Aromatase Inhibitors. Syntheses and Structure–Activity Studies of Novel Pyridyl-Substituted Indanones, Indans, and Tetralins

Rolf W. Hartmann,* Herbert Bayer,† and Gertrud Grün

Fachrichtung 12.1 Pharmazeutische Chemie, Universität des Saarlandes, P.O. Box 15 11 50, D-66041 Saarbrücken, Germany

The (*E*)-2-(4-pyridylmethylene)-1-indanones (*E*)-1–(*E*)-5 [(*E*)-1, H; (*E*)-2, 4-OCH₃; (*E*)-3, 5-OCH₃; (*E*)-4, 4-OH; (*E*)-5, 5-OH] were obtained by aldol condensation of the corresponding 1-indanones with 4-pyridinecarboxaldehyde, and in case of the OH compound (*E*)-4 subsequent ether cleavage of (*E*)-2. The synthesis of the (*Z*)-isomers (*Z*)-1–(*Z*)-3 [(*Z*)-1, H; (*Z*)-2, 4-OCH₃; (*Z*)-3, 5-OCH₃] was accomplished by UV irradiation of the corresponding (*E*)-isomers. Catalytic hydrogenation of (*E*)-1–(*E*)-3 gave the 2-(4-pyridylmethyl)-1-indanones **6–8** (6, H; 7, 4-OCH₃; 8, 5-OCH₃). The 2-(4-pyridylmethyl)-substituted indans **11–13** (11, H; 12, 4-OCH₃; 13, 5-OCH₃) and the tetralins **16–19** (16, H; 17, 5-OCH₃; 18, 6-OCH₃; 19, 7-OCH₃) were obtained by reduction of the corresponding ketones using N₂H₄/KOH. The 2-(4-pyridylmethyl)-substituted indanones **9** (4-OH) and **10** (5-OH), indans **14** (4-OH) and **15** (5-OH), and tetralins **20–22** (20, 5-OH; 21, 6-OH; 22, 7-OH) were synthesized by ether cleavage of the corresponding OCH₃ compounds. All compounds showed inhibition of human placental aromatase exhibiting relative potencies from 0.9 [(*E*)-4] to 163 [18; aminoglutethimide (AG) potency ≡ 1]. Compounds **13** and **18** showed competitive type of inhibition and a type II difference spectrum, indicating the interaction of the pyridyl-N with the central Fe(III) ion of the cytochrome P450 heme component. Only the OH-substituted indans and tetralins inhibited bovine adrenal desmolase with maximum activity shown by **20** and **22** (12% inhibition, 25 µM; AG, 53% inhibition, 25 µM). In vivo, however, all tested aromatase inhibitors (6, 8, 10, 14, 15, 18 and 20) were less active than AG concerning the inhibition of the uterotrophic activity of androstenedione (6, 8, 10, 15), the reduction of the plasma estradiol concentration (14, 20), and the mammary carcinoma (MC) inhibiting properties (18, 20; androstenedione-stimulated juvenile rats, pregnant mares' serum gonadotropin-primed rats as well as dimethylbenzanthracene-induced MC of the Sprague-Dawley rat, postmenopausal experiment). Since no affinity to the estrogen receptor was demonstrated (20), estrogenic effects as a cause for the poor tumor inhibiting activity have to be excluded.

Beside antiestrogens and progestins, aromatase inhibitors are used today for the endocrine treatment of advanced breast cancer of postmenopausal women.[1] Aromatase (P450 arom, CYP 19) is a cytochrome P450 enzyme which catalyses the conversion of androgens to estrogens.[1] In the premenopausal woman, the enzyme is mainly found in the ovaries, in the postmenopausal woman, in muscle and adipose tissue.[1] Presently only one nonsteroidal aromatase inhibitor is commercially available, aminoglutethimide [3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione, AG].[1] AG is far from being an optimal drug, as it also inhibits other P450 enzymes catalyzing steroid biosynthesis,[2,3] e.g., the cholesterol side chain cleavage enzyme (P450 scc, CYP 11 A1) leading to a depletion of corticosteroid production.[4] This disadvantage of AG brought us and other groups to develop more potent and selective compounds,[5–13] resulting in a series of compounds which are presently under clinical evaluation.[5,6] One of our attempts started from flavone and flavanone (Chart 1),[14] natural products inhibiting P450 arom with about ¹/₁₀ the activity of AG.[14,15] A strong increase in aromatase inhibition could be achieved by structural modifications leading to pyridyl-substituted tetralones (Chart 1).[11,14] The most active compounds were the 5-OH-substituted (pyridylmethylene)- and (pyridylmethyl)-1-tetralones, exhibiting relative potencies of 190 and 28, respectively (related to AG; relative potency of AG ≡ 1).[11] They are very selective compounds showing only a weak inhibition

Chart 1

flavone
flavanone

X: H, OCH₃, OH
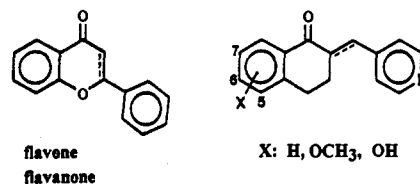
Chart 2
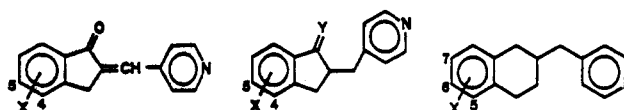
compd	1–5 X	compd	6–15 X	Y	compd	16–22 X
(*E*)-1	H	6	H	O	16	H
(*Z*)-1	H	7	4-OCH₃	O	17	5-OCH₃
(*E*)-2	4-OCH₃	8	5-OCH₃	O	18	6-OCH₃
(*Z*)-2	4-OCH₃	9	4-OH	O	19	7-OCH₃
(*E*)-3	5-OCH₃	10	5-OH	O	20	5-OH
(*Z*)-3	5-OCH₃	11	H	H, H	21	6-OH
(*E*)-4	4-OH	12	4-OCH₃	H, H	22	7-OH
(*E*)-5	5-OH	13	5-OCH₃	H, H		
		14	4-OH	H, H		
		15	5-OH	H, H		

of P450 scc. In vivo, however, the compounds are not superior to AG. The present paper describes structure–activity studies of the corresponding (pyridylmethylene)- and (pyridylmethyl)-substituted tetralins, indanones, and indans (compounds 1–22, Chart 2). In the following, their

Scheme 1
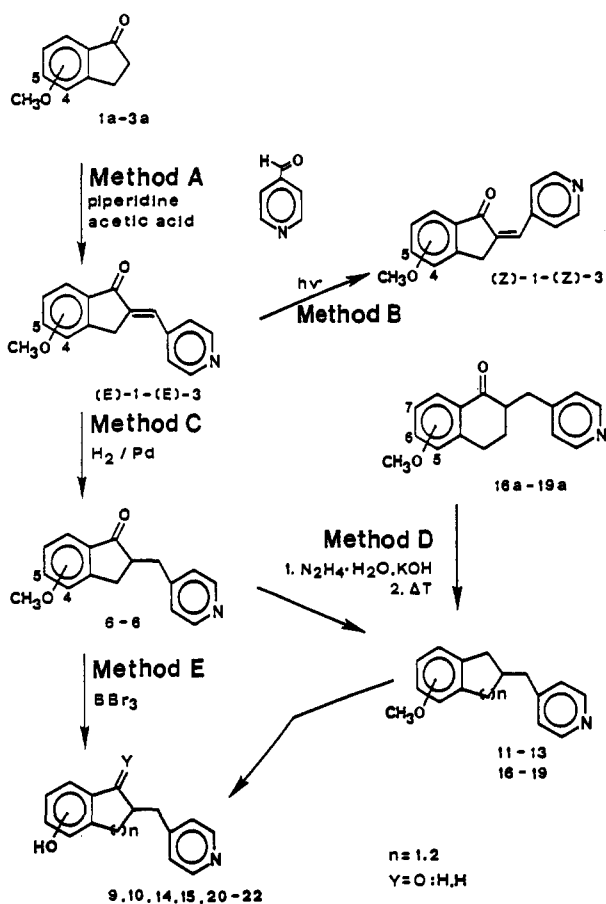
syntheses, the inhibitory activities toward P450 arom and P450 scc in vitro, the inhibition of the estrogen production, and the mammary tumor inhibiting activity in vivo will be described.

Chemistry

The starting materials for the syntheses of compounds 1–3 were the corresponding 1-indanones (1a-3a, Scheme 1). The substituted 2-(4-pyridylmethylene)-1-indanones (E)-1-(E)-3 (Table 1) were obtained by aldol condensation of the corresponding 1-indanones with 4-pyridinecarbox-aldehyde using piperidine/acetic acid as catalyst (method A, Scheme 1). The E configuration of the compounds was determined by ^{1}H-NMR spectroscopy according to the method previously reported by us for the analysis of pyridyl-substituted tetralones[11] (chemical shifts of vinyl-H singlets: δ (ppm) 7.55–7.89 for the (E)-isomers and 6.92–7.42 for the (Z)-isomers).

The preparation of the (Z)-isomers (Z)-1-(Z)-3 (Table 1) was accomplished by isolation from a mixture of the two geometrical isomers produced by UV irradiation of the corresponding (E)-isomers (E)-1-(E)-3 (method B, Scheme 1).

The 2-(4-pyridylmethyl)-1-indanones 6–8 (Table 1) were synthesized by catalytic hydrogenation of (E)-1-(E)-3 using palladium on charcoal (method C, Scheme 1). Reaction of compounds 6–8 with hydrazine hydrate and KOH gave the corresponding 2-(pyridylmethyl)indans 11–13 (method D, Table 1). The substituted tetralins 16–19 (Table 1) were obtained using the same method starting from the corresponding tetralones 16a–19a.

Ether cleavage of the OCH$_3$-substituted compounds (E)-2, 7, 8, 12, 13, and 17–19 was performed with BBr$_3$ (method

E), yielding the hydroxy compounds (E)-4 (Scheme 2, Table 1), 9, 10, 14, 15, and 20–22 (Scheme 1, Table 1). This method failed, however, in case of (E)-3. Therefore the synthesis of compound (E)-5 was accomplished by aldol condensation of 5-hydroxy-1-indanon (5a) with 4-pyridi-necarboxaldehyde and piperidine/acetic acid (method A, Scheme 2). Compound 5a was obtained by the reaction of 3-(3-hydroxyphenyl)propanoic acid (5b) with AlCl$_3$/ NaCl.

Biological Properties

Inhibition of Aromatase in Vitro. The inhibitory activities of the compounds toward aromatase were determined in vitro using human placental microsomes and [1β,2β-^{3}H]testosterone according to the method of Thompson and Siiteri.[17] The IC$_{50}$ values and the potencies of the compounds, relative to AG, are given in Table 2.
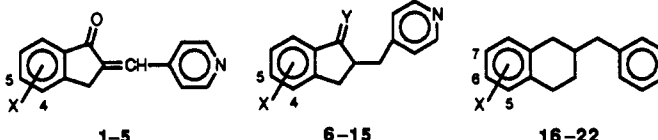
With the exception of the two hydroxy-substituted enones (E)-4 and (E)-5 [relative potency (rp) = 0.9 and < 1, respectively], all compounds exhibited a stronger inhibitory activity toward aromatase than AG (rp = 1).

Exhibiting relative potencies ranging from <1 to 11, the indanone derivatives 1–10 turned out to be less active than the corresponding tetralone derivatives (rp values of 2.2–190[11]). The (Z)-configurated 2-(4-pyridylmethylene)-1-indanones (Z)-1-(Z)-3 showed very similar rp values (6.7, 5.3, and 5.9). For these compounds the activity does not depend on the OCH$_3$ substituents. Except compound (Z)-3, the (Z)-isomers were more active than the corresponding (E)-isomers. The most active compound of the unsaturated indanones was compound (E)-3, exhibiting a rp value of 7.7. In accordance with the results observed with the tetralones,[11] hydrogenation of the unsaturated (E)-configurated indanones enhanced the rp values without exception; i.e., the 2-(4-pyridylmethyl)-substituted indanones 6–10 were more active than the corresponding pyridylmethylene compounds (E)-1-(E)-5. Exhibiting a rp value of 11, compound 8 was the most active indanone derivative of this series.

From the results listed in Table 2, it is demonstrated that the reduction of the (pyridylmethyl)-substituted indanones (6–10; rp values of 2.2–11) to the corresponding indans (11–15; rp values of 5.8–154) enhanced aromatase inhibition strongly. The same applies to the tetralins: they are very strong aromatase inhibitors (rp values of 8.6–163), and with the exception of compound 20 (rp = 70), they are more active than the corresponding tetralones (rp values of 4.4–17; rp of 5-hydroxy-2-(4-pyridylmethyl)-1-tetralone = 190).[11]

The wide range of rp values in most classes of aromatase inhibitors described in this paper indicates that the OCH$_3$ and OH substituents have a substantial influence on the aromatase inhibitory activity: In case of the indans a OCH$_3$ group in the 4-position decreased activity [rp values of 15 (11) and 5.8 (12), respectively], whereas the same substituent in the 5-position increased the activity strongly (13, rp = 154). In the case of the tetralins, 5-OCH$_3$ substitution (corresponding to 4-OCH$_3$ as for the indans) did not decrease but enhanced activity [rp values of 32 (16) and 63 (17), respectively] and 6-OCH$_3$ substitution increased activity even more (18, rp = 163). The same substituent in the 7-position decreased activity drastically (19, rp = 8.6).

The ether cleavage of the OCH$_3$ compounds 12, 13, and 17–19 to the corresponding OH derivatives 14, 15, and

Table 1. Substituted 2-(4-Pyridylmethylene)-1-indanones (1–5) and 2-(4-Pyridylmethyl)-1-indanones (6–10), -indans (11–15), and -tetralins (16–22)

compd	X	Y	mp, °C	formula[a]	method of prepn	recryst solvent	yield, %
(E)-1[b]	H		167–168	$C_{16}H_{11}NO$	AI	CH_3OH	34
(Z)-1	H		136–137.5	$C_{16}H_{11}NO$	B	acetone	22
(E)-2	4-OCH₃		195–196	$C_{16}H_{13}NO_2$	AII	CH_3OH	56
(Z)-2	4-OCH₃		137.5–138.5	$C_{16}H_{13}NO_2$	B	ethyl acetate	19
(E)-3	5-OCH₃		170.5–171	$C_{16}H_{13}NO_2$	AI	C_2H_5OH	52
(Z)-3	5-OCH₃		138–139	$C_{16}H_{13}NO_2$	B	acetone	25
(E)-4	4-OH		>220 (dec)	$C_{16}H_{11}NO_2$	E	c	76
(E)-5[b]	5-OH		>220 (dec)	$C_{15}H_{11}NO_2$	AI	d	52
6[b]	H	O	62–63	$C_{16}H_{13}NO$	C	petroleum ether (40–60 °C)	68
7	4-OCH₃	O	113–114	$C_{16}H_{15}NO_2$	C	C_2H_5OH	74
8	5-OCH₃	O	154–155	$C_{16}H_{15}NO_2$	C	C_2H_5OH	80
9	4-OH	O	219.5–221	$C_{16}H_{15}NO_2$	E	C_2H_5OH/H_2O	35
10	5-OH	O	195 (dec)	$C_{16}H_{13}NO_2$	E	H_2O	30
11	H	H, H	40–41	$C_{16}H_{15}N$	D	n-hexane[e]	46
12	4-OCH₃	H, H	46.5–47.5	$C_{16}H_{17}NO$	D	n-hexane[e]	42
13	5-OCH₃	H, H	43–44	$C_{16}H_{17}NO$	D	n-hexane[e]	49
14	4-OH	H, H	198.5–200	$C_{16}H_{15}NO$	E	C_2H_5OH	71
15	5-OH	H, H	202–204.5	$C_{16}H_{15}NO$	E	C_2H_5OH/H_2O	63
16	H		40–41	$C_{16}H_{17}N$	D	n-hexane	61
17	5-OCH₃		61–62	$C_{17}H_{19}NO$	D	n-hexane	64
18	6-OCH₃		oil	$C_{17}H_{19}NO$	D	f	68
19	7-OCH₃		80–81	$C_{17}H_{19}NO$	D	n-hexane	59
20	5-OH		203–204	$C_{16}H_{17}NO$	E	C_2H_5OH	85
21	6-OH		203–204	$C_{16}H_{17}NO$	E	C_2H_5OH	74
22	7-OH		195.5–197	$C_{16}H_{17}NO$	E	C_2H_5OH	78

[a] C, H, and N analyses were within ±0.4% of the theoretical values. [b] See ref 16. [c] The crude product was suspended in acetone and after filtration washed with acetone and Et₂O and dried in vacuo. [d] The same procedure outlined in footnote c was used, except that EtOH was used instead of acetone. [e] After some days at –20 °C. [f] Purification by bulb tube distillation.

Scheme 2
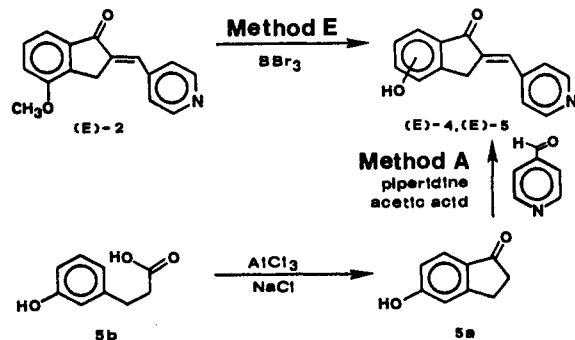
20–22 in most cases changed aromatase inhibitory activity considerably; i.e., the OH compounds exhibited both increased and diminished activity compared to the OCH₃ compounds. The differences are only marginal, however, if their activities are compared to those of the unsubstituted parent compounds (Table 2).

As some steroidal aromatase inhibitors show irreversible inhibition of this enzyme, this issue was examined with two select compounds (17 and 20) using the method of Brodie et al.[18] with modifications.[8] Human placental microsomes were incubated with NADPH and inhibitor (10 μM) for 30 min. After treatment with dextran-coated charcoal, enzyme activity was determined. None of the compounds caused a reduction of enzyme activity (data not shown). This is in accordance with previous observations of our group, implying that there is no nonsteroidal aromatase inhibitor showing irreversible enzyme inhibition.[8,11,13,14]

To get further insight into the mode of aromatase inhibition of this class of compounds, Lineweaver–Burk and difference spectroscopy experiments were performed with the highly active compounds 13 and 18. Both showed a competitive inhibition vs testosterone (figure not shown; apparent K_i values for 13 and 18 = 5 ± 2 and 4 ± 2 nM, respectively). The difference spectrum of 18 toward solubilized high spin aromatase is shown in Figure 1. Exhibiting a minimum at 390 nm and a maximum at 431 nm, the spectrum is typical of the interaction of a basic nitrogen (pyridyl-N) with the central Fe(III) ion of the cytochrome P450 heme component (type II difference spectrum).[19]

Inhibition of Desmolase in Vitro. The inhibitory activities of select indanones, indans, and tetralins toward desmolase were determined in vitro using bovine adrenal mitochondria and [26-¹⁴C]cholesterol according to the method of Hochberg et al.[20] The inhibition values of the test compounds in concentrations of 25 μM are presented in Table 3.

The (pyridylmethylene)- and (pyridylmethyl)-substituted indanones showed no inhibition. The unsubstituted and OCH₃-substituted indans and tetralins exhibited no effect on this enzyme either. The OH-substituted indans and tetralins, however, inhibited desmolase marginally (percent inhibition ≤ 12; Table 3).

Inhibition of Aromatase in Vivo. In order to elucidate whether these highly active in vitro aromatase inhibitors are capable of unfolding their activity in vivo as well, select compounds were further tested.

In juvenile female rats androstenedione treatment stimulates uterine weight strongly. This effect is caused by ovarian aromatization of the androgen and can be dose-

Table 2. Inhibition of Human Placental Aromatase by 2-(4-Pyridylmethylene)-1-indanones (1–5) and 2-(4-Pyridylmethyl)-1-indanones (6–10), -indans (11–15), and -tetralins (16–22)

compd	X	IC$_{50}$,^{a} μM	rp^b
		Indanones	
(E)-1	H	25	1.5
(Z)-1	H	5.5	6.7
(E)-2	4-OCH$_3$	23	1.6
(Z)-2	4-OCH$_3$	3.7	5.3
(E)-3	5-OCH$_3$	4.8	7.7
(Z)-3	5-OCH$_3$	6.3	5.9
(E)-4	4-OH	22^c	0.9
(E)-5	5-OH	>19^{c,d}	<1
6	H	17	2.2
7	4-OCH$_3$	14	2.6
8	5-OCH$_3$	3.4	11
9	4-OH	4.2	8.8
10	5-OH	9.3	4.0
		Indans	
11	H	2.4	15
12	4-OCH$_3$	6.4	5.8
13	5-OCH$_3$	0.24	154
14	4-OH	1.0	37
15	5-OH	1.9	20
		Tetralins	
16	H	1.2	32
17	5-OCH$_3$	0.59	63
18	6-OCH$_3$	0.23	163
19	7-OCH$_3$	4.3	8.6
20	5-OH	0.53	70
21	6-OH	0.75	49
22	7-OH	1.3	29

^{a} IC$_{50}$ is the concentration of inhibitor required to give 50% inhibition. Concentration of testosterone, 5 μM. The given values are mean values of at least three experiments. The deviations were within ±5%. ^{b} Relative potency, calculated from the IC$_{50}$ values and related to AG (IC$_{50}$ of AG = 37 μM). ^{c} Double the EtOH concentration was used compared to the standard assay (IC$_{50}$ of AG = 19 μM). ^{d} Exact determination not possible because of poor solubility.

dependently antagonized by aromatase inhibitors.[21] Table 4 shows the antiuterotrophic effects of the indanones 6, 8 and 10 and the indan 15. While compound 6 (rp = 2.2) shows no significant inhibition, the stronger aromatase inhibitors 10 (rp = 4), 8 (rp = 11), and 15 (rp = 20) exhibit significant effects (37, 46 and 49% inhibition respectively). No compound, however, was as potent as AG (rp = 1; 73% inhibition).

The effect of compound 14 and 20 on the plasma estradiol (E$_2$) level was determined using pregnant mares' serum gonadotropin (PMSG) stimulated female rats according to the procedure of Brodie et al.[22] The E$_2$ concentration lowering effect of the compounds was determined 1, 6, and 24 h after subcutaneous application of a single dose of inhibitor (1.94 and 2.06 mg/kg, respectively, equimolar to 2 mg/kg AG) using a conventional radioimmunoassay technique. In spite of a high activity in vitro (rp values of 37 and 70, respectively), neither compound 14 nor compound 20 showed strong inhibitory effects. (14: 1 h, 17% inhibition vs untreated control; 6 h, 15%; 24 h, −5%. 20: 1 h, 8%; 6 h, 32%; 24 h, −10%. Not significant ($p > 0.05$), $n = 5$ or 6, Student's t-test.) Aminoglutethimide (2 mg/kg) tested under the same experimental conditions shows a strong inhibitory effect (6 h, 67% inhibition).[8]

Antitumor Activity. The antitumor activity of compound 18 and 20 was determined using the dimethylbenzanthracene (DMBA) induced mammary carcinoma of the ovariectomized, testosterone propionate-treated Sprague-Dawley rat.[23] This model mimics the endocrine situation
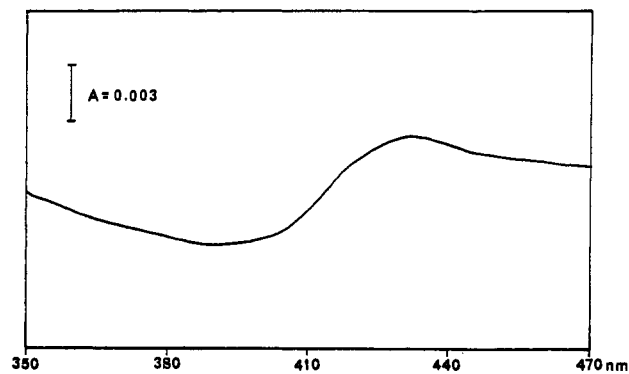
Figure 1. Difference spectrum obtained by the addition of compound 18 to solubilized low-spin aromatase (microsomal protein, 0.4 mg/mL; EtOH, 0.9%; compound 18, excess).

Table 3. Inhibition of Bovine Adrenal Desmolase by Select 2-(4-Pyridylmethylene)-1-indanones and 2-(4-Pyridylmethyl)-1-indanones, -indans, and tetralins

compd	X	% inhibn^{a,b}	compd	X	% inhibn^{a,b}
(E)-1	H	none^c	14	4-OH	11
(Z)-1	H	none	15	5-OH	9
(E)-3	5-OCH$_3$	none			
			16	H	none
8	5-OCH$_3$	none	17	5-OCH$_3$	none
9	4-OH	none	18	6-OCH$_3$	none
10	5-OH	none	20	5-OH	12
			21	6-OH	10
13	5-OCH$_3$	none	22	7-OH	12

^{a} Concentration of inhibitor, 25 μM. ^{b} Inhibition caused by AG = 53% (25 μM). ^{c} None means inhibition values ranging from +5 to −5%.

of a postmenopausal or ovariectomized woman. As can be seen from the percent change of tumor area in Table 5, the ovariectomy-induced regression can be overcome by the administration of testosterone. This stimulation of tumor growth is due to aromatization in peripheral tissues[23] and consequently can be dose-dependently inhibited by aromatase inhibitors.[23]

As can be seen from Table 5 the OH compound 20 shows no antitumor effect, whereas the OCH$_3$ compound 18 retards tumor growth, but is less active than AG.

In order to exclude estrogenic effects as a cause for the lack of tumor-inhibiting activity of compound 20, the relative binding affinity (RBA) of 20 (and 17) for the estrogen receptor was determined. Interaction with this receptor is a prerequisite for an estrogenic (or antiestrogenic) effect. The test was performed using calf uterine cytosol as the source of the receptor and the dextrancoated charcoal method.[24] None of the tested compounds showed detectable affinity for the estrogen receptor [maximum concentration tested, 10^{-4} M (range 10^{-4}–10^{-6} M); RBA in all cases <0.01; for comparison, RBA of E$_2$ = 100].

Discussion

The present paper demonstrates that the (pyridylmethylene)- and (pyridylmethyl)-substituted indanones show a reduced aromatase inhibition compared to the correspondingly substituted tetralones.[11] The indans and tetralins, however, exhibit strongly increased activity compared to the indanones and tetralones.[11] It is striking that the aromatase inhibitory properties of the pyridyl-substituted compounds not only depend on the type of benzocycloalkene moiety but also strongly on the position and the type of substituents (H, OH, or OCH$_3$) at the

Table 4. Effect of Select Aromatase Inhibitors on the Androgen-Stimulated Uterine Growth[a]

treatment group	effect[b] (means ± SD)	% inhibn
control	69 ± 12	
androstenedione[c]	175 ± 15	
AG[d]	97 ± 13[e,f]	73
control	88 ± 32	
androstenedione[c]	202 ± 42	
6[d]	181 ± 40	18
8[d]	150 ± 18[e,g]	46
10[d]	160 ± 12[e,h]	37
15[d]	147 ± 19[e,g]	49

[a] Immature female SD rats. [b] Uterus wet weight (mg)/body weight (g) × 100. [c] 30 mg/kg. [d] 0.043 mmol/kg + androstenedione 30 mg/kg. [e] Significantly different from androgen-stimulated control (Student's t-test). [f] $p < 0.001$. [g] $p < 0.05$. [h] $p < 0.1$.

Table 5. Effect of AG and Compounds 18 and 20 on the DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the Ovariectomized, Testosterone Propionate-Treated SD Rat (Postmenopausal Model)

treatment group[a]	dose mg/kg[b]	no. of tumors B[c]	no. of tumors NT[d]	% of tumors with CR[e]	% of tumors with PR[f]	% of tumors with NC[g]	% of tumors with P[h]	% change of tumor area[i,k]
control		28	0	82	18	0	0	−96
Tp		36	12	19	25	19	36	+141
Tp + AG	10.0	35	7	54	17	6	23	+4[l]
Tp + 18	10.9	31	3	29	19	19	32	+32
Tp + 20	10.3	37	4	24	16	19	41	+123

[a] Tp = testosterone propionate. [b] The animals received a single dose daily from Monday to Thursday, and a double dose on Friday, sc, as solution or suspension in olive oil. The compounds were administered equimolar to 10 mg/kg AG; dose of Tp, 23.5 mg/kg.[c] At the beginning of the test. [d] Occurring during the test. [e] CR = complete remission, tumor not palpable. [f] PR = partial remission, reduction of initial tumor size > 50%. [g] NC = no change, tumor size 51–150% of initial tumor size. [h] P = progression, tumor size >150% of initial tumor size. [i] Average on the 28th day of therapy. [k] The U test according to Wilcoxon, Mann, and Whitney was used. [l] Significantly different from the Tp group ($p < 0.05$).

benzene nucleus. The most active compounds are the 5-OCH$_3$-substituted (pyridylmethyl)indan 13 as well as the 6-OCH$_3$-substituted (pyridylmethyl)tetralin 18 exhibiting relative potencies of 154 and 163, respectively.

No X-ray structure exists of membrane (ER, endoplasmic reticulum) bound P450 arom. The knowledge of the active site comes from indirect experiments. On the basis of the competitive type of inhibition and the type II difference spectrum (shown in this paper) it has to be concluded that compounds 13 and 18 interact with the active site of aromatase with the pyridyl-N complexing the central Fe(III) ion of the cytochrome P450 component.

Bearing this in mind, one can suppose that the OCH$_3$ groups interact with the binding site of the enzyme for the 17-keto or 17β-hydroxy group of steroids. As a matter of fact, energy-minimized conformers of 13 and 18 and the steroidal substrates can be properly superimposed.[25] It is striking that the distance of the steroidal 19-CH$_3$ carbon to the Fe(III) ion in this model is very similar to the distance between the C-5 atom of camphor and the Fe(III) ion in the X-ray structure of the P450 cam–camphor complex[27] (in the P450 arom reaction the C-19 is hydroxylated, in the P450 cam reaction, the C-5 atom is).

This simple model, however, is not capable of explaining the finding that the hydroxy derivatives of 13 and 18, compounds 15 and 21, show a markedly reduced activity (by factors of 8 and 3). Using rigid high-activity templates, we presently are working on an aromatase binding model

which is also capable of explaining the data of the OCH$_3$ and OH compounds (13, 15, 18, and 21).

It is important for a possible therapeutic application that the compounds are selective with respect to inhibition of P450 scc, the key enzyme of steroidogenesis. Possible inhibition of further P450 enzymes is presently examined. In vivo, the compounds are poorly active and not superior to AG, though they had been much more active in vitro. The reason for this disappointing result remains to be clarified. It cannot be explained by an unsatisfactory uptake into intact cells, as compound 18 is 132 times as active as AG (on the basis of IC$_{50}$ values) as an aromatase inhibitor using human preadipocyte cell culture experiments (Löffler, unpublished results). It seems to be more probable that this type of inhibitor undergoes a fast metabolic degradation in rats.

Experimental Section

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytischen Laboratorien, University of Regensburg and University of the Saarland, and were within ±0.4% of the calculated values. ^{1}H-NMR spectra were measured on a Varian EM 360L (60 MHz), a Bruker WM 250 (250 MHz), and a AC 300 (300 MHz) spectrometer and are consistent with the assigned structures. Column chromatography was performed on Merck Kieselgel 60.

Method A. General Procedure for the Synthesis of the (E)-2-(4-Pyridylmethylene)-1-indanones 1–3 and 5. A.I. A mixture of 2.00 g (23.5 mmol) of piperidine, 2.00 g (33.3 mmol) of acetic acid, 16.1 g (150 mmol) of 4-pyridinecarboxaldehyde, and 100 mmol of the corresponding 1-indanones 1a–3a and 5a was heated at 130 °C for 1.5 h. After removal of the lower boiling materials under reduced pressure at temperatures not exceeding 130 °C, the residue was treated with CH$_2$Cl$_2$ [For the synthesis of (E)-3, the product was filtered off from the insoluble β-hydroxy ketone.] and extracted with 2 N HCl. The aqueous layer was neutralized with saturated NaHCO$_3$ solution and the crude product was collected, washed with water, and dried in vacuo. Recrystallization from a suitable solvent (Table 1) was carried out in the absence of light and yielded compounds (E)-1, (E)-3, and (E)-5.

A.II. As for method A.I., but after extraction of the CH$_2$Cl$_2$ phase with 2 N HCl, the hydrochloride was allowed to crystallize by cooling the aqueous layer. The solid was filtered, washed with 2 N HCl, and dissolved in water. The solution was neutralized by addition of a saturated NaHCO$_3$ solution. The crude product was isolated and purified as for method A.I. to yield compound (E)-2 (Table 1).

Method B. General Procedure for the Synthesis of the (Z)-2-(4-Pyridylmethylene)-1-indanones 1–3. Ten millimoles of the corresponding (E)-isomer was dissolved or suspended in 250 mL of EtOH (99%). The mixture was stirred and irradiated (λ = 200–600 nm) for 2 days. The isolation and purification of the product were performed in the absence of light. Compounds (Z)-1 and (Z)-3 were obtained by column chromatography (SiO$_2$; ethyl acetate), whereas compound (Z)-2 was isolated by fractional crystallization from MeOH. In the solid state the compounds are stable.

Method C. General Procedure for the Synthesis of the 2-(4-Pyridylmethyl)-1-indanones 6–8. A suspension of 50 mmol of the pyridylmethylene compound 1, 2, or 3 and 100 mg of palladium on charcoal (10%) in 250 mL of EtOH (99%) was shaken under a hydrogen atmosphere until the educt had completely dissolved and no more H$_2$ was accepted. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The crude product was recrystallized from a suitable solvent (Table 1) in the absence of light to give compound 6, 7, or 8.

Method D. General Procedure for the Synthesis of the Indans 11–13 and the Tetralins 16–19. A mixture of the ketones 6–8 and 16a–19a (15 mmol), 13.1 g KOH (234 mmol), 15 mL (247

mmol) of hydrazine hydrate (80%), and 100 mL of diethylene glycol was heated under reflux for 1.5 h. After removal of the water formed, the mixture was heated at 195 °C for an additional 4-h period. After cooling, the solution was diluted with H_2O and extracted with CH_2Cl_2. The organic extracts were washed with (H_2O) and dried (Na_2SO_4). The solvent was removed, the oily crude product purified by column chromatography (SiO_2, ethyl acetate) and recrystallized from n-hexane.

Method E. General Procedure for the Ether Cleavage Yielding 4, 9, 10, 14, 15, and 20–22. A solution of the methoxy compounds (E)-2, 7, 8, 12, 13, and 17–19 (5.0 mmol) in 150 mL of dry CH_2Cl_2, shielded from light, was cooled to –78 °C, and 4.38 g (17.5 mmol) of BBr_3 (for the synthesis of 10, 8.76 g) was added under N_2. After 30 min the cooling bath was removed and stirring was continued for 4 h (for the synthesis of 10, 3 h plus 1 h of refluxing). Hydrolysis was carried out by dropwise addition of 5 mL of MeOH (for the synthesis of 10, 10 mL) and was completed by stirring for 30 min. The mixture was concentrated to about one-half of the volume. After cooling, the solid was filtered and washed with CH_2Cl_2. If no or only small amounts of solid precipitated after hydrolysis, the reaction mixture was evaporated to dryness. The residue or the filtered solid was taken up in water and filtered from insoluble parts, and the product was precipitated by the addition of saturated $NaHCO_3$ solution. The solid was collected, washed with water, and dried in vacuo. Further purification was performed by recrystallization from a suitable solvent (Table 1). In the case of compound 10, the residue was suspended in CH_2Cl_2 and extracted with 1 N NaOH. The aqueous phase was acidified with 2 N HCl. The product was obtained by fractional crystallization after addition of 1 N NaOH.

5-Hydroxy-1-indanone (5a). A mixture of 50.0 g (375 mmol) of anhydrous $AlCl_3$ and 10.0 g (171 mmol) of NaCl was melted down in a porcelain cup. At a temperature of 140 °C, 10.0 g (60.2 mmol) of 3-(3-hydroxyphenyl)propanoic acid (5b) was added under stirring. The mixture was then quickly heated to 180–200 °C and kept there for 2 min. After cooling, hydrolysis was performed with ice and 2 N HCl. After extraction with ethyl acetate, the organic layer was washed with $NaHCO_3$ solution and H_2O and dried. After removal of the solvent, the residue was suspended in 200 mL of $CHCl_3$ and stirred for 1 h. The crude product was filtered off, washed with $CHCl_3$, and crystallized from ethyl acetate to yield 4.64 g (52%) of 5a: mp 183–184 °C (lit.[28] mp 184–185.5 °C); ^{1}H NMR (60 MHz, $CDCl_3$/TFA) δ 2.84–3.37 (m, 4H, CH_2), 6.89–7.13 (m, 2H, arom H), 7.81 (d, ^3J = 9 Hz, 1H, arom H-7).

Biological Methods. The preparation of aromatase and desmolase as well as the enzyme assays were performed as described previously.[8] The Lineweaver-Burk plot and the difference spectrum were obtained according to procedures published recently.[29]

Inhibition of the Androgen-Stimulated Uterine Growth. The method of Bhatnagar et al.[21] was used with minor modifications. Twenty-five-day-old female Sprague-Dawley (SD) rats were subcutaneously administered androstenedione (30 mg/kg) dissolved in olive oil (15 mg/mL) and test compound (0.043 mmol/kg) dissolved in a 1:4:5 mixture of ethanol, 1,2-propanediol, and saline (4.3 μmol/mL). One control group received vehicle only, the other androstenedione. Each group consisted of six or seven animals. The animals were injected daily for 4 days. On day 5 the animals were killed by CO_2 inhalation. The uteri were removed and dissected free from adhering adipose tissue, and the uterine wet weights were determined.

Inhibition of the Plasma E_2 Concentration. The test was performed as described[8] using female PMSG-primed SD rats. One, 6, and 24 h after subcutaneous application of a single dose of the test compound dissolved in olive oil, blood was taken by puncturing the right ventricle of the narcotized animal. The E_2 concentration in the plasma was measured by radioimmunoassay using the direct E_2 kit of DRG-Instruments (Marburg, FRG).

DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the Ovariectomized, Testosterone Propionate-Treated SD Rat. The antitumor effect of the test compounds was determined as described.[8,23] Animals bearing at least one tumor greater than 140 mm^2 were classified in groups of 10. The rats were ovariectomized and subcutaneously administered olive oil solutions of the test compounds and testosterone propionate.

Measurement of tumor size and determination of body weight were made once weekly. The therapy was continued for 28 days.

Estradiol Receptor Binding Assay. The test was performed as described.[30] The relative binding affinities were determined by measuring displacement of [³H]estradiol by the test compounds after incubation with cytosol from calf uteri at 4 °C for 16 h (DCC method).

Supplementary Material Available: ^{1}H NMR data of compounds 1–22 (4 pages). Ordering information is given on any current masthead page.

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