A. E. Bogden for providing us with the MXT tumor, M. Beer, C. Niel, K. Röhrl, and G. Tondl for skillful technical assistance and the Deutsche Krebshilfe e.V., Dr. Mildred Scheel-Stiftung, and the Deutsche Forschungsgemeinschaft (SFB 234) for financial support.

Registry No. 1, 3141-93-3; 2, 29955-23-5; 3, 4927-55-3; 4, 4927-54-2; 5, 84675-71-8; 6, 4927-53-1; 7, 35989-78-7; 8, 102520-52-5; 9, 102520-53-6; 10, 84675-72-9; 11, 84675-73-0; 12, 84675-74-1; 13, 102520-54-7; 14, 102520-55-8; 15, 4131-03-7; (*Z*)-16, 102520-56-9; (*E*)-16, 102520-57-0; 17, 102520-58-1; (*Z*)-18, 102520-59-2; (*E*)-18, 102520-60-5; 19, 102520-61-6; (*Z*)-20, 102520-62-7; (*E*)-20, 102520-63-8; (*Z*)-21, 102520-64-9; (*E*)-21, 102520-65-0; (*Z*)-22, 102520-66-1; (*E*)-22, 102520-67-2; (*Z*)-23, 102520-68-3; (*E*)-23, 102520-69-4; (*Z*)-24, 102520-70-7; (*E*)-24, 102520-71-8; (*Z*)-25, 102520-72-9; (*E*)-25, 102520-73-0; (*Z*)-26, 102520-74-1; (*E*)-26, 102520-75-2; 27, 102520-76-3; (*Z*)-28, 102520-77-4; (*E*)-28, 102520-78-5; 29, 102520-79-6; (*Z*)-30, 102520-80-9; (*E*)-30, 102520-81-0; (*Z*)-31, 102520-82-1; (*E*)-31, 102520-83-2; (*Z*)-32, 102520-84-3; (*E*)-32, 102520-85-4; (*Z*)-33, 102520-86-5; (*E*)-33, 102520-87-6; (*Z*)-34, 102520-88-7; (*E*)-34, 102520-89-8; (*Z*)-35, 102520-90-1; (*E*)-35, 102520-91-2; 1,1-diphenyl-2-(3,4-dimethoxyphenyl)butan-1-ol, 102520-92-3; bromobenzene, 108-86-1; 4-bromo-1,2-dimethoxybenzene, 2859-78-1; 1-bromo-4-methoxybenzene, 104-92-7; propiophenone, 93-55-0.

Supplementary Material Available: Tables V–XI giving analytical data of compounds 1–35 (10 pages). Ordering information is given on any current masthead page.

Aromatase Inhibitors. Synthesis and Evaluation of Mammary Tumor Inhibiting Activity of 3-Alkylated 3-(4-Aminophenyl)piperidine-2,6-diones

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The synthesis and biological evaluation of 3-alkyl-substituted 3-(4-aminophenyl)piperidine-2,6-diones as inhibitors of estrogen biosynthesis are described [H (1), methyl (2), ethyl (3), *n*-propyl (4), isopropyl (5), *n*-butyl (6), isobutyl (7), *sec*-butyl (8), *n*-pentyl (9), isopentyl (10), 2-methylbutyl (11), *sec*-pentyl (12), *n*-hexyl (13), *n*-heptyl (14)]. In vitro compounds 4–14 showed a stronger inhibition of human placental aromatase compared to aminoglutethimide (AG, compound 3), which recently has become used for the treatment of hormone-dependent breast cancer. The most active derivative, compound 10, showed a 93-fold stronger inhibition than AG. With the exception of 5, 7, and 8, all other compounds exhibited similar or decreased inhibition of bovine adrenal desmolase compared to AG. Compounds 4 and 6–12 showed a stronger inhibition of the plasma estradiol concentration of pregnant mare serum gonadotropin (PMSG) primed Sprague–Dawley (SD) rats compared to the parent compound. Compounds 4, 6–8, 10, and 12 inhibited the testosterone-stimulated tumor growth of ovariectomized 9,10-dimethyl-1,2-benzanthracene (DMBA) tumor-bearing SD rats more strongly than AG. Being stronger and more selective inhibitors of the estrogen biosynthesis than AG, some of the newly developed derivatives of AG might be better candidates for the treatment of the hormone-dependent human breast cancer.

Recently the use of inhibitors of estrogen biosynthesis has become a new strategy in the treatment of metastatic hormone-dependent breast cancer.[1] As aromatization is the last reaction in the biosynthesis of estrogens, compounds interacting exclusively with the aromatizing enzyme might be very specific drugs. The only nonsteroidal compound clinically used so far, aminoglutethimide (AG, compound 3, 3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione) has shown benefit in many trials with postmenopausal and ovariectomized premenopausal women.[1-5] AG exerts its mammary tumor inhibitory activity principally by reducing the extraglandular peripheral aromatization of the adrenal androgens androstenedione and testosterone into estrone and estradiol (E_2), respectively,[1,6] and patients treated with AG exhibit response rates comparable to

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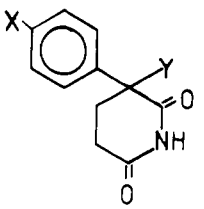
Table I. Substituted 3-Alkyl-3-phenylpiperidine-2,6-diones



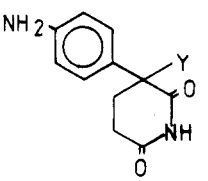
compd	X	Y	synth method[a]	yield,[b,c] %	mp, °C	recrystn solvent[d]	formula[e]
1b[f]	H	H	C	24	142–143	F	$C_{11}H_{11}NO_2$
1	NH_2	H	D, E	44	139–141	F	$C_{11}H_{12}N_2O_2$
2b[f]	H	CH_3	C	71	104–105	G	$C_{12}H_{13}NO_2$
2[g]	NH_2	CH_3	D, E	21	144–145	F	$C_{12}H_{14}N_2O_2$
3b[h]	H	C_2H_5	C	59	86–88	G	$C_{13}H_{15}NO_2$
3[g]	NH_2	C_2H_5	D, E	28	149–150	F	$C_{13}H_{16}N_2O_2$
4b[i]	H	$(CH_2)_2CH_3$	C	57	95–97	G	$C_{14}H_{17}NO_2$
4	NH_2	$(CH_2)_2CH_3$	D, E	36	190–192	F	$C_{14}H_{18}N_2O_2$
5b[k]	H	$CH(CH_3)_2$	C	68	114–115	G	$C_{14}H_{17}NO_2$
5	NH_2	$CH(CH_3)_2$	D, E	36	154–155	G	$C_{14}H_{18}N_2O_2$
6b	H	$(CH_2)_3CH_3$	C	57	103–104	H	$C_{15}H_{19}NO_2$
6	NH_2	$(CH_2)_3CH_3$	D, E	24	146–147	F	$C_{15}H_{20}N_2O_2$
7b[k]	H	$CH_2CH(CH_3)_2$	C	42	78–81	H	$C_{15}H_{19}NO_2$
7	NH_2	$CH_2CH(CH_3)_2$	D, E	17	139–141	J	$C_{15}H_{20}N_2O_2$
8b[l]	H	$CHCH_3C_2H_5$	C	56	*m*	G	$C_{15}H_{19}NO_2$
8[l]	NH_2	$CHCH_3C_2H_5$	D, E	24	*m*	J	$C_{15}H_{20}N_2O_2$
9b[k]	H	$(CH_2)_4CH_3$	C	49	43–45	H	$C_{16}H_{21}NO_2$
9	NH_2	$(CH_2)_4CH_3$	D, E	14	oil	J	$C_{16}H_{22}N_2O_2$
10b[k]	H	$(CH_2)_2CH(CH_3)_2$	C	46	65–68	K	$C_{16}H_{21}NO_2$
10	NH_2	$(CH_2)_2CH(CH_3)_2$	D, E	35	123–125	J	$C_{16}H_{22}N_2O_2$
11b[l]	H	$CH_2CHCH_3C_2H_5$	C	32	oil	K	$C_{16}H_{21}NO_2$
11[l]	NH_2	$CH_2CHCH_3C_2H_5$	D, E	41	oil	J	$C_{16}H_{22}N_2O_2$
12b[l]	H	$CHCH_3(CH_2)_2CH_3$	C	56	oil	K	$C_{16}H_{21}NO_2$
12[l]	NH_2	$CHCH_3(CH_2)_2CH_3$	D, E	24	oil	J	$C_{16}H_{22}N_2O_2$
13b	H	$(CH_2)_5CH_3$	C	53	oil	K	$C_{17}H_{23}NO_2$
13	NH_2	$(CH_2)_5CH_3$	D, E	27	oil	J	$C_{17}H_{24}N_2O_2$
14b	H	$(CH_2)_6CH_3$	C	51	oil	K	$C_{18}H_{25}NO_2$
14	NH_2	$(CH_2)_6CH_3$	D, E	25	oil	J	$C_{18}H_{26}N_2O_2$

[a] Capital letters refer to synthetic methods C, D, and E under the Experimental Section. [b] Yield of analytically pure product; no effort made to optimize yields. [c] In cases of compounds 1–14 the overall yield for the nitration and subsequent reduction is given, for a mixture of the corresponding para and meta nitro compounds was used for the hydrogenation. [d] F = MeOH; G = MeOH/H_2O; H = MeOH/ether; J = column chromatography (SiO_2; CH_2Cl_2:ethyl acetate = 1:1); K = column chromatography (SiO_2; CH_2Cl_2:ethyl acetate = 9:1). [e] All compounds were analyzed for C and H within ±0.40% of the calculated values. [f] See ref 10. [g] See ref 11. [h] See ref 12. [i] See ref 13. [k] See ref 14. [l] Mixture of diastereomers. [m] Melting point not given because of mixture.

antiestrogen treatment[1,2,4] or ablative surgery[1,2,4,5] (e.g., tamoxifen therapy or adrenalectomy, respectively). As AG is also an inhibitor of adrenal steroidogenesis—its principal effect being the inhibition of the conversion of cholesterol into pregnenolone, mediated by the enzyme desmolase[7]—the biosyntheses of other adrenal steroids are also affected by this compound.[2] Above all, corticosteroid production is depleted,[1,2] and consequently patients receiving AG require hydrocortisone as replacement therapy to prevent the reflex rise in ACTH and the subsequent adrenal overstimulation.[1,2] Hence it might be advantageous to use a drug showing less inhibition of desmolase.

Attempts to separate the inhibitory actions in analogues of AG have already been undertaken.[8,9] Foster et al. showed that 1-amino-3-ethyl-3-phenylpiperidine-2,6-dione is a strong and selective inhibitor of the cholesterol side-chain cleavage enzyme.[8] The same group found that replacement of the 4-aminophenyl substituent of AG by a 4-pyridyl group, on the other hand, led to a compound [3-ethyl-3-(4-pyridyl)piperidine-2,6-dione] showing no inhibition of desmolase.[9] But like all previous structural modifications of AG, this manipulation also resulted in a decrease of inhibitory potency toward aromatase.[9]

Chart I



compd	Y	compd	Y
1	H	8	$CHCH_3C_2H_5$
2	CH_3	9	$(CH_2)_4CH_3$
3	C_2H_5	10	$(CH_2)_2CH(CH_3)_2$
4	$(CH_2)_2CH_3$	11	$CH_2CHCH_3C_2H_5$
5	$CH(CH_3)_2$	12	$CHCH_3(CH_2)_2CH_3$
6	$(CH_2)_3CH_3$	13	$(CH_2)_5CH_3$
7	$CH_2CH(CH_3)_2$	14	$(CH_2)_6CH_3$

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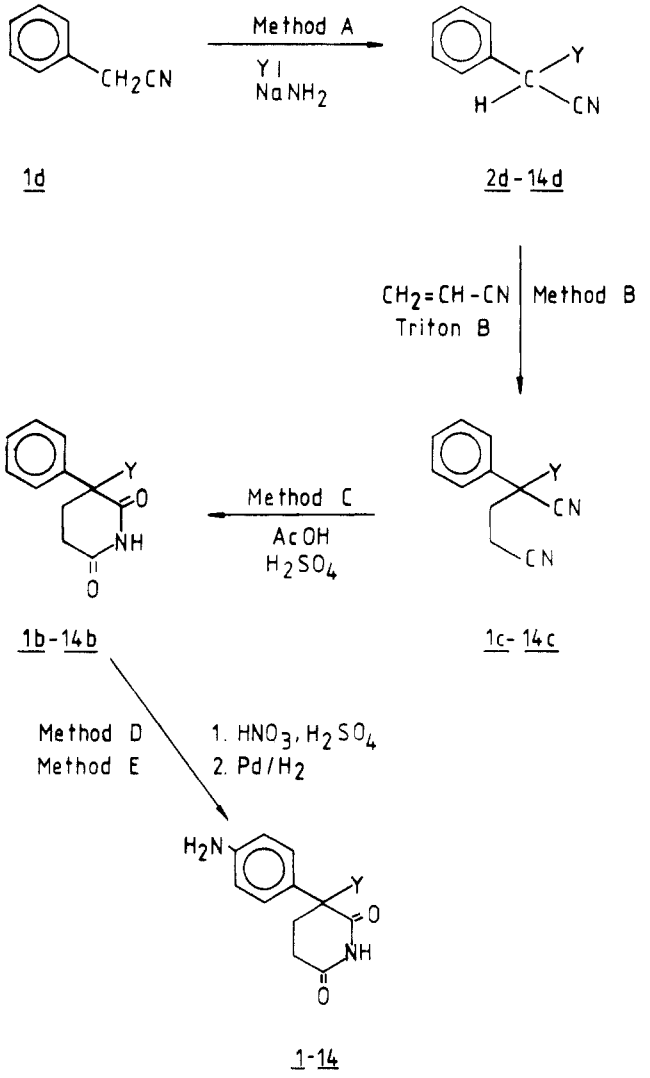
Scheme I



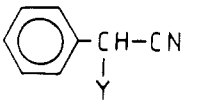
This paper describes the attempt to obtain stronger inhibitors of aromatase exhibiting less inhibition of desmolase than AG. In the following, the synthesis, the inhibitory activities toward both enzymes, the inhibition of estrogen production in vivo, and the mammary tumor inhibiting activity of new 3-alkyl-substituted 3-(4-aminophenyl)piperidine-2,6-diones will be described (Chart I).

Chemistry. The synthesis of the 3-alkyl-3-phenylpiperidine-2,6-diones (compounds **1b–14b**, Table I) started from phenylacetonitrile (Scheme I). The latter compound was converted with an alkyl iodide and sodamide in benzene to the corresponding 2-alkyl-substituted phenylacetonitriles (**2d–14d**, Table II, method A).

Michael reaction of compounds **1d–14d** with acrylonitrile using Triton B (trimethylbenzylammonium hydroxide; method B) as catalyst and cyclization of the resulting 2-alkyl-2-phenylglutarodinitriles (**1c–14c**, Table III) with acetic acid and concentrated H_2SO_4 (method C) yielded the corresponding 3-alkyl-3-phenylpiperidine-2,6-diones (**1b–14b**).

Nitration of compounds **1b–14b** was performed according to the method of Hoffmann and Urech[11] using concentrated H_2SO_4 and HNO_3 (method D). A mixture of the corresponding isomeric 2-alkyl-2-(nitrophenyl)-glutarimides was obtained, as was shown by ^1H NMR spectroscopy. In the case of the nitration of glutethimide (**3b**) it has already been described[8] that mixtures of isomers were isolated when published procedures were followed. The major byproduct was found to be the meta analogue (meta:para = 1:2),[17] while the ortho derivative constituted only about 1% of the nitro isomers[8] formed under standard conditions.[11] Attempts to separate the 3-alkyl-3-(4-nitrophenyl)piperidine-2,6-diones (**1a–14a**) from the corresponding meta nitro analogues proved to be very difficult. Efforts to obtain the pure isomers by TLC and HPLC failed. Fractional crystallization provided the para nitro compounds in very poor yields. The 3-ethyl-, 3-isopropyl-,

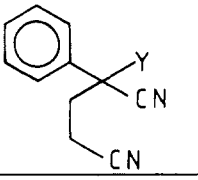
Table II. 2-Alkyl-Substituted 2-Phenylacetonitriles



compd	Y	synth method[a]	yield,[b] %	formula
2d	CH_3	A	74	C_9H_9N
3d	C_2H_5	A	73	$C_{10}H_{11}N$
4d	$(CH_2)_2CH_3$	A	86	$C_{11}H_{13}N$
5d	$CH(CH_3)_2$	A	85	$C_{11}H_{13}N$
6d	$(CH_2)_3CH_3$	A	63	$C_{12}H_{15}N$
7d	$CH_2CH(CH_3)_2$	A	72	$C_{12}H_{15}N$
8d[c]	$CHCH_3C_2H_5$	A	64	$C_{12}H_{15}N$
9d	$(CH_2)_4CH_3$	A	49	$C_{13}H_{17}N$
10d	$(CH_2)_2CH(CH_3)_2$	A	47	$C_{13}H_{17}N$
11d[c]	$CH_2CHCH_3C_2H_5$	A	45	$C_{13}H_{17}N$
12d[c]	$CHCH_3(CH_2)_2CH_3$	A	41	$C_{13}H_{17}N$
13d	$(CH_2)_5CH_3$	A	50	$C_{14}H_{19}N$
14d	$(CH_2)_6CH_3$	A	45	$C_{15}H_{21}N$

[a] A refers to synthetic method A under the Experimental Section. [b] Yield of analytically pure (TLC) product; no effort made to optimize yields. [c] Mixture of diastereomers.

Table III. 2-Alkyl-2-phenylglutarodinitriles



compd	Y	synth method[a]	yield,[b] %	formula
1c[c]	H	B	21	$C_{11}H_{10}N_2$
2c	CH_3	B	51	$C_{12}H_{12}N_2$
3c[d]	C_2H_5	B	53	$C_{13}H_{14}N_2$
4c	$(CH_2)_2CH_3$	B	30	$C_{14}H_{16}N_2$
5c	$CH(CH_3)_2$	B	51	$C_{14}H_{16}N_2$
6c	$(CH_2)_3CH_3$	B	39	$C_{15}H_{18}N_2$
7c	$CH_2CH(CH_3)_2$	B	32	$C_{15}H_{18}N_2$
8c[e]	$CHCH_3C_2H_5$	B	37	$C_{15}H_{18}N_2$
9c	$(CH_2)_4CH_3$	B	39	$C_{16}H_{20}N_2$
10c	$(CH_2)_2CH(CH_3)_2$	B	33	$C_{16}H_{20}N_2$
11c[e]	$CH_2CHCH_3C_2H_5$	B	37	$C_{16}H_{20}N_2$
12c[e]	$CHCH_3(CH_2)_2CH_3$	B	46	$C_{16}H_{20}N_2$
13c	$(CH_2)_5CH_3$	B	43	$C_{17}H_{22}N_2$
14c	$(CH_2)_6CH_3$	B	47	$C_{18}H_{24}N_2$

[a] B refers to synthetic method B under the Experimental Section. [b] Yield of analytically pure (TLC) product; no effort made to optimize yields. [c] See ref 15. [d] See ref 16. [e] Mixture of diastereomers.

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Table IV. Inhibition of Human Placental Aromatase by 3-Alkyl-3-(4-aminophenyl)piperidine-2,6-diones

compd	IC_{50},[a,b] μM	rel potency[c]	compd	IC_{50},[a,b] μM	rel potency[c]
1	>300	<0.1	8	3.0	12
2	135	0.3	9	1.3	29
3	37	1	10	0.4	93
4	11	3.4	11	1.1	34
5	16	2.3	12	0.6	62
6	2.3	16	13	1.5	25
7	3.0	12	14	1.7	22

[a] Concentration of testosterone: 5 μM. [b] IC_{50} is the concentration of inhibitor required to give 50% inhibition. [c] Calculated from the IC_{50} values and relative to aminoglutethimide (compound 3).

and 3-*n*-butyl-substituted 3-(4-nitrophenyl)piperidine-2,6-diones (**3a**, **5a**, and **6a**) were obtained in yields of 9, 6, and 8%, respectively.

Separation of the isomers was less difficult after a single crystallization of the crude product from MeOH and subsequent reduction of the isomeric mixture (para and meta) to the corresponding 3-(aminophenyl)piperidine-2,6-diones by catalytic hydrogenation using palladium on charcoal (method E). Fractional crystallization or column chromatography gave compounds **1–14** (Table I) in satisfying yields.

Compounds **8**, **11**, and **12** were prepared as mixtures of diastereomers. A separation was not possible by fractional crystallization, TLC, or HPLC.

Biological Properties. The activities of the test compounds **1–14** as inhibitors of aromatase were determined in vitro using human placental microsomes and [1β,2β-^{3}H]testosterone. The potencies of the derivatives, relative to AG (compound **3**) are given in Table IV.

It becomes apparent from the low relative potencies of compounds **1** and **2** that the shortening of the Et group of AG is unsuitable for enhancing inhibitory activity.

On the other hand, an elongation of the Et group leads to dramatic increases of the relative potencies. In the case of the *n*-alkyl derivatives, the propyl compound **4** shows a 3.4-fold higher potency and the butyl compound **6** a 16-fold higher potency. The maximum potency is found with the pentyl compound (**9**, relative potency (rp) = 29), whereas in the case of the hexyl and heptyl compounds **13** and **14** a small decrease of inhibitory activity is observed.

The introduction of a CH_3 group into the α-position of the *n*-alkyl substituents leads to a further increase of the enzyme inhibition of the corresponding compound. Exhibiting relative potencies of 2.3, 12, and 62, compounds **5**, **8**, and **12** show 2–4-fold higher activities compared to the corresponding *n*-alkyl compounds **3**, **4**, and **6** (rp = 1, 3.4, and 16). The displacement of the CH_3 group of the *sec*-butyl compound **8** from the α-position into the β-position does not change the activity (isobutyl compound **7**, rp = 12). In contrast to this the displacement of the CH_3 group of the *sec*-pentyl compound **12** alters the inhibitory activity. In the case of the 2-methylbutyl compound **11** the activity is reduced, but in the case of the isopentyl compound **10** the activity is increased. Thus the latter compound is the most active one of this series, exhibiting a relative potency of 93.[18]

As it is known from studies on steroidal inhibitors of aromatase that some of them lead to an irreversible inhibition of the enzyme,[19] we examined whether our compounds show this effect too. Human placental microsomes were incubated with test compounds for 15, 30, and 60 min according to the procedure of Brodie et al.[20] After removal of the test compounds by charcoal the enzyme activity was monitored. None of the test compounds showed a reduction of enzyme activity at any time point (data not given).

Table V. Inhibition of Bovine Adrenal Desmolase and Human Placental Aromatase by 3-Alkyl-3-(4-aminophenyl)piperidine-2,6-diones

compd	% inhibn of aromatase[a,b]	% inhibn of desmolase[a]	compd	% inhibn of aromatase[a,b]	% inhibn of desmolase[a]
1	<10	<10	8	88	73
2	13	20	9	>95	51
3	43	57	10	>95	25
4	78	45	11	>95	28
5	62	81	12	>95	21
6	92	54	13	>95	31
7	88	74	14	>95	30

[a] Concentration of inhibitor: 25 μM. [b] Concentration of testosterone: 5 μM.

The inhibitory activities of the 3-alkyl-3-(4-aminophenyl)piperidine-2,6-diones **1–14** toward desmolase were determined in vitro using bovine adrenal mitochondria and [26-^{14}C]cholesterol. The inhibition values of the test compounds in concentrations of 25 μM are given in Table V. For comparative purposes the percent inhibition of aromatase by the test compounds in the same concentrations is given too.

As was observed in the case of aromatase inhibition, substitution of the 3-Et group in AG by H or CH_3 (compounds **1** and **2**, respectively) also leads to a strong decrease of inhibitory activity toward desmolase.

In contrast to the properties shown toward aromatase, the *n*-alkyl homologues of AG show no stronger inhibition of desmolase than the parent compound. The inhibitory activity of compounds **4**, **6**, and **9** is slightly diminished, while that of the hexyl and heptyl derivatives (**13** and **14**) is more strongly reduced.

The introduction of a CH_3 group in AG and the *n*-propyl derivative **4** leads to an increase of inhibitory activity (compounds **5**, **7**, and **8**), whereas the same structural modification reduces the inhibitory potency of the *n*-butyl compound **6** (compounds **10–12**).

An examination of the inhibitory effects of the test compounds toward aromatase *and* desmolase suggests that the *n*-propyl, butyl, and pentyl derivatives **4**, **6**, and **9** might have a therapeutic advantage compared to AG, since they exhibit a stronger inhibition of aromatase (especially compond **9**) and a similar (**6** and **9**) or a smaller (**4**) inhibition of desmolase. In spite of their stronger inhibition of aromatase, the isopropyl and iso- and *sec*-butyl derivatives **5**, **7**, and **8** are less interesting because of their relatively strong inhibition of desmolase. Compounds **10–12** seem to be even more interesting than the *n*-pentyl compound **9**. Exhibiting a similar (compound **11**) or even a stronger (compounds **10** and **12**) inhibition of aromatase, these three compounds show a considerably reduced inhibition of desmolase compared to compound **9**. The

(18) The corresponding CH_3-substituted pentyl compounds should show higher relative potencies, but we have refrained from synthesizing these compounds, because of the in vivo data obtained from the CH_3-substituted butyl compounds **10–12**.

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Table VI. Effect of Aromatase Inhibitors on Estradiol Concentration in the PMSG-Primed SD Rat[a,b]

	plasma obtained from			
	right ventricle		ovarian vein	
compd[c]	pg/mL	% inhibn	pg/mL	% inhibn
control	797		2413	
3	260	67	796	67
4	137	83	183	92
5	248	69	720	70
6	133	83	156	94
7	100	87	167	93
8	123	85	185	92
9	187	77	514	79
10	140	82	248	90
11	154	81	239	90
12	146	82	219	91
13	287	64	751	69
14	310	61	838	65

[a] The compounds were applied in doses equimolar to 2 mg/kg aminoglutethimide. [b] Blood samples were taken 6 h after application of inhibitor. [c] Each treatment group consisted of five animals.

n-hexyl and *n*-heptyl derivatives **13** and **14** on the other hand show a reduced inhibition of aromatase and a slightly enhanced inhibition of desmolase in comparison with the *n*-pentyl derivative.

In order to determine the effects of the test compounds on the E_2 concentration in vivo, the following experiments were performed by using pregnant mare serum gonadotropin (PMSG) primed Sprague–Dawley (SD) rats.[21] After a single dose of AG (50 mg/kg) the E_2 concentration in the plasma was measured at different times. Already 4 h after application of AG the E_2 concentration was strongly reduced and remained suppressed for more than 24 h (% inhibition of the E_2 concentration 4 h after AG application: ovarian vein 96, right ventricle 97; 8 h: 94, 95; 12 h: 93, 94; 24 h: 93, 93). The following data were obtained by testing AG at different doses: % inhibition of the E_2 concentration 6 h after application of 50 mg/kg AG: ovarian vein 95, right ventricle 95; 20 mg/kg: 93, 74; 10 mg/kg: 79, 68; 5 mg/kg: 74, 72; 2 mg/kg: 63, 57. The test compounds that had shown an aromatase inhibiting potency exceeding that of AG (rp > 1) were tested in doses equimolar to 2 mg/kg AG (Table VI).

The *n*-propyl compound **4** causes a much stronger decrease of the E_2 concentration than observed after AG treatment. The *n*-butyl and *n*-pentyl derivatives **6** and **9**, which were stronger inhibitors of aromatase than compound **4**, were only similarly active (**6**) or even less potent (**9**) in reducing the E_2 concentration. Compounds **13** and **14** show a further decrease of activity leading to similar E_2 concentrations as observed after AG. The introduction of a CH_3 group into compounds **3** and **4**—also a structural manipulation leading to an increase of inhibitory activity toward aromatase—does not significantly change the effect of the parent compounds on the E_2 concentration (compounds **5**, **7**, and **8**). In the case of the *n*-butyl derivative **6**, introduction of the CH_3 group even leads to a decrease of the E_2 concentration inhibiting potency (compounds **10–12**).[22]

As some steroidal inhibitors of aromatase also reduce the activity of this enzyme in the ovary in vivo,[23] we have determined the activity of the ovarian aromatase in the latter experiment. In accordance with the in vitro test the enzyme activity of rats treated with any test compound was in no case reduced compared to that of the untreated control (data not given).

The hormone-dependent mammary tumor of the SD rat was used for the further evaluation of the test compounds. This experimental tumor is similar to the human carcinoma[24] and has been frequently used for the evaluation of antiestrogens by us[25] and others. AG and the *n*-propyl derivative **4**, however, show only a moderate antitumor activity on this tumor model (% change of tumor area at the end of treatment: control, 298; **3**, 50 mg/kg 199; **4**, 53 mg/kg 175). Others also have found only poor antitumor activity with AG using similar experimental conditions.[26] An explanation for this phenomenon might be the increased LH secretion observed with AG treatment, which promotes ovarian growth and synthesis of aromatase, counteracting the inhibitory action of this compound to some extent.[26,27]

However, the test compounds inhibit the testosterone-stimulated tumor growth of ovariectomized tumor-bearing rats (Table VII).

This model is based on the observation that application of testosterone can prevent the tumors from ovariectomy-induced regression (Table VII).[28] This effect of testosterone can completely or partially be inhibited by the test compounds. While AG exerts a very strong effect in a dose of 50 mg/kg, this compound is less active in a dose of 20 mg/kg. Compound **4**—tested in a dose of 10.6 mg/kg—shows a stronger testosterone-inhibiting antitumor effect than AG in the 20 mg/kg dose and exhibits a similar activity compared to AG in the 50 mg/kg dose. The test compounds **4**, **6–8**, **10**, and **12**, which had shown promising properties in the preceding tests, were similarly active.

Testosterone also stimulates the uterine weights of the ovariectomized, tumor-bearing rats strongly. This effect can also be inhibited by the test compounds. In contrast to the tumors, however, the values of the ovariectomized control group are not reached (Table VII).

Discussion

The aim of this study—the synthesis of compounds that inhibit the aromatase stronger than AG—has been reached by elongation of the 3-Et group. Compounds **4–14** are the first analogues of AG showing a stronger inhibition of the estrogen synthetase than the parent compound. Showing a relative potency of 93, the most active compound of this series—the isopentyl derivative **10**—is even more active than 4-hydroxyandrostenedione (rp = 30[29]). Compound **10** also exceeds the most active nonsteroidal inhibitors of aromatase 4-cyclohexylaniline and 7,8-benzoflavone, compounds which have been reported to be about 10 times more potent than AG,[30,31] and the antimycotic compound miconazole, which is 73-fold more potent than AG.[32]

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Table VII. Effect of Aromatase Inhibitors on the Ovariectomized, Testosterone-Treated SD Rat Bearing DMBA Tumors

treatment gp[a]	dose of inhibn,[b] mg/kg	% of tumors with				% change of tumor area[g,h]	uterine wt effect,[i,h] means ± SD
		CR[c]	PR[d]	NC[e]	P[f]		
control		85	15	0	0	−97	9.9 ± 2.3[l]
T		36	31	10	23	−37[k]	23.3 ± 2.7[k]
T + **3**	50	79	21	0	0	−95[l]	17.3 ± 2.2[k,l]
control		88	6	6	0	−96	11.2 ± 3.1[l]
T		36	18	28	18	−24[k]	29.3 ± 3.7[k]
T + **3**	20	86	5	5	4	−81[l]	22.4 ± 4.3[k,l]
T + **4**	21.2	80	20	0	0	−94[l]	23.7 ± 5.0[k,l]
T + **4**	10.6	88	8	4	0	−96[l]	24.4 ± 2.3[k,l]
control		92	8	0	0	−97	10.5 ± 2.0[l]
T		22	19	53	6	−29[k]	34.7 ± 2.6[k]
T + **4**	10.6	74	9	13	4	−90[l]	22.8 ± 4.3[k,l]
T + **6**	11.2	84	13	3	0	−92[l]	24.8 ± 3.9[k,l]
T + **7**	11.2	67	24	9	0	−85[l]	29.0 ± 2.0[k,l]
T + **8**	11.2	61	24	15	0	−88[l]	28.4 ± 1.5[k,l]
T + **10**	11.8	75	19	6	0	−86[l]	26.6 ± 3.7[k,l]
T + **12**	11.8	48	36	12	4	−86[l]	28.6 ± 2.8[k,l]

[a] T, testosterone. [b] Dose of T: 20 mg/kg. [c] CR, complete remission, tumor not palpable. [d] PR, partial remission, reduction of initial tumor size ≥50%. [e] NC, no change, tumor size 51–150% of initial tumor size. [f] P, progression, tumor size >150% of initial tumor size. [g] Average on the 28th day of therapy. [h] The U test according to Wilcoxon, Mann, and Whitney was used. [i] Uterus dry weight (milligrams)/body weight (grams) × 100. [k] Significantly different from the control group ($\alpha = 0.01$). [l] Significantly different from the T group ($\alpha = 0.01$).

In contrast to the latter compound, which is a stronger inhibitor of desmolase than AG,[32] some of the test compounds exhibit a similar or a decreased inhibitory activity toward this enzyme compared to the parent compound. This means that in the class of the 3-alkyl-substituted 3-(4-aminophenyl)piperidine-2,6-diones the maxima of aromatase inhibition and desmolase inhibition do not coincide. This has already been observed by Foster et al., who synthesized an AG derivative, selectively inhibiting desmolase.[8] In the class of the *n*-alkyl homologues the 3-Et derivate (AG) already shows a maximum inhibition of desmolase and is only surpassed by the CH_3-substituted 3-Et and 3-propyl derivatives, whereas the maximum of aromatase inhibition is shifted toward the compounds with a longer alkyl chain.

This increase of aromatase inhibition might be due to the enhanced lipophilicity of these compounds. Lipophilicity as being important for inhibition of aromatase has already been supposed by Foster et al.,[8] who explained the poor activity of AG derivatives containing a OH group or an additional NH_2 group by the enhanced hydrophylicity of these compounds.

The different maxima of aromatase and desmolase inhibition of the 3-alkyl-substituted 3-(4-aminophenyl)-piperidine-2,6-diones confer a greater selectivity of action of the corresponding compounds. This is an important prerequisite for a possible therapeutic use.

Most of the AG derivatives, which had shown a stronger inhibition of the aromatase in vitro, were found to be superior to AG considering the inhibition of the E_2 concentration in vivo. It is striking that the strong effect of the *n*-propyl compound **4** is not or only slightly exceeded by compounds with a longer alkyl chain, though the latter compounds had shown a much stronger inhibitory potency toward aromatase in vitro.

A reason for this phenomenon might be the increased lipophilicity of these compounds leading to an enhanced binding on plasma proteins and an increased distribution in favor of adipose tissue, thus decreasing the concentration of unbound and therefore available compound.

If it is true that the plasma concentration of free compound is significantly reduced in the case of homologues with a longer alkyl chain, it would mean that such a compound should show a reduced inhibition of desmolase and therefore less side effects. This would be an advantage for a possible use in therapy because the compound had shown a similar reduction of the E_2 concentration.

To examine this further, experiments are in preparation to monitor the influence of the test compounds on the plasma progesterone concentration of rats as a measure of their inhibitory activity against desmolase, as well as to test the ability of the compounds to induce abortions in pregnant rats. Pregnancy is known to be maintained by plasma progesterone, and AG itself apparently induces abortions by inhibiting desmolase and, hence, depleting progesterone precursors.[33]

The present data confirm the finding[26] that the intact DMBA-tumor-bearing SD rat is not a suitable test model for the evaluation of aromatase inhibitors of the AG type. It seems that only irreversible inhibitors (e.g., 4-hydroxyandrostenedione) show strong antitumor activity on this experimental tumor.[26] On the other hand, the new test model presented seems to be appropriate for the evaluation of reversible inhibitors of aromatase. It will be described in more detail in a subsequent publication. This test model is based on the finding that application of testosterone can prevent the tumors from ovariectomy-induced regression. This has already been observed and explained to be the result of interaction of testosterone with the estrogen receptor,[20] i.e., an estrogenic effect of this androgen. The argument against this explanation, however, is the fact that this testosterone-stimulated effect can be fully inhibited by the aromatase inhibitors and that the latter compounds do not show antiestrogenic activity in the antiuterotrophic test; i.e., they are not able to antagonize the uterine growth-stimulating effect of estrone in the immature mouse (unpublished data).

The finding of the PMSG test that the test compounds are superior to AG was confirmed in this tumor test. They showed the same antitumor effect tested in smaller doses compared to the parent compound.

The present data give rise to the hope that among the newly synthesized 3-alkyl-substituted 3-(4-aminophenyl)piperidine-2,6-diones there are more effective drugs or at least similarly effective compounds with reduced side effects compared to AG. Nevertheless one has to bear in

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mind that AG formerly was in use as an anticonvulsant drug. The new derivatives of AG only then mean a progress in the treatment of hormone-dependent breast cancer or other estrogen-dependent disease conditions, if they do not show stronger depressant action on the CNS than the parent compound. Studies examining this question are presently in progress.

Experimental Section

General Procedures. TLC of each compound was performed on Merck F 254 silica gel or Merck F 254 neutral Al_2O_3 60 plates, respectively. Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, Universität Regensburg. The structures of all compounds were confirmed by their IR (Beckman Acculab 3) and ^1H NMR spectra (Varian EM 390, 90 MHz).

The following compounds are representatives of synthetic methods A–E reported in Tables I–III.

Method A. 2-Ethyl-2-phenylacetonitrile (3d). To a mixture of phenylacetonitrile (11.7 g, 0.1 mol) and EtI (16.0 g, 0.1 mol) in 30 mL of dry benzene was slowly added a suspension of $NaNH_2$ (3.90 g, 0.1 mol) in toluene at 80 °C. After 1 h the mixture was cooled and water was added. The organic layer was separated, washed with water, and dried (Na_2SO_4). The solvent was removed and the product distilled under reduced pressure to give 9.71 g of **3d**.

Method B. 2-Ethyl-2-phenylglutarodinitrile (3c). Acrylonitrile (5.31 g, 0.1 mol) and **3d** (13.3 g, 0.1 mol) were dissolved in 100 mL of dry dioxane. At 100 °C a solution (40%) of Triton B (trimethylbenzylammoniumhydroxide; 16.7 g, 0.1 mol) in methanol was added slowly with stirring. The mixture was refluxed for 15 h. The solvent was removed and the residue dissolved in ether. The solution was washed with water and dried (Na_2SO_4). The ether was evaporated, and the resulting crude product was purified by column chromatography (SiO_2; CH_2Cl_2:ethyl acetate = 99:1) to yield 10.5 g of **3c**.

Method C. 3-Ethyl-3-phenylpiperidine-2,6-dione (3b). A mixture of **3c** (19.8 g, 0.1 mol), 150 mL of concentrated H_2SO_4, and 500 mL of acetic acid was heated in a water bath for 6 h. The resulting dark-brown liquid was poured onto crushed ice and extracted with CH_2Cl_2. The organic layer was repeatedly washed with a saturated Na_2CO_3 solution and water and dried (Na_2SO_4). After removal of the solvent the resulting crude product was recrystallized from MeOH/H_2O to give 12.8 g of **3b**.

Method D. 3-Ethyl-3-(4-nitrophenyl)piperidine-2,6-dione (3a). At −10 °C a mixture of 11.0 g of concentrated H_2SO_4 and 11.0 g of HNO_3 (63%) was slowly added to a suspension of **3b** (21.7 g, 0.1 mol) in 80.0 g of concentrated H_2SO_4. After stirring for 2 h at 0 to −10 °C, the reaction mixture was decomposed with ice and extracted with CH_2Cl_2. The organic layer was washed and dried (Na_2SO_4). After evaporation of the solvent the crude product was crystallized from MeOH/H_2O to yield 2.36 g (9%) of **3a** (mp 141–142 °C).

3-Isopropyl-3-(4-nitrophenyl)piperidine-2,6-dione (5a). The crude product was crystallized from MeOH to yield 1.66 g (6%) of **5a** (mp 133–134 °C).

3-*n*-Butyl-3-(4-nitrophenyl)piperidine-2,6-dione (6a). The crude product was crystallized from MeOH/ether to yield 2.32 g (8%) of **6a** (mp 108–109 °C).

Method E. 3-(4-Aminophenyl)-3-ethylpiperidine-2,6-dione (3). Palladium on charcoal (10%, 0.1 g) was added to a solution of **3a** (2.62 g, 0.01 mol) in 100 mL of EtOH. The suspension was shaken under a hydrogen atmosphere until no more H_2 was accepted. The reaction mixture was filtered. The solvent was removed, and the crude product was recrystallized from MeOH to give 2.11 g (91%) of **3**.

Biological Methods

Enzyme Assays. The following applies to the enzyme assays described below. Each time point of control or inhibitor incubation was run in triplicate. The amount of product formed was averaged. At each concentration of inhibitor, the samples were removed from each assay tube at three time points. The results were plotted on a graph of product against time of incubation. The resulting linear graph was used to determine the inhibition of enzyme reaction at the corresponding concentration of inhibitor by comparing the values to those of control samples (no inhibitor) that were run simultaneously. The inhibition values are given in Table V or were used to calculate the IC_{50} values of Table IV. For the determination of the latter values each inhibitor was tested in six appropriate concentrations. The percent inhibition was plotted vs. the concentration of inhibitor on a semilog plot. From this the molar concentration causing 50% inhibition was calculated. Protein concentration was determined by the method of Lowry.

Preparation of Aromatase. The enzyme was obtained from the microsomal fraction of freshly delivered human term placental tissue according to the procedure of Thompson and Siiteri.[34] The isolated microsomes were suspended in a minimum volume of phosphate buffer (0.05 M, pH 7.4) and stored at −30 °C as described.[34] No loss of activity was observed within 4 months.

Inhibition of Aromatase. This assay was performed similar to described methods[8,35] monitoring enzyme activity by measuring the ^3H_2O formed from [1β,2β-^{3}H]testosterone during aromatization. Each incubation tube contained 0.225 μCi of [1β,2β-^{3}H]testosterone, 5 μM unlabeled testosterone, 2 mM NADPH, 20 mM glucose-6-phosphate, 1 EU glucose-6-phosphate dehydrogenase, and inhibitor (0–250 μM) in phosphate buffer (0.05 M, pH 7.4). The test compounds had been dissolved in EtOH and diluted with buffer. The final EtOH concentration of control and inhibitor incubation was 2%. Each tube was preincubated for 5 min at 30 °C in a shaking water bath. Microsomal protein (0.5 mg) was added to start the reaction. The total volume for each incubation was 0.5 mL. The reaction was terminated by withdrawing 100-μL aliquots at 0, 7, 14, and 21 min and pipetting them into 200 μL of a cold 1 mM $HgCl_2$ solution. After addition of 200 μL of an aqueous dextran-coated charcoal (DCC) suspension (2%), the vials were shaken for 20 min and centrifuged at 1500*g* for 5 min to separate the charcoal-adsorbed steroids. Aliquots of the supernatant were assayed for ^3H_2O by counting in a scintillation mixture in a Beckman liquid scintillation spectrometer (LS 8000).

Irreversible Inhibition of Aromatase. This assay was performed similar to the procedure of Brodie et al.[20] Each incubation tube contained phosphate buffer, NADPH, NADPH-generating system, and microsomal protein in the concentrations described above, as well as inhibitor (0 or 50 μM), the total volume being 0.5 mL. The tubes were incubated for 15, 30, and 60 min at 30 °C. After addition of 200 μL of an aqueous DCC suspension (2%) the vials were shaken for 10 min and centrifuged at 1500*g* to separate the charcoal-adsorbed inhibitor. The supernatant was then incubated with NADPH, NADPH-generating system, 0.225 μCi of [1β,2β-^{3}H]testosterone, and 5 μM unlabeled testosterone for 30 min. The reaction was terminated and the assay processed as described above.

Preparation of Desmolase. This enzyme was obtained from the mitochondrial fraction of bovine adrenal cortex according to the method of Hochberg et al.[36] After resuspension in a minimum volume of buffered sucrose, the isolated mitochondria were stored at −70 °C, as described.[8] Enzyme activity remained stable within 3 months.

Inhibition of Desmolase. After thawing, the mitochondrial suspension was sonicated with a microtip at 0 °C, 3 times for 10 s each time with intervals of 1 min. The resulting suspension was clarified by centrifugation at 8000*g* for 15 min. The supernatant was used in the assay described below. The enzyme activity was assayed similar to the described method[36] using [26-^{14}C]cholesterol as substrate and measuring the [^{14}C]isocaproic acid released. Each incubation tube contained 0.168 μCi of [26-^{14}C]cholesterol, 10 μg of Tween 80 (necessary for the solubilization of the cholesterol, which was performed according to ref 36), 1 mg of mitochondrial protein, and inhibitor (0 or 25 μM) in Tris-HCl buffer (0.1 M $MgCl_2$, 0.01 M, pH 7.4). The test compounds had been dissolved in EtOH and diluted with buffer. The final EtOH concentration

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of control and inhibitor incubation was 1%. Each tube was preincubated for 3 min at 30 °C in a shaking water bath. The reaction was started by the addition of NADPH (1 mM) and NADPH-generating system (10 mM glucose-6-phosphate and 1 EU glucose-6-phosphate dehydrogenase). The total volume for each incubation was 1 mL. The reaction was stopped by withdrawing 200-μL aliquots at 0, 3, 6, and 9 min and pipetting them into 1.5 mL of cold glycin–$HgCl_2$–buffer (0.05 M, $HgCl_2$ 1 mM, pH 9.5). The diluted aliquots were then filtered through a microcolumn of Al_2O_3 (neutral, thin-layer grade, mesh <40 μm, Merck, Darmstadt, FRG), which was prepared by scooping 500 ± 100 mg of Al_2O_3 into a Pasteur pipet plugged with glass wool. The percolation was complete by the next morning. Aliquots of the eluate were pipetted into scintillation mixture and counted.

In Vivo Inhibition of E_2 Secretion and Ovarian Aromatase Activity. Three-month-old female Sprague–Dawley rats were obtained from the Zentralinstitut für Versuchstierzucht Hannover. The animals were primed with pregnant mare serum gonadotropin (PMSG) similar to the method of Brodie et al.[21] Subcutaneous injections of 100 IU of PMSG in 500 μL of saline were applied for 12 days on alternate days. On day 12 the rats received a single sc injection of olive oil (control) or of an olive oil solution of the text compound: 2 mg/kg of compound **3** or equimolar doses of the other compounds. After 6 h the animals were anesthetized and blood was taken from the ovarian vein and the right ventricle of the heart. The ovaries were excised, weighed, and processed in ice-cold phosphate buffer (0.1 M, pH 7.4).

A. Determination of E_2 Concentration. The plasma was obtained by centrifugation at 1500*g* using 125 IU of heparin-Na/1 mL of blood. The E_2 concentration was determined by radioimmunoassay (RIA) technique using the direct E_2 assay of DRG-Instruments, Marburg, FRG.

B. Determination of Ovarian Aromatase Activity. After pooling of both ovaries of each animal, ovarian microsomes were prepared as described.[21] The incubation was performed as described under "Inhibition of Aromatase" with the exception of using 1 μCi of [1β,2β-^{3}H]testosterone. Neither inhibitor nor EtOH was present in the incubation mixture. Samples were removed at 10, 20, and 30 min. Further processing was identical with that described under Inhibition of Aromatase.

Mammary Tumor Studies. The methods used for tumor induction and assignment to treatment groups were similar to those formerly described by us.[37] At the age of 50 days, female SD rats were administered a single dose of 20 mg of 9,10-dimethyl-1,2-benzanthracene (DMBA) in 1 mL of olive oil by gastric incubation. Rats were palpated twice weekly beginning 4 weeks later. They were assigned to groups of 10 animals, when at least one tumor reached an area of 140 mm^2. The tumor area is the product of measurements of two perpendicular diameters, one across the longest dimension. The maximum number of tumors per animal was 6; on an average one rat beared three tumors at the start of the treatment. Each group consisted of rats with approximately the same total number of tumors and total tumor area. In groups with ovariectomy as part of the treatment scheme, the ovaries of the tumor-bearing hosts were removed through incisions in the lumbar region of the back one day before the start of the treatment. The animals were subcutaneously administered olive oil (control) or olive oil solutions of the test compounds 6 times a week for 4 weeks. Measurement of tumor size and determination of body weight were made weekly. At the 29th day after initiation of the therapy the animals were killed, and the uteri were removed and dissected free from fat, fixed with Bouin's fluid, and processed as formerly described by us.[38] Finally the uterine weights were determined.

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Registry No. **1**, 92137-90-1; **1b**, 14149-34-9; **1c**, 91137-66-5; **2**, 100134-86-9; **2b**, 14149-35-0; **2c**, 102746-75-8; **2d**, 1823-91-2; **3**, 125-84-8; **3b**, 77-21-4; **3c**, 74220-50-1; **3d**, 769-68-6; **4**, 102746-46-3; **4b**, 21389-09-3; **4c**, 102746-76-9; **4d**, 5558-78-1; **5**, 102746-47-4; **5b**, 102746-57-6; **5c**, 102746-77-0; **5d**, 5558-29-2; **6**, 102746-48-5; **6b**, 21389-10-6; **6c**, 102746-78-1; **6d**, 3508-98-3; **7**, 102746-49-6; **7b**, 102746-58-7; **7c**, 102746-79-2; **7d**, 5558-31-6; **8** (isomer 1), 102746-50-9; **8** (isomer 2), 102746-64-5; **8b** (isomer 1), 102746-59-8; **8b** (isomer 2), 102746-63-4; **8c** (isomer 1), 102746-80-5; **8c** (isomer 2), 102868-99-5; **8d** (isomer 1), 102746-69-0; **8d** (isomer 2), 102746-72-5; **9**, 102746-51-0; **9b**, 21389-11-7; **9c**, 102746-81-6; **9d**, 5558-33-8; **10**, 102746-52-1; **10b**, 102746-60-1; **10c**, 102746-82-7; **10d**, 5558-34-9; **11** (isomer 1), 102746-53-2; **11** (isomer 2), 102746-66-7; **11b** (isomer 1), 102746-61-2; **11b** (isomer 2), 102746-65-6; **11c** (isomer 1), 102746-83-8; **11c** (isomer 2), 102746-87-2; **11d** (isomer 1), 102746-70-3; **11d** (isomer 2), 102746-73-6; **12** (isomer 1), 102746-54-3; **12** (isomer 2), 102746-68-9; **12b** (isomer 1), 102746-62-3; **12b** (isomer 2), 102746-67-8; **12c** (isomer 1), 102746-84-9; **12c** (isomer 2), 102746-88-3; **12d** (isomer 1), 102746-71-4; **12d** (isomer 2), 102746-74-7; **13**, 102746-55-4; **13b**, 21389-12-8; **13c**, 102746-85-0; **13d**, 5558-35-0; **14**, 102746-56-5; **14b**, 21389-13-9; **14c**, 102746-86-1; **14d**, 5558-36-1; CH_3I, 74-88-4; C_2H_5I, 75-03-6; $I(CH_2)_2CH_3$, 107-08-4; $ICH(CH_3)_2$, 75-30-9; $I(CH_2)_3CH_3$, 542-69-8; $ICH_2CH(CH_3)_2$, 513-38-2; $ICH(CH_3)C_2H_5$, 513-48-4; $I(CH_2)_4CH_3$, 628-17-1; $I(CH_2)_2CH(CH_3)_2$, 541-28-6; $ICH_2CH(CH_3)C_2H_5$, 616-14-8; $ICHCH_3(CH_2)_2CH_3$, 637-97-8; $I(CH_2)_5CH_3$, 638-45-9; $I(CH_2)_6CH_3$, 4282-40-0; aromatase, 9039-48-9; desmolase, 101149-96-6; phenylacetonitrile, 140-29-4; acrylonitrile, 107-13-1.

Supplementary Material Available: ^{1}H NMR data (Tables VIII–X) of compounds **1d–14d**, **1c–14c**, **1b–14b**, **1–14** (9 pages). Ordering information is given on any current masthead page.

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