New Aromatase Inhibitors. Synthesis and Biological Activity of Pyridyl-Substituted Tetralone Derivatives

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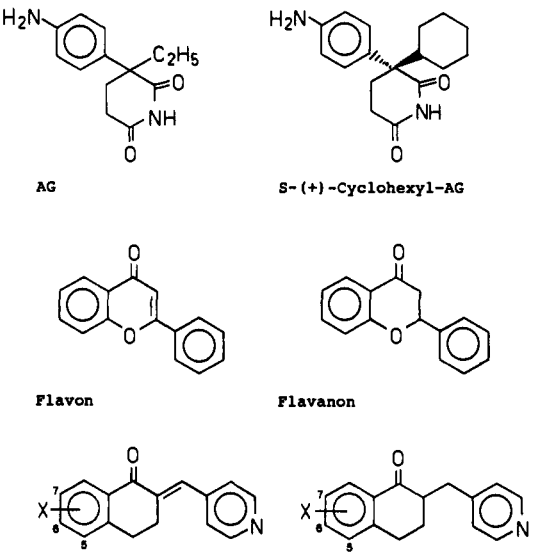
The (*E*)-2-(4-pyridylmethylene)-1-tetralones 1–7 (1, H; 2, 5-OCH_3; 3, 6-OCH_3; 4, 7-OCH_3; 5, 5-OH; 6, 6-OH; 7, 7-OH) were obtained by aldol condensation of the corresponding 1-tetralones with 4-pyridinecarboxaldehyde, and in the case of the OH compounds 5 and 7 subsequent ether cleavage of the OCH_3-substituted 2-(4-pyridylmethylene)-1-tetralones. Catalytic hydrogenation of 1–4 gave the 2-(4-pyridylmethyl)-1-tetralones 8–11 (8, H; 9, 5-OCH_3; 10, 6-OCH_3; 11, 7-OCH_3). Subsequent ether cleavage of 9–11 led to the corresponding OH compounds 12–14 (12, 5-OH; 13, 6-OH; 14, 7-OH). The enantiomers of 11 and 12 were separated semipreparatively by HPLC on triacetylcellulose. All compounds (1–14) showed an inhibition of human placental aromatase exhibiting relative potencies from 2.2 to 213 [compounds 6 and (+)-12, respectively; aromatase inhibitory potency of aminoglutethimide (AG) ≡ 1]. The compounds exhibited no or only a weak inhibition of desmolase [cholesterol side chain cleavage enzyme; maximum activity shown by 12, 23% inhibition (25 μM); AG, 53% inhibition (25 μM)]. In vivo, however, the compounds were not superior to AG as far as the reduction of the plasma estradiol concentration and the mammary carcinoma (MC) inhibiting properties are concerned (PMSG-primed SD rats as well as DMBA-induced MC of the SD rat, pre- and postmenopausal experiments, and the transplantable MXT-MC of the BD2F1 mouse). This is due to a fast decrease of the plasma E_2 concentration inhibiting effect as could be shown by a kinetic experiment. In addition, select compounds inhibited rat ovarian aromatase much less than human placental aromatase (12, factor of 10). Estrogenic effects as a cause for the poor in vivo activity of the test compounds could be excluded, since they did not show affinity for the estrogen receptor.

The use of aromatase inhibitors has recently become a new strategy in the treatment of estrogen-dependent diseases, such as the mammary carcinoma.[1-3] The only nonsteroidal compound commercially available, aminoglutethimide (AG, Chart I), has been shown to be of benefit in many trials with postmenopausal or ovariectomized premenopausal women.[4] But AG is far from being an optimal drug, as it also inhibits the enzyme desmolase [cholesterol side chain cleavage enzyme], leading to a depletion of corticosteroid production, and in addition causes severe side effects, such as depression, ataxia, and somnolence.[5] Attempts of our group to optimize AG have been very successful: (*S*)-(+)-cyclohexyl-AG shows aromatase inhibition exceeding that of AG by a factor of more than 200 [relative potency (RP) >200], whereas the inhibition of desmolase is decreased and the compound shows no CNS-depressive activity.[6] Another attempt to develop more potent and selective aromatase inhibitors started from an observation of Kellis and Vickery, who had reported on a weak aromatase inhibitory action of flavone and flavanone (RP values about 0.1).[7] An increase in aromatase inhibition could be achieved by structure modifications of these natural products leading to compounds 1 and 8 (RP values 4.0 and 8.6, respectively).[8] This paper describes the attempt to obtain even stronger aromatase inhibitors, exhibiting no or only weak desmolase inhibition by the introduction of methoxy or hydroxy substituents in position 5, 6, or 7 of the 1-tetralone moiety of compounds 1 and 8. In the following, the synthesis, the inhibitory activities toward both enzymes, the inhibition of the estrogen production in vivo, and the mammary tumor inhibiting activity using three experimental tumors will be described (Chart I).

Chart I



compd	X	compd	X
1	H	8	H
2	5-OCH_3	9	5-OCH_3
3	6-OCH_3	10	6-OCH_3
4	7-OCH_3	11	7-OCH_3
5	5-OH	12	5-OH
6	6-OH	13	6-OH
7	7-OH	14	7-OH

Chemistry

The methoxy-substituted 2-(4-pyridylmethylene)-1-tetralones 2–4 (Table I) were obtained by a method described previously (compound 1),[8] implying an aldol condensation of the corresponding methoxy-1-tetralone with

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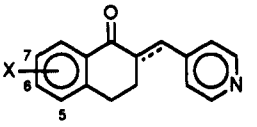
Table I. Substituted 2-(4-Pyridylmethylene)- and 2-(4-Pyridylmethyl)-1-tetralones



compd	X	C=C	mp, °C	formula[a]	method of prepn	recryst solvent	yield, %
1[b]	H	(E)-C=C	113–114	$C_{16}H_{13}NO$	A.I	i-PrOH	75
2	5-OCH_3	(E)-C=C	143–144	$C_{17}H_{15}NO_2$	A.II	MeOH	58
3	6-OCH_3	(E)-C=C	126–128	$C_{17}H_{15}NO_2$	A.I	EtOH	38
4[c]	7-OCH_3	(E)-C=C	135–136	$C_{17}H_{15}NO_2$	A.I	ethyl acetate	57
5	5-OH	(E)-C=C	208–210	$C_{16}H_{13}NO_2$	C	EtOH	64
6	6-OH	(E)-C=C	>220	$C_{16}H_{13}NO_2$	A.I	EtOH[d]	29
7	7-OH	(E)-C=C	219–220	$C_{16}H_{13}NO_2$	C	MeOH	57
8[b]	H	CH—CH	69–70	$C_{16}H_{15}NO$	B	petroleum ether (40–60 °C)	86
9	5-OCH_3	CH—CH	101–102	$C_{17}H_{17}NO_2$	B	*n*-hexane	86
10	6-OCH_3	CH—CH	80–81	$C_{17}H_{17}NO_2$	B	*n*-hexane	80
11	7-OCH_3	CH—CH	89–90	$C_{17}H_{17}NO_2$	B	*n*-hexane	81
(+)-11[e]	7-OCH_3	CH—CH	89–92	$P = 1$	D	–	–
(−)-11[e]	7-OCH_3	CH—CH	86–90	$P = 0.9$	D	–	–
12	5-OH	CH—CH	194–196	$C_{16}H_{15}NO_2$	C	–	70
(+)-12[e]	5-OH	CH—CH	175–184	$P = 1$	D	–	–
(−)-12[e]	5-OH	CH—CH	180–199	$P = 0.9$	D	–	–
13	6-OH	CH—CH	>220	$C_{16}H_{15}NO_2$	C	–	38
14	7-OH	CH—CH	192–194	$C_{16}H_{15}NO_2$	C	–	54

[a] C, H, and N analyses were within ±0.4% of the theoretical values; P = enantiomeric purity. [b] Previously reported; see ref 8. [c] See CA 82: 57687b. [d] The crude product was suspended in EtOH. [e] Measured at 365 nm.

Table II. Determination of the Configuration of 2–7 by ^1H NMR Spectroscopy

compd	vinyl-H: δ (ppm)[a] prior to *hν*	after *hν*[b,c]	configuration
1[d]	7.73	6.73	*E*
2	7.71	6.70	*E*
3	7.70	6.69–6.79[e]	*E*
4	7.75	6.77	*E*
5	8.00	7.12	*E*
6	8.00	7.12	*E*
7	8.03	7.17	*E*

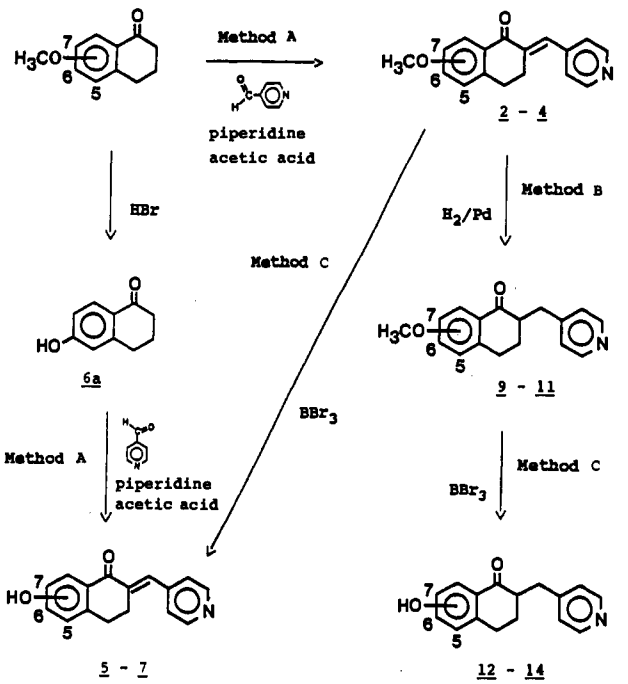
[a] 60 MHz, $CDCl_3$/TMS (2–4) and 250 MHz, $CDCl_3$/TFA/TMS (5–7), respectively. [b] Additional signal. [c] λ = 200–600 nm; time of irradiation 2 days; solvent $CDCl_3$ (3, 4) or EtOH (2, 5–7); concentration 80 mg/mL; ethanolic samples were evaporated to dryness before measurement. [d] Previously reported; see ref 8. [e] Exact determination not possible, as signal is masked by aromatic peaks.

4-pyridinecarboxaldehyde using piperidine/acetic acid as catalyst (method A; Scheme I).

By ^1H NMR spectroscopy it was shown that compounds 2–4 are the *E* isomers. Due to the diamagnetic anisotropy of the carbonyl group, the vinyl proton of the *E* isomer gives a signal at a greater chemical shift than the vinyl proton of the *Z* isomer, for the latter is more remote from the carbonyl group. The conversion of the *E* isomers into the *Z* isomers was accomplished by UV irradiation. Table II shows the chemical shifts of the vinyl protons before and after irradiation. Catalytic hydrogenation of 2–4, using palladium on charcoal, gave the corresponding (pyridylmethyl)-1-tetralones 9–11 (method B; Scheme I).

Ether cleavage of the methoxy group of 2, 4, 9, and 11 was performed with BBr_3, yielding the hydroxy-substituted compounds 5, 7, 12, and 14 (Table I). The 6-methoxy compounds 3 and 10 did not react with the Lewis acid

Scheme I



under these conditions. Whereas in the case of compound 10 a greater excess of BBr_3 and a higher temperature made the conversion to compound 13 (Table I) possible, in the case of compound 3 this procedure also led to an addition of HBr to the double bond. For this reason the ether cleavage was carried out with 6-methoxy-1-tetralone. 6-Hydroxy-1-tetralone (6a, Scheme I) was obtained in satisfactory yields by using concentrated hydrobromic acid. Subsequent aldol condensation with 4-pyridinecarboxaldehyde provided compound 6 (Table I). Just like the methoxy derivatives 2–4, the hydroxy compounds 5–7 show *E* configuration, too (Table II).

Because of the chirality of C-2, compounds 8–14 are racemic mixtures. An analytical separation of the enantiomers of compounds 8–12 was successfully performed by HPLC using triacetylcellulose[9] as optically active sorbent

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Table III. Inhibition of Human Placental Aromatase by 2-(4-Pyridylmethylene)- and 2-(4-Pyridylmethyl)-1-tetralones

compd	X	IC_{50},[a] μM	RP[b]	compd	X	IC_{50},[a] μM	RP[b]
1[c]	H	9.3	4.0	8[c]	H	4.3	8.6
2	5-OCH_3	6.3	5.8	9	5-OCH_3	2.2	17
3	6-OCH_3	8.9	4.2	10	6-OCH_3	3.3	11
4	7-OCH_3	13	2.8	11	7-OCH_3	7.9	4.7
				(+)-11	7-OCH_3	8.5	4.4
				(-)-11	7-OCH_3	9.6	3.9
5	5-OH	0.69[d]	28	12	5-OH	0.20	190
				(+)-12	5-OH	0.17	213
				(-)-12	5-OH	0.21	175
6	6-OH	8.6[d]	2.2	13	6-OH	6.2	5.9
7	7-OH	3.0[d]	6.3	14	7-OH	4.9	7.6

[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 ±10%. [b] Relative potency, calculated from the IC_{50} values and related to AG (AG: IC_{50} = 37 μM). [c] Previously reported; see ref 1. [d] Double the EtOH concentration was used compared to the standard assay (IC_{50} of AG: 19 μM).

and EtOH (96%) as eluent (8, k_+ = 0.66, k_- = 1.12; 9, k_+ = 0.62, k_- = 1.01; 10, k_+ = 0.75, k_- = 1.77; 11, k_+ = 0.69, k_- = 0.91; 12, k_+ = 0.49, k_- = 0.77).[10] Repeated LPLC injections were used for preparing milligram quantities of the enantiomers of compounds 11 and 12 (method D).

Biological Properties

1. **Inhibition of Human Placental Aromatase.** The inhibitory activities of the test compounds 1–14 toward aromatase were determined in vitro using human placental microsomes and [1β,2β-^{3}H]testosterone according to the method of Thompson and Siiteri.[11] The IC_{50} values and the potencies of the derivatives, relative to AG, are given in Table III.

All compounds proved to be more potent aromatase inhibitors than AG, exhibiting relative potencies from RP = 2.2 to RP = 213. The hydrogenated compounds 8–14 show a stronger enzyme inhibition than the corresponding α,β-unsaturated ketones 1–7. Further, the inhibitory potency strongly depends on the kind and the position of the substituent. Comparing the unsubstituted tetralones 1 and 8 with the corresponding 5-, 6-, and 7-methoxy derivatives 2–4 and 9–11, it becomes apparent that the introduction of this group into the 5-position (2, 9) leads to a strong increase in enzyme inhibition, whereas introduction into the 6-position (3, 13) leads only to weak increase. A methoxy substituent in position 7 (4, 11), however, decreases inhibitory activity. Exchanging the methoxy group for a hydroxy substituent results in a marked decrease of inhibitory potency in case of the 6-substituted compounds (3, 10 in comparison to 6, 13), whereas in the case of the 5- and 7-substituted tetralones the hydroxy derivatives (5, 12 and 7, 14) are more potent than the corresponding methoxy compounds (2, 9 and 4, 11). Compound 12 was the most active aromatase inhibitor, exhibiting a relative potency of 190. The determination of aromatase inhibitory potency of the enantiomers of 12, (+)-12 and (-)-12, showed no strong difference compared to the racemic mixture. The same result was found with the enantiomers of compound 11.

From studies on steroidal inhibitors of aromatase it is known that some of them show an irreversible inhibition of this enzyme. For the evaluation of the tetralones 5, 8, 9, and 12 the method of Brodie et al.[12] was used with modifications.[13] Human placental microsomes were incubated with NADPH and inhibitor (10 μM) for 30 min. After treatment with dextran-coated charcoal, the enzyme activity was determined. None of the compounds caused a reduction of enzyme activity. In a further experiment, compound 12 showed no decrease in aromatase activity even after an incubation time of 1 h (data not given).

Table IV. Inhibition of Bovine Adrenal Desmolase by Select 2-(4-Pyridylmethylene)- and 2-(4-Pyridylmethyl)-1-tetralones

compd	X	% inhibn[a,b]	compd	X	% inhibn[a,b]
1[c]	H	none[d]	9	5-OCH_3	none[d]
2	5-OCH_3	none[d]	12	5-OH	23
5	5-OH	12	13	6-OH	none[d]
8[c]	H	none[d]	14	7-OH	20

[a] Concentration of inhibitor: 25 μM. [b] Inhibition caused by AG: 53% (25 μM). [c] Previously reported; see ref 8. [d] The inhibition values range from +2 to −3%.

2. **Inhibition of Desmolase.** The inhibitory activities of the select compounds 2, 5, 9, and 12–14 toward desmolase were determined in vitro using bovine adrenal mitochondria and [26-^{14}C]cholesterol according to the method of Hochberg et al.[14] The inhibition values of the test compounds in concentrations of 25 μM are presented in Table IV.

Just as the unsubstituted compounds 1 and 8, the 5-methoxy-substituted derivatives 2 and 9 did not inhibit desmolase either. Only the hydroxy compounds 5, 12, and 14 show a weak inhibition of desmolase. The inhibition values range from 12 to 23%, whereas AG shows an inhibition of 53%.

3. **Inhibition of Rat Ovarian Estrogen Secretion.** In order to determine the effects of the tetralone derivatives on the E_2 concentration in vivo, pregnant mares' serum gonadotropin (PMSG)-primed Sprague–Dawley (SD) rats[15] were used. Six hours after subcutaneous application of a single dose of the test compounds 5, 8, 9, and 12 (equimolar to 2 mg/kg AG), the E_2 concentration in the plasma was measured by radioimmunoassay (RIA).

As shown in Table V, a reduction of the E_2 level was observed only in plasma obtained from the right ventricle, not in the ovarian vein samples. Although the rank order of IC_{50} values with in vivo efficacy is the same (12, RP = 190, E_2 inhibition 39%; 5, RP = 28, E_2 inhibition 33%; 9, RP = 17, E_2 inhibition 30%; 8, RP = 8.6, E_2 inhibition 8%), there is no correlation of these two parameters. In spite of excellent results in vitro, the four test compounds were less active in vivo compared to AG (E_2 inhibition 67%[13]).

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Table V. Effect of Compounds 5, 8, 9, and 12 on Estradiol Concentration in the PMSG-Primed SD Rat[a-c]

compd		ovarian vein: plasma-E_2 conc (pg/mL) mean ± SE	ovarian vein: inhibn (%)	right ventricle: plasma-E_2 conc (pg/mL) mean ± SE	right ventricle: inhibn (%)
control		3695 ± 431	-	778 ± 214	-
8[d]	H	3696 ± 1086	0	717 ± 155	8
9[d]	5-OCH_3	3949 ± 495	0	542 ± 98	30[f]
5[e]	5-OH	4039 ± 598	0	524 ± 160	33[f]
12[e]	5-OH	3697 ± 788	0	472 ± 111	39[f]

[a] The compounds were applied in doses equimolar to 2 mg/kg AG. [b] Each treatment group consisted of 5–7 animals. [c] Blood samples were taken 6 h after application of inhibitor. [d] Applied as solution in olive oil. [e] Applied as suspension in olive oil. [f] Significantly different from control group ($p < 0.05$).

Table VI. Time-Dependent Inhibition of the E_2 Concentration of PMSG-Primed SD Rats after Subcutaneous (sc) and Intraperitoneal (ip) Application of Compound 9[a,b]

route of appl	time after appl (h)	right ventricle: plasma-E_2 conc (pg/mL) mean ± SE	right ventricle: inhibn (%)	time after appl (h)	right ventricle: plasma-E_2 conc (pg/mL) mean ± SE	right ventricle: inhibn (%)
sc	0	1462 ± 371		0	902 ± 250	
	1	576 ± 311	61[d]	2	458 ± 126	49[d]
	4	1044 ± 245	29[d]			
ip	0	990 ± 348		0	1247 ± 383	
	1	639 ± 241	35[d]	2	882 ± 276	29[d]
	4	894 ± 247	10	8	851 ± 187	32[d]
AG[c] (sc)	0	843 ± 299		0	500 ± 213	
	1	272 ± 112	68[d]	8	164 ± 96	67[d]

[a] Dosage equimolar to 2 mg/kg AG. [b] Each treatment group consisted of 7–8 animals. [c] For comparison. [d] Significantly different from control group ($p < 0.05$).

The plasma E_2 concentration was also measured 1, 2, 4 (and 8 h) after sc or ip administration of compound 9 (Table VI). One hour after sc application of 9, the E_2 concentration is reduced to about the same extent as observed after AG treatment (61% vs 68% inhibition). While the E_2 level remains suppressed for 8 h after AG, the inhibition of the E_2 concentration after application of 9 decreases within this time to a value of 17%. After 1, 2, and 4 h the application of 9 through the ip route reduces plasma estradiol concentration to a less extent than observed after sc administration. Maximal inhibition of the E_2 secretion amounts to only 35% after 1 h.

4. Inhibition of Rat Ovarian Aromatase. To investigate the reason for the surprisingly low in vivo activities of these compounds, the two select compounds 9 and 12 were examined for their inhibitory potency toward rat ovarian aromatase. It is known that human placental and rat ovarian aromatase differ in some properties.[16-19] The results compared to those obtained using human placental aromatase are shown in Table VII.

It becomes apparent that AG inhibits rat ovarian and human placental aromatase to nearly the same extent.[20] Compounds 9 and 12, however, show strongly decreased inhibitory activities toward aromatase obtained from rat ovaries. The OH derivative 12 inhibits rat ovarian aromatase 10 times less than human placental aromatase (RP = 19 compared to RP = 190).

5. Antitumor Activity in Hormone-Dependent Mammary Carcinoma Models. For the evaluation of the tumor inhibiting effect, three different hormone-dependent mammary tumor models were used:

Table VII. Comparison of the Inhibitory Effect of Compounds 9, 12, and AG toward Aromatase Obtained from the Ovaries of PMSG-Primed SD Rats and Human Placenta, Respectively

compd	rat ovarian aromatase: IC_{50},[a] μM	rat ovarian aromatase: RP[b]	human placental aromatase: IC_{50},[a] μM	human placental aromatase: RP[b]
AG	34	1.1	37	≡1
9	8	4	2.2	17
12	2	19	0.195	190

[a,b] See Table III. The relative potencies are related to human placental aromatase.

Compounds 1 (RP = 4.0) and 4 (RP = 2.8) were tested on the DMBA-induced mammary tumor of the SD rat.[21] Similar to AG,[13] both compounds (dose: 6 × 20 mg/kg per week) show only a moderate antitumor activity (% change of tumor area at the end of a 4-week treatment period: control, 446; 1, 201; 4, 199). Both the uterine and the ovarian weights of the treated rats do not significantly differ from those of the control group (data not given).

No significant antitumor activity was observed testing compound 9 (dose: 3 × 75 mg/kg per week) on the transplantable, hormone-dependent MXT mammary tumor of the BD2F1 mouse[22,23] (med tumor weight: treated/control group = 0.93 after 6 weeks of therapy). At a dosage of 3 × 100 mg/kg, AG also shows only marginal antitumor activity (med tumor weight: treated/control group = 0.48). Neither AG nor compound 9 had any influence on the uterine weights of the treated mice (data not given).

Compounds 1, 2, 8, 9, and 12 were examined in doses equimolar to 10 mg/kg AG on the DMBA-induced mammary carcinoma of the ovariectomized, testosterone pro-

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Table VIII. Effect of AG and Compounds 1, 2, 8, 9, and 12 on the DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the Ovariectomized, Testosterone Propionate Treated SD Rat (Postmenopausal Model)

treatment group[a]	dose of inhibn,[b] mg/kg	no. of tumors		% of tumors with				% change of tumor area[i,k]
		B[c]	NT[d]	CR[e]	PR[f]	NC[g]	P[h]	
control	–	28	0	82	18	0	0	–96
Tp	–	36	12	19	25	19	36	+141
Tp + AG[l]	10.0	35	7	54	17	6	23	+4[n]
Tp + 1[m]	10.1	30	6	40	20	20	20	+2[n]
Tp + 2[l]	11.4	28	9	11	11	29	50	+115
Tp + 8[m]	10.2	31	8	45	13	10	32	+41
Tp + 9[m]	11.5	34	11	12	29	24	35	+50
Tp + 12[l]	10.9	33	3	21	6	27	45	+61

[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: 20 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] Dose received as suspension (see *b*). [m] Dose received as solution (see *b*). [n] Significantly different from the Tp group ($\alpha = 0.025$).

pionate treated SD rat.[6,13] This model mimicks the endocrine situation of a postmenopausal or ovariectomized woman, thus representing an appropriate tumor model for this group of patients. As can be seen from Table VIII and Figure 1, ovariectomy-induced regression of the tumors can be prevented by administering testosterone propionate.[24] Thus the tumor area increases to nearly double the initial value (% change of tumor area at the end of a 4-week treatment period: +92%). This stimulating effect of testosterone propionate must be due to aromatization in peripheral tissues and will be subject of a subsequent publication.[25] The test compounds inhibit this stimulation differently: While the unsubstituted enone 1 (RP = 4.0) exerts an antitumor effect which is comparable to AG (Figure 1), the corresponding 5-OCH_3 substituted compound 2 (RP = 5.8) is completely inactive. The pyridylmethyl derivatives 8 (RP = 8.6), 9 (RP = 17), and 12 (RP = 190) only weakly inhibit the testosterone propionate stimulated tumor growth showing lower inhibitory effects than AG. These results confirm the findings of the PMSG experiment. In this experiment, too, the three test compounds were less active than AG.

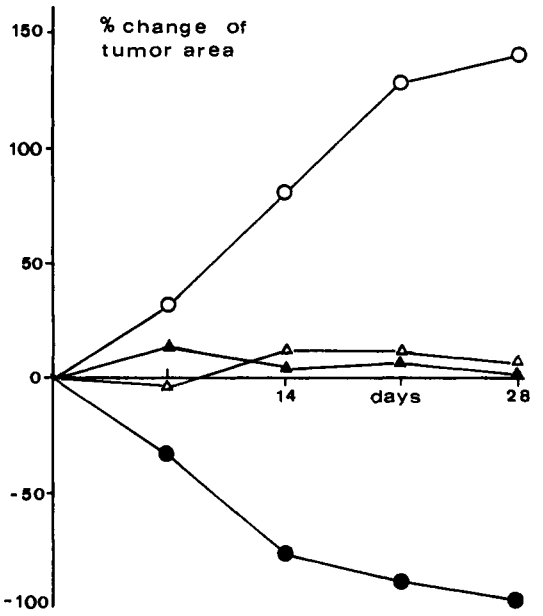

Figure 1. Effect of AG and compound 1 on the tumor area of the DMBA-induced hormone-dependent mammary carcinoma of the ovariectomized testosterone propionate (Tp) treated SD rat (postmenopausal model): control (●–●), Tp (○–○), Tp + AG (△–△), Tp + 1 (▲–▲) [dosage and application schedule see Table VIII].

6. Binding Affinity for the Estrogen Receptor. In order to exclude possible estrogenic side effects, as a cause of the weak tumor-inhibiting activity, the relative binding affinities (RBA) of the four select compounds 5, 8, 9, and 12 for the estrogen receptor were determined. Interaction with this receptor is a prerequisite for an estrogenic (or antiestrogenic) effect. The test was performed using calf uterine cytosol as source of the receptor and the dextran-coated charcoal method.[26] None of the tested compounds showed detectable affinity for the estrogen receptor [maximum concentration tested: 100 μM (range 1–100 μM); RBA in all cases <0.01; for comparison, E_2 RBA ≡ 100].

Discussion

The present work shows that an enhancement of the aromatase inhibitory activity of compounds 1 and 8 can be achieved by introducing methoxy or hydroxy groups into the aromatic ring of the tetralone structure. Maximum activity is shown by compounds bearing those groups in position 5. This might be indicative of a hydrophilic interaction between the inhibitor and the enzyme at that position. The most active compound was the 5-OH derivative 12 being 190 times as active as AG.

Except for the 6-hydroxy compounds 6 and 13, the phenols proved to be more effective in inhibiting aromatase than the corresponding methoxy derivatives. This might be due to the property of the former to act as hydrogen bonding donors. The lower activity of the OCH_3 derivatives due to a steric hindrance by the methoxy groups, however, cannot be excluded. The fact that the 7-methoxy derivatives 7 and 14 show markedly decreased inhibition values compared to the unsubstituted compounds 1 and 8 indeed indicates that there might be a steric hindrance at that position.

The strikingly low aromatase inhibitory properties of the 6-OH compounds 6 and 13 might be due to the conjugation of the 6-hydroxyphenyl nucleus with the carbonyl group, leading to an increased acidity of these phenols.

The finding that the α,β-unsaturated ketones exhibit a lower inhibition of aromatase compared to the pyridylmethyl derivatives shows that the fixation of the pyridine nitrogen into the plane of the tetralone structure is not suitable for a maximum inhibitory effect. The more

(24) A similar tumor stimulation is obtained by androstenedione, whereas the nonaromatizable androgen dihydrotestosterone shows no effect. The testosterone (androstenedione) stimulation is dose-dependently inhibited by aromatase inhibitors (ref 25).

(25) Hartmann, R. W.; Batzl, C. *Pharm. Pharmacol. Lett.*, submitted.

(26) Korenman, S. G. *J. Clin. Endocrinol. Metab.* **1968**, *28*, 127.

flexible hydrogenated compounds, however, are able to attach to the enzyme in a way being more favorable to a strong inhibition. Surprisingly the two enantiomers of 11 and 12 show similar inhibitory potencies. Studies using Dreiding models indicate that there exists a conformation for each enantiomer in which the pyridyl group and the tetralone moiety occupy very similar positions (Hartmann, Bartz unpublished data). This might indicate that the enantiomers interact with the enzyme in different conformations regarding the cyclohexenone moiety. Molecular modeling studies are presently being performed to further elucidate this issue.

The inhibition of the desmolase seems to depend on the presence of a hydroxy group. The uneffectiveness of compound 13 might as well be due to the electronic properties of the (4-hydroxyphenyl)carbonyl moiety as stated above. It seems possible that besides the unsubstituted parent compounds 1 and 8, the OCH_3-substituted derivatives are selective inhibitors of the aromatase, too.

Although being superior to AG in the aromatase assay in vitro, the test compounds show in contrast to AG, only a moderate inhibition of the E_2 concentration in vivo. To what extent phenomena concerning the resorption and distribution of the compounds may play a role is not known. One reason for the dissappointing in vivo activity might be a rapid metabolic inactivation. The fast restoration of the E_2 concentration is indicative of this statement. Besides, the lower inhibitory activity of the tetralone derivatives toward rat ovarian aromatase compared to human placental aromatase may also play an important role. This implies that the tetralone derivatives might exert a stronger effect in man than in rats.

The weak or lack of effect on the two premenopausal tumor models is not only due to that low in vivo activity of these compounds but also to that sensitive hormonal regulatory mechanism. Thereby, a decrease of the E_2 level leads to an ovarian overstimulation and by this means to an intensified estrogen biosynthesis, thus counteracting the action of the weak aromatase inhibitor.[18] These premenopausal tumor models are primarily appropriate tests for those steroidal inhibitors which show no negative feedback mechanism on hypothalamus and pituitary. A proper test model for the evaluation of hormonally nonactive aromatase inhibitors is the DMBA-induced mammary carcinoma of the SD rat in the postmenopausal arrangement. The significant tumor inhibiting activity of compound 1 demonstrates that further structural modifications might lead to even more active compounds, appropriate candidates for the treatment of postmenopausal breast cancer. One important step in this direction could be the development of metabolically stable derivatives.

Experimental Section

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, University of Regensburg, and were within ±0.4% of the calculated values. ^1H NMR spectra were measured on a Varian EM 360L (60 MHz) and a Bruker WM 250 (250 MHz; by Dr. T. Burgemeister and his staff, University of Regensburg) spectrometer and are consistent with the assigned structures. Column chromatography was performed on Merck Kieselgel 60. For the separation of the enantiomers on triacetylcellulose (8–15 μm) by HPLC, a Model 6000 A pump and a Model U6K injector from Waters were used. The separation on triacetylcellulose (20–30 μm) by LPLC was performed with the pump Duramat from Chemie und Filter, Heidelberg. In each case UV detection was carried out at 254 nm with an ERC 7210 from ERMA Optical Works. The rotation angles were measured at 365 nm on a PE 241 polarimeter from Perkin-Elmer.

6-Hydroxy-1-tetralone (6a). A solution of 17.6 g (100 mmol) of 6-methoxy-1-tetralone in 220 mL of aqueous HBr (47%) was heated under reflux for 5 h. After cooling in an ice bath, the precipitate was collected, washed with water, and finally recrystallized from water to yield 12.2 g (75%) of beige crystals: mp 155–156 °C (lit.[27] mp 156–157 °C); ^1H NMR (60 MHz, $CDCl_3$) δ 1.87–2.29 (m, 2 H, $CH_2CH_2CH_2$), 2.53–3.00 (m, 4 H, $CH_2CH_2CH_2$), 6.72–6.90 (m, 2 H, arom H), 7.15 (br s, 1 H, OH), 7.99 (d, J = 8 Hz, 1 H, arom H-8).

Method A. General Procedure for the Synthesis of the 2-(4-Pyridylmethylene)-1-tetralones 1–4 and 6. 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-tetralone 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_2Cl_2 (for the synthesis of 3: filtered off from 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 I), carried out in the absence of light, yielded 1, 3, 4, or 6. **A.II.** As for method A.I, but after extraction of the CH_2Cl_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, and the solution was neutralized by addition of saturated $NaHCO_3$ solution. The crude product was isolated and purified as for method A.I yielding compound 2 (Table I).

Method B. General Procedure for the Synthesis of the 2-(4-Pyridylmethyl)-1-tetralones 8–11. A suspension of 50.0 mmol of 1, 2, 3, or 4 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 I) in the absence of light to give 8, 9, 10, or 11.

Method C. General Procedure for Ether Cleavage Yielding 5, 7, and 12–14. A solution of the methoxy-substituted 1-tetralones 2, 4, 9, 10, or 11 (5.00 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 13: 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 13: 3 h plus 1 h refluxing). Hydrolysis was carried out by dropwise addition of 5 mL of MeOH (for the synthesis of 13: 10 mL) and was completed by stirring for 30 min. In the case of a precipitating hydrobromide the mixture was concentrated to about half 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 I).

Method D. Separation of the Enantiomers of 11 and 12. The separation was performed by LPLC on triacetylcellulose (EtOH 96%) in the absence of light.

Biological Methods. The preparation of aromatase and desmolase as well as the enzyme assays were performed as described previously[13] except for the following modifications. **Irreversible inhibition of aromatase:** the inhibitors were tested at concentrations of 10 μM each. **Inhibition of rat ovarian aromatase:** 0.9 μM testosterone and 2.55 μCi [1β,2β-^3H]testosterone were used.

Inhibition of Ovarian E_2 Secretion. The test was performed as described[13] using female PMSG-primed SD rats. Six hours after sc application of a single dose of the test compounds, the E_2 concentration in the plasma was measured by radioimmunoassay (RIA) using the direct E_2 assay of DRG-Instruments, Marburg, FRG. In a time-dependent study the E_2 concentration

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in the plasma was additionally measured 0, 1, 2, 4, and 8 h after sc or ip application of the test compound. From each rat, blood (0.5 mL) was taken at three time points (0, 1, 4 h and 0, 2, 8 h, respectively) by puncturing the right ventricle of the narcotized animal.

Hormone-Dependent, Transplantable MXT Mammary Tumor of the BD2F1 Mouse. The method is identical with that described.[28] Tumor-bearing mice were randomly distributed into groups of 10. The test compounds were applied in olive oil sc three times a week for 6 weeks. At the end of the test, the animals were killed and the wet weights of the tumors and the dry weights of the uteri were determined.

DMBA-Induced, Hormone-Dependent Mammary Carcinoma of the SD Rat. The test was accomplished as described previously.[29] Animals bearing at least one tumor greater than 140 mm^2 were classified in groups of 10. Compounds were dissolved in olive oil and applied sc. Measurement of tumor size and determination of body weight were performed once a week. The treatment was continued for 28 days.

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.[13] Animals bearing at least one tumor greater than 140 mm^2 were classified in groups of 10. The rats were ovariectomized and sc administered olive oil solutions of the test compounds and testosterone propionate. Measurement of tumor size and determination of body weight were made once a week. The therapy was continued for 28 days.

Estradiol Receptor Binding Assay. The test was performed as described.[28] The relative binding affinities (RBA) were determined by displacement of [^{3}H]estradiol by the test compounds after incubation with cytosol from calf uteri at 4 °C for 16 h (DCC method).

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Registry No. 1, 135075-22-8; 2, 135075-23-9; 3, 135075-24-0; 4, 135075-25-1; 5, 135075-26-2; 6, 135075-27-3; 7, 135075-28-4; 8, 135075-29-5; 9, 135075-30-8; 10, 135075-31-9; 11, 135075-32-0; (+)-11, 135075-36-4; (−)-11, 135075-37-5; 12, 135075-33-1; (+)-12, 135075-38-6; (−)-12, 135075-39-7; 13, 135075-34-2; 14, 135075-35-3; 4-pyridinecarboxaldehyde, 872-85-5; 1-tetralone, 529-34-0; 5-methoxy-1-tetralone, 33892-75-0; 6-methoxy-1-tetralone, 1078-19-9; 7-methoxy-1-tetralone, 6836-19-7; 6-hydroxy-1-tetralone, 3470-50-6; aromatase, 9039-48-9.

Supplementary Material Available: ^{1}H NMR data (Table IX) of compounds 1–14 (3 pages). Ordering information is given on any current masthead page.

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