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Short communication

Synthesis and evaluation of 4-alkylanilines as mammary tumor inhibiting aromatase inhibitors

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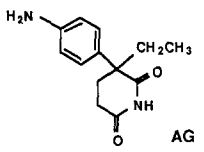
Summary — The 4-alkylanilines **1–20** were synthesized to elucidate the importance of the glutarimide moiety for the aromatase inhibiting activity of aminoglutethimide [3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione, AG], the only non-steroidal aromatase inhibitor which is commercially available at present. The most interesting compounds were the (4-aminophenyl)cycloalkanes **4–6** (**4**, c-pentyl; **5**, c-hexyl; **6**, c-heptyl) and the 1-alkyl-1-(4-aminophenyl)cyclohexanes **1–3** (**1**, CH_3; **2**, C_2H_5; **3**, n-C_3H_7). Derivatives **1–6** are stronger inhibitors of human placental aromatase than AG exhibiting relative potencies from 1.5 to 2.7 (AG≡1). For selectivity of action, the inhibition of desmolase (cholesterol side chain cleavage enzyme) was determined. Compounds **1–3** showed an inhibition comparable to AG, whereas compounds **4–6** exhibited no effect on desmolase. Being more potent and selective aromatase inhibitors *in vitro*, compounds **4–6**, however, were not superior to AG *in vivo*, when the reduction of plasma estradiol concentration and the tumor inhibiting activity (PMSG-primed SD rats and DMBA-induced mammary carcinoma of the SD rat, postmenopausal model) were concerned.

alkylaniline / aromatase inhibitor / desmolase / mammary tumor inhibiting activity / plasma estradiol concentration / structure–activity relationship

Introduction

Aromatase inhibitors have aroused considerable interest as promising therapeutics for the treatment of estrogen dependent breast cancer. So far the only commercially available aromatase inhibitor is aminoglutethimide [3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione, AG]. Being a relatively weak and less selective aromatase inhibitor, AG was the starting point of numerous structure modifications in order to improve aromatase inhibition and reduce inhibition of other cytochrome P-450 dependent enzymes involved in steroidogenesis.



AG

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Investigations of our group showed that the elongation of the ethyl substituent and the introduction of a CH_3 residue into the *n*-alkyl chains leads to a dramatic increase of both efficacy and selectivity [1]. The replacement of the 4-aminophenyl substituent of AG by a 4-pyridyl group results in a decrease of inhibitory potency toward aromatase but also in an increase of selectivity [2]. As in the case of AG, efficacy can be enhanced by introduction of longer alkyl chains in the 1- or 3-position of the molecule [3].

Structural modifications concerning the glutarimide moiety of AG have also been undertaken. Analogues in which the piperidine-2,6-dione residue is replaced by pyrrolidinedione are also selective for aromatase, but the compounds are less active aromatase inhibitors than AG [4]. 4-Cyclohexylaniline, in which the 3-ethyl substituent as well as the glutarimide moiety are replaced by a cyclohexyl substituent proved to be an effective aromatase inhibitor *in vitro* [5]. To further elucidate the importance of the glutarimide moiety for the aromatase inhibiting activity, a series of compounds has been synthesized, which lack the piperidine-2,6-dione ring.

This paper describes the synthesis and the aromatase inhibiting activities of (4-aminophenyl)cycloalkanes (compounds **1–6**) and (4-aminophenyl)-alkanes (compounds **7–20**; scheme 1). Inhibition of the cholesterol side chain cleavage enzyme (desmolase) *in vitro* as well as inhibition of estrogen production and mammary tumor inhibiting activity *in vivo* of the most interesting compounds will be presented.

1 - 6 7 - 20

Scheme 1.

Compd	*Y*	*n*	*Compd*	*Y*
1	CH_3	1	**11**	$CH(CH_3)_2$
2	C_2H_5	1	**12**	$(CH_2)_3CH_3$
3	$(CH_2)_2CH_3$	1	**13**	$CH_2CH(CH_3)_2$
4	H	0	**14**	$CHCH_3C_2H_5$
5	H	1	**15**	$C(CH_3)_3$
6	H	2	**16**	$(CH_2)_4CH_3$
7	H	–	**17**	$(CH_2)_2CH(CH_3)_2$
8	CH_3	–	**18**	$(CH_2)_5CH_3$
9	C_2H_5	–	**19**	$CH_2CH(CH_2)_4$
10	$(CH_2)_2CH_3$	–	**20**	$CH_2CH(CH_2)_5$

Chemistry

Compounds **5**, **7–12** and **15** were commercially available. The remaining compounds were synthesized as follows.

The synthesis of the 1-alkyl-1-phenylcyclohexanes **1b–3b** (scheme 2) started from cyclohexanone, which was converted with alkylmagnesium iodide in a Grignard reaction to the corresponding 1-alkyl-cyclohexanols. Friedel-Crafts reaction of the latter compounds with benzene using concentrated H_2SO_4 as catalyst [6] yielded compounds **1b–3b**.

For the preparation of phenylcyclopentane (**4b**) and phenylcycloheptane (**6b**; scheme 2), Friedel-Crafts alkylation was performed using $AlCl_3$ catalysis. In the case of the cycloheptyl compound milder conditions, namely the method of Sidorova and Zukervanik [7], were applied to prevent skeleton isomerisation and the formation of phenylmethylcyclohexanes [8].

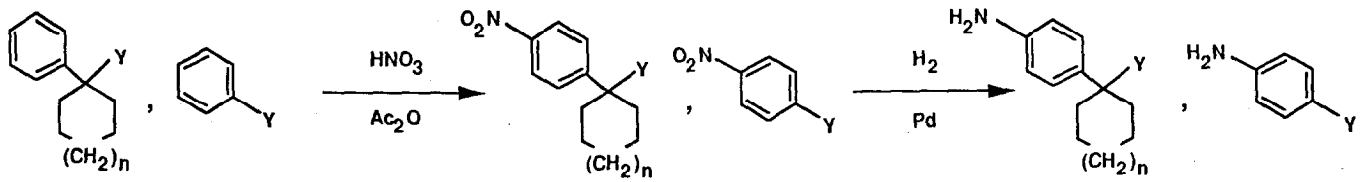
The cycloalkylphenylmethanes **19b** and **20b** (scheme 2) were synthesized by Friedel-Crafts acylation of benzene with the corresponding cycloalkyl carboxylic acid chloride using $AlCl_3$ as catalyst and subsequent Wolff-Kishner reduction of the resulting cycloalkylphenyl ketones.

The phenylalkanes **13b**, **14b**, and **16b–18b** were commercially available.

Nitration of all compounds was performed according to the method of Hahn *et al* [9] using HNO_3/acetic anhydride. Following this procedure, the para-nitro compounds **1a–4a**, **6a**, **13a**, **14a** and **16a– 20a** were obtained in satisfying amounts (scheme 2). Catalytic hydrogenation using palladium on charcoal and purification by column chromatography gave the 4-aminophenyl derivatives **1–4**, **6**, **13**, **14** and **16–20** (scheme 2).

Biological results

To get a first insight into the aromatase inhibiting activity, the compounds were tested in concentrations corresponding to the 10- and 50-fold substrate concentration, respectively. As can be seen from the inhibition values given in tables I and II, the 1-alkyl-1-(4-aminophenyl)cyclohexanes **1–3** and the (4-aminophenyl)cycloalkanes **4–6** are stronger inhibitors *in vitro* than is AG. The potencies of the latter compounds are given in table I. The strongest activity is shown by (4-aminophenyl)cyclohexane (**5**, relative potency (rp) compared to AG = 2.7). Ring diminution as well as ring extension results in reduced activities (cyclopentyl compound **4**: rp = 1.5; cycloheptyl compound **6**: rp = 2.0). Neither the introduction of an alkyl group in the 1-position of the cyclohexane deri-



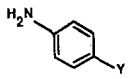
1b - 4b, 6b | **13b , 14b, 16b - 20b** | **1a - 4a, 6a** | **13a , 14a, 16a - 20a** | **1 - 4, 6** | **13 , 14, 16 - 20**

Scheme 2.

Table I. Inhibition of human placental aromatase by 1-alkyl-1-(4-aminophenyl)cyclohexanes and (4-aminophenyl)cycloalkanes. Concentration of the substrate testosterone: 5 μM. IC_{50}: inhibitor concentration required for 50% inhibition. The given values are mean values of at least three experiments. The deviations were within ± 10%. Relative potency is calculated from the IC_{50} values and relative to AG.

Compd	*Inhibition at 50 μM (%)*	*IC_{50} μM*	*Relative potency*
1	85	20.0	1.9
2	73	23.5	1.6
3	77	21.5	1.7
4	72	24.0	1.5
5	93	13.5	2.7
6	91	18.5	2.0
AG	68	37.0	1.0

Table II. Inhibition of human placental aromatase by (4-aminophenyl)alkanes and (4-aminophenyl)cycloalkylmethanes. Concentration of testosterone: 5 μM. The given values are mean values of at least three experiments.



Compound	*Y*	*Inhibition (%) 50 μM*	*250 μM*
7	H	< 10	< 10
8	CH_3	< 10	< 10
9	C_2H_5	15	40
10	$(CH_2)_2CH_3$	20	70
11	$CH(CH_3)_2$	18	62
12	$(CH_2)_3CH_3$	45	92
13	$CH_2CH(CH_3)_2$	43	94
14	$CHCH_3C_2H_5$	39	89
15	$C(CH_3)_3$	17	47
16	$(CH_2)_4CH_3$	24	86
17	$(CH_2)_2CH(CH_3)_2$	26	89
18	$(CH_2)_5CH_3$	18	72
19	$CH_2CH(CH_2)_4$	50	–[a]
20	$CH_2CH(CH_2)_5$	34	–[a]

[a]Compound not soluble in this concentration.

vative **5** is an appropriate modification to enhance inhibitory potency. The 1-alkylcyclohexanes **1–3** exhibit similar effects regardless of the length of the alkyl chain [CH_3 (**1**): rp = 1.9; C_2H_5 (**2**): rp = 1.6; n-C_3H_7 (3): rp = 1.7].

In the series of the *n*-alkyl substituted anilines there is a trend toward increasing inhibition values with alkyl chain length being maximal for the butyl derivative **12** and decreasing thereafter (table II). The introduction of a CH_3 group into the α-position of the *n*-alkyl substituent results in an increased inhibition. Isopropyl-(**11**) and sec-butylaniline (compound **14**) are stronger inhibitors than the corresponding ethyl (**9**) and *n*-propyl analogues (**10**). However, the introduction of a second CH_3 group into the α-position of compound **11** leads again to a decrease of activity (t-butyl derivative (**15**)). As observed with the *n*-alkyl compounds, maximum activity in the isoalkyl series is seen with the butyl analogue **13**.

As can be seen from the inhibition values of the (4-aminophenyl)cycloalkylmethanes (compounds **19–20**, table II), the insertion of a CH_2 group into the corresponding (4-aminophenyl)cycloalkanes (compounds **1** and **2**; table I) results in a reduction of the inhibitory activity. It is striking that all compounds containing a cycloalkyl structure exhibit stronger inhibitory effects than the corresponding acyclic derivatives with the same number of carbon atoms (we have refrained from testing the *n*-heptyl derivative because of the decrease in the inhibition values from the *n*-butyl-(**12**) to the *n*-hexylaniline (**18**)).

In order to examine whether the test compounds also cause irreversible inhibition of aromatase, human placental microsomes were preincubated with (4-aminophenyl)cyclohexane **5** and the corresponding 1-*n*-propyl analogue **3**, respectively, as described in *Experimental protocols*. In no case was a reduced enzyme activity observed (data not shown).

Compounds **1–6**, being stronger inhibitors of aromatase than AG, were further examined for selectivity of inhibition. At a concentration of 25 μM the compounds were tested for inhibition of desmolase (table III). In the series of the 1-alkyl-1-(4-aminophenyl)cyclohexanes the methyl (**1**) and the ethyl derivative (**2**) both show strong inhibitory effects toward this enzyme, whereas the corresponding *n*-propyl compound **3** causes a slightly weaker inhibition of desmolase than does AG. All derivatives

Table III. Inhibition of bovine adrenal desmolase by 1-alkyl-1-(4-aminophenyl)cyclohexanes and (4-aminophenyl)-cycloalkanes. The given values are mean values of at least three experiments.

Compound	*Inhibition (%) 25 μM*
1	67
2	78
3	39
AG	57
4	< 10
5	< 10
6	< 10

lacking the alkyl group (compounds **4–6**) did not show inhibition of desmolase up to 25 μM. Being more potent and selective inhibitors of aromatase than AG, the latter compounds were further tested *in vivo*.

According to the method of Brodie *et al* [11], the effects of the test compounds on the plasma estradiol level of adult female rats, which had been pretreated with pregnant mares' serum gonadotropin (PMSG), was measured. Six h after subcutaneous application of compounds **4–6**, which were applied in doses equimolar to 2 mg/kg AG, the estradiol (E_2) levels in the ovarian and cardiac blood were determined by radioimmunoassay (fig 1). Surprisingly none of the test compounds, which proved to be stronger inhibitors of human placental aromatase *in vitro* (table I), was able to suppress E_2 concentration to the same extent as did AG. Furthermore, in contrast to the results obtained in the *in vitro* assay, maximum activity in lowering E_2 concentration in rats, is shown by the cyclopentyl compound **4**.

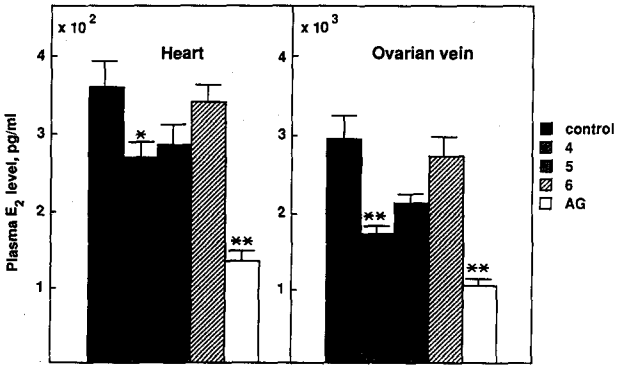


Fig 1. Effect of (4-aminophenyl)cycloalkanes on estradiol (E_2) concentration of PMSG-primed SD rats. Values shown are mean ($n = 5$). *$P < 0.05$, **$2P < 0.05$ (paired Student's t-test). The compounds were applied in doses equimolar to 2 mg/kg AG. Blood samples were taken 6 h after subcutaneous application of test compounds.

The mammary tumor inhibiting activity of compound **5** was determined using our postmenopausal model [1,12] of the estrogen-dependent DMBA induced mammary tumor of the SD rat. As reported earlier by us, the application of testosterone or testosterone propionate to ovariectomized DMBA tumor-bearing rats prevents tumor regression and causes stimulation of uterine weights [1, 12, 13]. This effect is not due to testosterone itself but to its aromatization product estradiol. As a consequence, this stimulation can be antagonized by aromatase inhibitors in a dose-dependent manner. Table IV shows the mammary tumor reducing effect of (4-aminophenyl)cyclohexane (compound **5**) compared to AG. The test compound **5** exhibits a similar antitumor effect to AG. Inhibition of the testosterone-stimulated uterine weights is only observed in the AG treated animals.

Discussion

The present structure-activity relationship clearly demonstrates that the glutarimide moiety of AG is not an essential structural feature for the aromatase inhibiting effect of the drug. The synthesized cycloalkylanilines **1–6** are even stronger aromatase inhibitors than AG. Some of these derivatives, namely the compounds lacking the second alkyl substituent (compounds **4–6**), in addition do not inhibit the cholesterol side chain cleavage enzyme in concentrations up to 25 μM, thus being also more selective inhibitors of the aromatase enzyme system. On the other hand, in spite of being more active *in vitro*, compounds **4–6** were not superior to AG *in vivo* concerning the E_2 lowering effect in PMSG-pretreated

Table IV. Effect of (4-aminophenyl)cyclohexane (compound **5**) on DMBA-induced, hormone-dependent mammary tumors of ovariectomized, testosterone-treated SD rats.

Treatment group[a]	*Dose of inhibitor*[b] *mg/kg*	*No of tumors*		*Percentage of tumors with*				*Percent change of tumor area*[i,k]	*Effect on uterine weight means ± SD*[l,k]
		B[c]	*NT*[d]	*CR*[e]	*PR*[f]	*NC*[g]	*P*[h]		
Control		36	0	94	6	0	0	–99	11.1 ± 1.7
T		38	0	22	19	53	6	–29[m]	38.0 ± 3.7[m]
T + 5	7.6	37	0	65	19	16	0	–81[n]	37.2 ± 4.1
T + AG	10	33	0	76	21	3	0	–86[n]	32.1 ± 4.2[n]

[a]T: testosterone. [b]Dose of T: 20 mg/kg. [c]At the beginning of the experiment. [d]Occurring during the experiment. [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 treatment. [k]The U-test of Wilcoxon, Mann and Whitney was used. [l]Uterus dry weight (mg/body weight (g) x 100). [m]Significantly different from the control group ($\alpha = 0.01$). [n]Significantly different from the T group ($\alpha = 0.01$).

adult rats and the mammary tumor inhibiting activity in ovariectomized, testosterone-treated rats. This is probably due to unfavourable pharmacokinetic properties of this class of compounds. For this reason, though being more potent and selective aromatase inhibitors than AG *in vitro*, the (4-aminophenyl)cycloalkanes **4–6** can not be taken into account for clinical use. At present, there are several aromatase inhibitors known, which are highly selective and much more active than AG *in vitro* and *in vivo* [1, 14, 15].

On the other hand, the presented structure-activity study provides helpful background information on the inhibitor enzyme interaction. The finding that the introduction of alkyl substituents in the 1-position of 1-(4-aminophenyl)cyclohexane does not influence the inhibitory activity towards aromatase considerably, is in contrast to our previous findings in the class of 3-alkyl-3-(4-aminophenyl)piperidine-2,6-diones. In the case of AG, shortening of the 3-ethyl group strongly reduces activity, the compound lacking the 3-alkyl moiety being inactive, whereas elongation of the alkyl substituent leads to a dramatic increase in inhibitory potency [1]. This indicates that the cycloalkyl anilines interact with the aromatase enzyme in a different way than do AG and the structure-related piperidinediones. Presumably, it is not the piperidinedione moiety which is mimicked by the cycloalkyl substituent of the aniline derivatives but the 3-ethyl group of AG, with the piperidinedione binding region in the active site of aromatase being left unoccupied. Based on this hypothesis, the finding of the presented structure–activity study that all compounds containing a cycloalkyl group, exhibit stronger inhibitory activities than the corresponding acyclic derivatives, was of utmost importance for our further design of new aromatase inhibitors of the AG-type. Exchange of the 3-ethyl group by cycloalkyl substituents should result in even stronger inhibitors than the acyclic substituted AG derivatives with the same number of carbon atoms reported earlier [1]. Meanwhile this hypothesis could be verified. The synthesis and the biological evaluation of the 3-(4-aminophenyl)-3-cycloalkylpiperidine-2,6-diones will be the subject of a following report.

Experimental protocols

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 as well as the boiling points uncorrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium, Universität Regensburg. All compounds were analyzed for C and H and were within ± 0.4% of the calculated values. ^{1}H-NMR (Varian EM 360 L, 60 MHz) and IR (Beckman Acculab 3) data were recorded for each compound and were consistent with the assigned structures. The given yield is the yield of analytically pure product. No effort was made to optimize yields.

Method A

*1-n-Propyl-1-phenylcyclohexane (**3b**)*
A solution of 1-*n*-propylcyclohexanol (12.8 g, 0.1 mol) in benzene was added dropwise at 0–5°C to 50 ml of concentrated H_2SO_4. The mixture was stirred at room temperature for 3 h. The organic layer was separated, washed with water, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography (silica gel, petrol ether) to give 10.7 g (53%) of **3b**, bp 142°C (6 mm); Anal ($C_{15}H_{22}$) C, H; ^{1}H-NMR ($CDCl_3$), δ: 0.80 (t, J = 7 Hz, 3H, CH_3), 1.00–2.30 [m, 14H, $(CH_2)_5$ and $(CH_2)_2$ CH_3], 7.00–7.40 (m, 5H, arom).

*1-Methyl-1-phenylcyclohexane (**1b**) [16]*
Purification by silica gel flash chromatography (petrol ether: CH_2Cl_2 = 3:1) gave 9.76 g (56%); oil, bp 110°C (12 mm). Anal $C_{13}H_{18}$ (C, H).

*1-Ethyl-1-phenylcyclohexane (**2b**) [8]*
Purification by silica gel flash chromatography as described for **1b** gave 10.9 g (58%); oil, bp 134°C (10 mm) ([8]; 257–258°C). Anal $C_{14}H_{20}$ (C, H).

Method B

*Phenylcyclopentane (**4b**) [17]*
To a cooled suspension of anhydrous $AlCl_3$ (13.4 g, 0.1 mol) in 100 ml of dry benzene cyclopentanol (8.61 g, 0.1 mol) was slowly added. The mixture was stirred for 16 h at ambient temperature and poured onto ice. The organic layer was washed with water and a saturated Na_2CO_3 solution and dried ($MgSO_4$). After removal of the solvent the crude product was distilled under reduced pressure to yield 11.4 g (78%) of **4b**; oil, bp 115–117°C (23 mm) [17]: 118°C (25 mm). Anal $C_{11}H_{14}$ (C, H).

Method C

*Phenylcycloheptane (**6b**) [7]*
Anhydrous $AlCl_3$ (9.38 g 0.07 mol) was added slowly to 11.4 g (0.1 mol) of cycloheptanol in 250 ml of dry benzene over a period of 2 h. The mixture was stirred at ambient temperature for 5 days, heated up to 40–55°C for 3–4 h and then to 70–80°C for a few minutes. The mixture was cooled to room temperature and treated with 10% aqueous HCl. The organic layer was separated, washed with water, and dried ($MgSO_4$). The oily crude product was distilled under water-jet vacuum to give 12.4 g (71%) of **6b**; bp 123–125°C (23 mm). Anal $C_{13}H_{18}$ (C, H).

Method D

*Cyclopentylphenylmethane (**19b**) [7]*
A mixture of cyclopentylphenylketone (8.71 g, 0.05 mol, obtained by Friedel-Crafts acylation of benzene with cyclopentyl carboxylic acid chloride using *method B*), KOH (43.8 g, 0.78 mol), and 50 ml of hydrazine hydrate (85%) in 400 ml of diethylene glycol was refluxed for 1.5 h. After the formed water was removed, the mixture was heated at 195°C for an additional 4 h. After cooling, the solution was diluted with H_2O

and extracted with ether. The combined ether extracts were washed, dried ($MgSO_4$), and evaporated. The residue was flash chromatographed on silica gel (CH_2Cl_2) to give 7.21 g (90%) of **19b**; oil, bp 92°C (15 mm). Anal $C_{12}H_{16}$ (C, H).

*Cyclohexylphenylmethane (**20b**) [7]*
Yield 7.41 g (85%); oil, bp 105°C (12 mm). Anal $C_{13}H_{18}$ (C, H). The phenyl alkanes **13b**, **14b** and **16b–18b** were available from Janssen Chimica, Brugge, Belgium.

Method E

*1-(4-Nitrophenyl)-1-n-propylcyclohexane (**3a**)*
To a solution of **3b** (20.2 g, 0.1 mol) in 100 ml of acetic anhydride was added dropwise a solution of 0.05 mol HNO_3 (100%) in 10 ml of acetic anhydride at 0–10°C. The mixture was stirred at room temperature for 2 h, poured onto ice and extracted with ether. The combined ether extracts were washed with a $NaHCO_3$ solution and water and dried ($MgSO_4$). The solvent was removed under reduced pressure and the oily crude product was purified by flash chromatography on silica gel (petrol ether: CH_2Cl_2 = 3: 1) to yield 14.6 g (59%) of **3a**; mp 51–52°C; Anal $C_{15}H_{21}NO_2$ (C, H); ^{1}H-NMR ($CDCl_3$), δ: 0.74 (t, J = 7 Hz, 3H, CH_3), 0.88–2.33 [m, 14 H, $(CH_2)_5$ and $(CH_2)_2CH_3$], 7.40–8.33 (AA'BB', 4H, arom).

*1-Methyl-1-(4-nitrophenyl)cyclohexane (**1a**) [16]*
Yield 14.9 g (68%); oil, bp 122°C (2 mm). Anal $C_{13}H_{17}NO_2$ (C, H).

*1-Ethyl-1-(4-nitrophenyl)cyclohexane (**2a**)*
Yield 17.5 g (75%); bp 146°C (2 mm). Anal $C_{14}H_{19}NO_2$ (C, H); ^{1}H-NMR ($CDCl_3$), δ: 0.55 (t, J = 7 Hz, 3H, CH_3), 0.77–2.16 [m, 12 H $(CH_2)_5$ and CH_2CH_3], 7.29–8.28 (AA'BB', 4H, arom).

*(4-Nitrophenyl)cyclopentane (**4a**) [9]*
Yield 12.4 g (65%); oil, bp 110–114°C (5 mm). Anal $C_{11}H_{13}NO_2$ (C, H).

*(4-Nitrophenyl)cycloheptane (**6a**) [7]*
Yield 11.4 g (52%); oil, bp 139°C (2 mm). Anal $C_{13}H_{17}NO_2$ (C, H).

*2-Methyl-1-(4-nitrophenyl)propane (**13a**) [18]*
Yield 10.4 g (58%); oil, bp 110°C (4 mm). Anal $C_{10}H_{13}NO_2$ (C, H).

*2-(4-Nitrophenyl)butane (**14a**) [18]*
Yield 9.14 g (51%); oil, bp 130°C (5 mm). Anal $C_{10}H_{13}NO_2$ (C, H).

*1-(4-Nitrophenyl)pentane (**16a**) [19]*
Yield 9.47 g (49%); oil, bp 138°C (5 mm). Anal $C_{11}H_{15}NO_2$ (C, H).

*2-Methyl-1-(4-nitrophenyl)butane (**17a**) [20]*
Yield 10.4 g (54%); oil, bp 145°C (5 mm) ([20]; 110°C (0.5 mm)). Anal $C_{11}H_{15}NO_2$ (C, H).

*1-(4-Nitrophenyl)hexane (**18a**)*
Yield 12.6 g (61%); oil, bp 135°C (1 mm). Anal $C_{12}H_{17}NO_2$ (C, H); ^{1}H-NMR ($CDCl_3$), δ: 0.66–2.14 [m, 11H, $(CH_2)_4CH_3$], 2.93 (t, J = 7 Hz, 2H, $ArCH_2$), 7.65–8.85 (AA'BB', 4H, arom).

*Cyclopentyl-(4-nitrophenyl)methane (**19a**)*
Yield 13.3 g (65%); oil, bp 132°C (0.5 mm). Anal $C_{12}H_{15}NO_2$ (C, H); ^{1}H-NMR ($CDCl_3$), δ: 0.75–2.00 [m, 9H, $(CH_2)_4$ and CH], 2.72 (d, J = 7 Hz, $ArCH_2$), 7.25–8.32 (AA'BB', 4H, arom).

*Cyclohexyl-(4-nitrophenyl)methane (**20a**) [7]*
Yield 10.7 g (49%); oil, bp 140°C (0.5 mm). Anal $C_{13}H_{17}NO_2$ (C, H).

Method F

*1-(4-Aminophenyl)-1-n-propylcyclohexane (**3**)*
Palladium on charcoal (10%, 0.1 g) was added to a solution of **3a** (12.4 g, 0.05 mol) in 500 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 flash chromatographed on silica gel ($CHCl_3$) to give 10.0 g (92%) of **3**; mp 44–45°C; Anal $C_{15}H_{23}N$ (C, H); ^{1}H-NMR ($CDCl_3$), δ: 0.73 (t, J = 7 Hz, 3H, CH_3), 0.90–2.22 [m, 14 H $(CH_2)_5$ and $(CH_2)_2CH_3$]; 3.46 (s, 2H, NH_2), 6.53–7.22 (AA'BB', 4H, arom).

*1-(4-Aminophenyl)-1-ethylcyclohexane (**2**)*
Purification by silica gel flash chromatography (petrol ether: CH_2Cl_2 = 4:1) gave 9.4 g (92%); mp 33–35°C; Anal $C_{14}H_{21}N$ (C, H); ^{1}H-NMR ($CDCl_3$), δ: 0.53 (t, J = 7 Hz, 3H, CH_3), 0.74–2.12 [m, 12H $(CH_2)_5$ and CH_2CH_3], 3.43 (s, 2H, NH_2), 6.42–7.11 (AA'BB', 4 H, arom).

*1-(4-Aminophenyl)-1-methylcyclohexane (**1**) [16]*
Purification by silica gel flash chromatography (petrol ether: CH_2Cl_2 = 3:1) gave 8.33 g (88%); oil, bp 129°C (5 mm). Anal $C_{13}H_{19}N$ (C, H).

*(4-Aminophenyl)cyclopentane (**4**) [21]*
Yield 7.33 g (91%); oil, bp 93°C (10 mm) ([21]; 215–217°C). Anal $C_{11}H_{15}N$ (C, H).

*(4-Aminophenyl)cycloheptane (**6**) [7]*
Yield 8.90 g (94%); oil, bp 113°C (5 mm). Anal $C_{13}H_{19}N$ (C, H).

*1-(4-Aminophenyl)-2-methylpropane (**13**) [18]*
Yield 6.94 g (93%); oil, bp 99°C (8 mm). Anal $C_{10}H_{15}N$ (C, H).

*2-(4-Aminophenyl)butane (**14**) [18]*
Yield 6.56 g (88%); oil, bp 121°C (5 mm). Anal $C_{10}H_{15}N$ (C, H).

*1-(4-Aminophenyl)pentane (**16**) [22]*
Yield 7.68 g (94%); oil, bp 129°C (5 mm). Anal $C_{11}H_{17}N$ (C, H).

*1-(4-Aminophenyl)-2-methylbutane (**17**) [20]*
Yield 7.76 g (95%); oil, bp 127°C (4 mm) ([20]; 96°C (1 mm)). Anal $C_{11}H_{17}N$ (C, H).

*1-(4-Aminophenyl)hexane (**18**) [10]*
Yield 7.7 g (87%); oil, bp 140°C (4 mm) ([10]; 270–310°C). Anal $C_{12}H_{19}N$ (C, H).

*(4-Aminophenyl)cyclopentylmethane (**19**)*
Yield 8.0 g (91%); oil, bp 138°C (5 mm). Anal $C_{12}H_{17}N$ (C, H); ^{1}H-NMR ($CDCl_3$), δ: 1.07–2.07 [m, 9H, $(CH_2)_4$ and CH], 2.65 (d, J = 7 Hz, 2H, $ArCH_2$), 3.77 (s, 2H, NH_2), 6.87–7.49 (AA'BB', 4H, arom).

*(4-Aminophenyl)cyclohexylmethane (**20**) [7]*
Yield 8.23 g (87%); oil, bp 148°C (8 mm). Anal $C_{13}H_{19}N$ (C, H). Compounds **5**, **7–12** and **15** were commercially available.

Biological methods

Enzyme preparation and assay procedures
The compounds were tested for their inhibitory activity against aromatase and desmolase according to the procedure published previously [1]. The microsomal fraction of freshly delivered human term placenta provided the source of the aromatase enzyme. Desmolase was obtained from the mitochondrial fraction of bovine adrenal cortex. Each time point of control or inhibitor incubation was run in triplicate.

Aromatase inhibition in vitro
Inhibition of aromatase by the test compounds was determined by measuring the ^3H_2O formed during the conversion of [1β,2β-^{3}H] testosterone to estradiol with or without inhibitor. Separation of the ^3H_2O from the steroids was performed by adding Dextran-coated charcoal. After centrifugation, the radioactivity of a 200-μl supernatant aliquot was counted. Inhibition values for each compound were determined at two concentrations (50 and 250 μM). Compounds showing a stronger inhibition than AG were tested in 6 appropriate concentrations to determine the IC_{50} values. The percent inhibition was plotted *vs* the concentration of inhibitor on a semilog plot. From this the molar concentration causing 50% inhibition was determined.

Desmolase inhibition in vitro
Only compounds showing a stronger inhibition of aromatase than AG were tested for their inhibitory effects against desmolase. The enzyme activity was assayed at 37°C by measuring the [^{14}C]isocaproic acid released from the substrate [26-^{14}C] cholesterol. Separation of the isocaproic acid from the substrate was achieved by chromatography on alumina mini-columns. Aliquots of the eluate were counted. The inhibition values given in table III were determined at an inhibitor concentration of 25 μM.

Irreversible inhibition of aromatase
In this assay microsomal protein (1 mg) was preincubated for 30 min with NADPH (0.5 μM), glucose-6-phosphate (1 μM), 1 EU glucose-6-phosphate dehydrogenase, and inhibitor (0–50 μM) as described in [1]. The total volume was 0.5 ml. After separation of the inhibitor by the addition of Dextran-coated charcoal, aromatase activity in the supernatant was measured as reported previously [1].

Reduction of E_2 level in vivo
Eight- to ten-week-old female Sprague-Dawley (SD) rats (Zentralinstitut für Versuchstierzucht, Hannover) were primed with pregnant mares' serum gonadotropin (PMSG; Sigma St Louis, MO) similar to the method of Brodie *et al* [11]. Subcutaneous (sc) injections of 100 IU of PMSG in 500 μl of saline were applied every other day for 11 days. On day 12 the animals received a single sc injection of olive oil (control) or of an olive oil solution of the test compound: 2 mg/kg of AG or equimolar doses of the other compounds. After 6 h the rats were anesthetized and blood was taken from the ovarian vein and the heart. The plasma E_2 level was determined as described previously [1].

Mammary tumor studies (postmenopausal model)
The tumor inhibiting effect was determined by using the DMBA-induced, hormone-dependent mammary adenocarcinoma of the SD rat. The methods applied for tumor induction and assignment to treatment groups have been described previously [23]. Animals bearing at least one tumor greater than 140 mm^2 were classified in groups of 10. One day before the start of the treatment the ovaries of the tumor bearing rats were removed through incisions in the lumbar region of the back. Compounds were dissolved in olive oil and applied sc 6 times a week. The therapy was continued for 28 days. Measurement of tumor size and determination of body weight were made weekly. At the end of the therapy the uteri were removed, fixed with Bouin's solution, washed, dried, and weighed as described formerly [24].

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