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Dibenzo[*a,f*]quinolizines: syntheses and cytostatic activity in estrogen-sensitive tumor cells

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Summary: A number of methoxy-substituted 7,11b,12,13-tetrahydro-6*H*-dibenzo-[*a,f*]quinolizines with short alkyl groups in position 6 or 12 were synthesized by the Bischler–Napieralski reaction using the appropriate starting material followed by a second ring closure reaction involving a base-generated benzyne intermediate. The methoxy functions in positions 2 or 3 and 9 were cleaved with BBr_3 and the free hydroxy groups converted into the acetates. The enantiomers of two of these derivatives were separated by liquid chromatography on triacetylcellulose. Compounds with alkyl substituents bind strongly to the estrogen receptor except those with a *cis*-orientation at the central ring connection. The RBA values ranged from 2.2–10.8 (17β-estradiol: RBA = 100). There was no major difference in binding between the (+) and (−)-enantiomers. The 3,9-diacetoxy-6-alkyl derivatives also showed binding affinity for the progesterone receptor (RBA: 1.2–3.1). The 2,9-diacetoxy-dibenzoquinolizines *trans*-**6l** and **-6m** with ethyl and propyl respectively in position 12 strongly inhibited the growth of hormone-sensitive MCF-7 breast cancer cells at concentrations of 10^{-6} M and higher but were inactive in hormone-independent MDA-MB 231 breast cancer cells. Preliminary tests with hormone-dependent MXT mouse mammary tumors as model showed that these compounds have also antineoplastic activity *in vivo*. Derivative *trans*-**6l** at a dose of 20 mg/kg body weight, administered 3 times/week, inhibited the growth of these tumors by 78% (tamoxifen: 76% inhibition). Studies on the estrogenic and antiestrogenic properties of these agents in mice revealed that they are mixed agonists/antagonists with strong antiestrogenic activity at low doses but significant estrogenic effects at higher doses.

Key words: cytostatic activity/dibenzo[*a,f*]quinolizines/estrogen receptor affinity/synthesis

Introduction

Tetracyclic systems based on the isoquinoline structure such as indolo[*2,1-a*]isoquinoline (Polossek *et al.*, 1992; Ambros *et al.*, 1989) and dibenzo[*a,g*]-quinolizin-8-ones (von Angerer *et al.*, 1992) have been shown to possess cytostatic activity. When acetoxy groups were introduced into appropriate positions of the benzene rings in these structures, significant binding affinities for estrogen receptors were observed. The receptor binding

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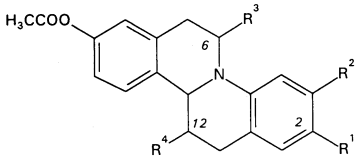
can be improved by the introduction of short alkyl groups as a result of an enhanced lipophilicity in the central part of the molecule. Endocrine studies revealed that some of these derivatives can be considered as antiestrogens and might be useful for the endocrine therapy of hormone-dependent malignancies such as mammary and endometrium carcinomas.

In order to evaluate the therapeutic potential of these tetracyclic systems we extended our studies to acetoxy-substituted 7,11b,12,13-tetrahydro-6*H*-dibenzo[*a,f*]quinolizines with alkyl substituents at C-6 and C-12 (Structures 1). The main differences between this structure and the series of compounds we studied previously are a basic nitrogen and a tetrahedral carbon adjacent to the nitrogen which gives rise to two different orientations of the ring connection in the quinolizidine moiety which can be discriminated when a substituent at C-12 is present. In this report, we describe the syntheses of a number of tetrahydro-6*H*-dibenzo[*a,f*]quinolizines with acetoxy groups in positions 2 (3) and 9 and short alkyl chains either at C-6 or at C-12, their binding affinities for calf uterine estrogen and progesterone receptors, their cytostatic activity in human breast cancer cells, and their endocrine properties in the mouse uterine weight test.



R^1, R^2 = H, $OCOCH_3$
R^3, R^4 = H, CH_3, C_2H_5, C_3H_7

Structures 1

Materials and methods

Melting points (m.p.) were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed by Mikroanalytisches Laboratorium, University of Regensburg. ^{1}H NMR spectra were obtained on a Varian EM 390 and a Bruker WM 250 spectrometer and are consistent with the assigned structures.

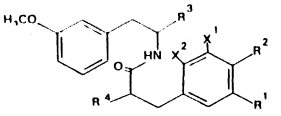
Reagents

[2,4,6,7-^{3}H(N)]Estradiol (110 Ci/mmol) was obtained from New England Nuclear (Dreieich, Germany), [^{3}H]Org 2058 ((16α-ethyl)-21-hydroxy-19-norpregn-4-en-3,20-dione) from Amersham, Braunschweig, Germany. Hormones and biochemicals were purchased from Sigma (München, Germany). TEA [Tris buffer (10 mM, pH 7.4) supplemented with EDTA (1.5 mM) and NaN_3 (3 mM)] was used as buffer.

General procedure for the synthesis of the phenylpropionic acid phenethylamides **3a–m**

Phenylpropionic acid **2** (38.6 mmol) (Seidl, 1990) was dissolved in a threefold excess of $SOCl_2$. The reaction was started by addition of a few drops of DMF at room temperature and completed by heating at 60 °C until the gas evolution ceased. The excess of $SOCl_2$ was removed *in vacuo*. The crude acid chloride was dissolved in dry CH_2Cl_2 (40 ml) and added slowly to a mixture of the respective phenylethyl amine **1** (38.6 mmol) and triethylamine (38.6 mmol) in 40 ml of CH_2Cl_2. After stirring for 1 h at room temperature, the mixture was poured into 2N HCl and extracted with CH_2Cl_2. The organic layer

Table I 3-(Brom-methoxyphenyl)-N-[2-(3-methoxyphenyl)ethyl]propionic acid amides **3**



Compound	R^1	R^2	R^3	R^4	X^1	X^2	*Formula*[a]	*m.p.*[b] (°C)
3a	OCH_3	H	H	H	H	Br	$C_{19}H_{22}BrNO_3$	96
3b	H	OCH_3	H	H	Br	H	$C_{19}H_{22}BrNO_3$	112
3c	OCH_3	H	CH_3	H	H	Br	$C_{20}H_{24}BrNO_3$	91
3d	H	OCH_3	CH_3	H	Br	H	$C_{20}H_{24}BrNO_3$	94
3e	OCH_3	H	C_2H_5	H	H	Br	$C_{21}H_{26}BrNO_3$	74
3f	H	OCH_3	C_2H_5	H	Br	H	$C_{21}H_{26}BrNO_3$	47.5
3g	OCH_3	H	C_3H_7	H	H	Br	$C_{22}H_{28}BrNO_3$	72
3h	H	OCH_3	C_3H_7	H	Br	H	$C_{22}H_{28}BrNO_3$	oil
3i	OCH_3	H	H	CH_3	H	Br	$C_{20}H_{24}BrNO_3$	70–72
3k	H	OCH_3	H	CH_3	Br	H	$C_{20}H_{24}BrNO_3$	113
3l	OCH_3	H	H	C_2H_5	H	Br	$C_{21}H_{26}BrNO_3$	77.5
3m	OCH_3	H	H	C_3H_7	H	Br	$C_{22}H_{28}BrNO_3$	57

[a] Analyzed for C and H within ±0.4% of the calculated values
[b] Recrystallized from EtOAc/Et_2O mixtures

was dried and the solvent evaporated. The residues were purified by crystallization from appropriate solvents or by Kugelrohr distillation. Yields were in the range 70–90%. M.p. are reported in Table I.

General procedure for the synthesis of 1,2,3,4-tetrahydro-1-(phenylethyl)isoquinolines **4a–m**

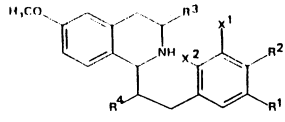
A mixture of the phenylpropionic acid amide **3** (46 mmol) in 50 ml of dry MeCN and 12 ml of $POCl_3$ was refluxed for 3 h under nitrogen. The volume of the mixture was reduced to 15 ml *in vacuo*. In the refrigerator, the product usually crystallized; sometimes a small volume of dry Et_2O had to be added. The crystals were separated by filtration and washed with ice-cold dry acetone. An aqueous solution of the crystals was made alkaline with NaOH (40%) and extracted with CH_2Cl_2. After drying, the organic solvent was removed *in vacuo* to give the dihydroisoquinoline as an oily residue.

Without further purification, 16.3 mmol of the dihydroisoquinoline was dissolved in MeOH (70 ml). At 0°C, 2 g of $NaBH_4$ was added in small portions with stirring. Stirring was continued for 1 h at 0°C, 30 min at room temperature, and 30 min with boiling under reflux. The volume was reduced to 10 ml. After addition of 80 ml of water, the mixture was extracted with CH_2Cl_2. The organic layer was washed with water and dried. The residue obtained after evaporation of the solvent was purified by chromatography (SiO_2; EtOAc). Solid products were crystallized from Et_2O, oils were distilled *in vacuo*. Yields were 80–90%. M.p. are reported in Table II.

General procedure for the synthesis of 7,11b,12,13-tetrahydro-methoxy-6H-dibenzo[a,f]quinolizines **5a–m**

A solution of the bromotetrahydro(phenylethyl)isoquinoline **4** (12.6 mmol) in 50 ml of DMSO was added slowly to a solution of sodium methylsulfinylmethanide prepared from 2.5 g (84 mmol) of NaH (80% in oil dispersion) and 50 ml of DMSO. After stirring for

Table II 1,2,3,4,-Tetrahydro-6-methoxy-1-(phenylethyl)isoquinolines **4**



Compound	R^1	R^2	R^3	R^4	X^1	X^2	*Formula*[a]	*m.p.*[b] (°C)
4a	OCH_3	H	H	H	H	Br	$C_{19}H_{22}BrNO_3$	76.5
4b	H	OCH_3	H	H	Br	H	$C_{19}H_{22}BrNO_3$	oil
4c[c]	OCH_3	H	CH_3	H	H	Br	$C_{20}H_{24}BrNO_3$	95–96
4d	H	OCH_3	CH_3	H	Br	H	$C_{20}H_{24}BrNO_3$	oil
4e[c]	OCH_3	H	C_2H_5	H	H	Br	$C_{21}H_{26}BrNO_3$	69
4f	H	OCH_3	C_2H_5	H	Br	H	$C_{21}H_{26}BrNO_3$	oil
4g[c]	OCH_3	H	C_3H_7	H	H	Br	$C_{22}H_{28}BrNO_3$	71.5
4h	H	OCH_3	C_3H_7	H	Br	H	$C_{22}H_{28}BrNO_3$	oil
4i[d]	OCH_3	H	H	CH_3	H	Br	$C_{20}H_{24}BrNO_3$	oil
4k[d]	H	OCH_3	H	CH_3	Br	H	$C_{20}H_{24}BrNO_3$	oil
4l[d]	OCH_3	H	H	C_2H_5	H	Br	$C_{21}H_{26}BrNO_3$	oil
4m[d]	OCH_3	H	H	C_3H_7	H	Br	$C_{22}H_{28}BrNO_3$	oil

[a] Analyzed for C and H within ±0.4% of the calculated values
[b] Recrystallized from Et_2O
[c] Predominantly one diastereomer (95%)
[d] Mixture of diastereomers, ratio approximately 7:3

12 h, the mixture was poured into 400 ml of ice water containing an excess of NH_4Cl and extracted with CH_2Cl_2. The organic layer was washed with water and saline. After drying (Na_2SO_4) and evaporation of the solvent, an oil was obtained. After chromatography (SiO_2; $CHCl_3/Et_2O$ 19:1) solid products were crystallized from MeOH and oils were distilled. Diastereomeric mixtures **5i–m** were separated by an additional chromatography (SiO_2) with a different solvent system ($CHCl_3/Et_2O$ 9:1). Yields and m.p. are reported in Table III.

General procedure for the synthesis of acetoxy-7,11b,12,13-tetrahydro-6H-dibenzo[a,f]quinolizines **6a–m**

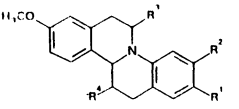
BBr_3 (0.42 ml) was added to a solution of methoxy-dibenzoquinolizine **5** (0.65 mmol) in 15 ml of dry $CHCl_3$ at 0°C. After stirring for 10 min at 0°C and 10 min at room temperature, the mixture was refluxed for 2 h and stirred at room temperature for 12 h. With cooling, 15 ml of dry MeOH were added. After evaporation of the solvent, dry acetone was added to crystallize the product. The crystals were separated by filtration and dried.

Under nitrogen, the crystals were heated in a mixture of Ac_2O (12 ml) and 2 drops of pyridine until a homogeneous solution was obtained. After stirring for additional 2 h, the solution was poured into ice-water and neutralized with NaOH (40%). The aqueous phase was extracted with CH_2Cl_2. After drying, the organic solvent was evaporated *in vacuo*. The residue was purified by chromatography (SiO_2; $CHCl_3/Et_2O$ 9:1). The yields ranged from 60–70%. M.p. are given in Table IV, NMR data in Table V.

Chromatographic separation of the enantiomers of **6l** *and* **6m**

The enantiomers of **6l** and **6m** were separated semipreparatively by liquid chromatography on triacetylcellulose (20–30 μm)(Koller *et al.*, 1983) with EtOH as eluent at 22°C

Table III 7,11b,12,13-Tetrahydro-dimethoxy-6*H*-dibenzo[*a,f*]quinolizines **5**



Compound	R^1	R^2	R^3	R^4	*% Yield*	*Formula*[a]	*m.p.*[b] *(°C)*
5a	OCH_3	H	H	H	64	$C_{19}H_{21}NO_2$	151
5b	H	OCH_3	H	H	60	$C_{19}H_{21}NO_2$	oil
5a	OCH_3	H	CH_3	H	51	$C_{20}H_{23}NO_2$	151
5d	H	OCH_3	CH_3	H	54	$C_{20}H_{23}NO_2$	114
5e	OCH_3	H	C_2H_5	H	49	$C_{21}H_{25}NO_2$	99.5
5f	H	OCH_3	C_2H_5	H	60	$C_{21}H_{25}NO_2$	oil
5g	OCH_3	H	C_2H_5	H	55	$C_{22}H_{27}NO_2$	81.5
5h	H	OCH_3	C_3H_7	H	45	$C_{22}H_{27}NO_2$	oil
***trans*-5i**[c]	OCH_3	H	C_3H_7	CH_3	15	$C_{20}H_{23}NO_2$	158.5
***cis*-5i**[d]	OCH_3	H	H	CH_3	36	$C_{20}H_{23}NO_2$	86.5
***trans*-5k**[c]	H	OCH_3	H	CH_3	17	$C_{20}H_{23}NO_2$	oil
***cis*-5k**[d]	H	OCH_3	H	CH_3	34	$C_{20}H_{23}NO_2$	118
***trans*-5l**[c]	OCH_3	H	H	C_2H_5	18	$C_{21}H_{25}NO_2$	106
***cis*-5l**[d]	OCH_3	H	H	C_2H_3	32	$C_{21}H_{25}NO_2$	oil
***trans*-5m**[c]	OCH_3	H	H	C_3H_7	15	$C_{22}H_{27}NO_2$	109
***cis*-5m**[d]	OCH_3	H	H	C_3H_7	36	$C_{22}H_{27}NO_2$	oil

[a] Analyzed for C and H within ±0.4% of the calculated values
[b] Recrystallized from MeOH
[c] *Trans* refers to the linkage of B and C rings
[d] *Cis* refers to the linkage of B and C rings

(2 bar, flow rate 3–4 ml/min). All [α] values refer to 22°C.

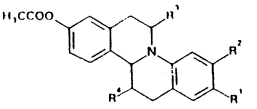
(+)-*trans*-**6l**: $k^a = 1.2$; $[\alpha]_{365} = +790 \pm 60$, $[\alpha]_{436} = +400 \pm 40$, $[\alpha]_{546} = +210 \pm 30$, $[\alpha]_{578} = +180 \pm 30$ (c = 1.1 mg/ml; CH_3CN); $P^b = 1.0$.
(−)-*trans*-**6l**: $k = 2.1$; $[\alpha]_{365} = -670 \pm 50$, $[\alpha]_{436} = -360 \pm 40$, $[\alpha]_{546} = -170 \pm 30$, $[\alpha]_{578} = -150 \pm 30$ (c = 1.1 mg/ml; CH_3CN); $P \approx 0.83$.
(+)-*trans*-**6m**: $k = 1.0$; $[\alpha]_{365} = +930 \pm 100$, $[\alpha]_{436} = +460 \pm 70$, $[\alpha]_{546} = +230 \pm 50$, $[\alpha]_{578} = +200 \pm 50$, $[\alpha]_{589} = +200 \pm 50$ (c = 0.6 mg/ml; CH_3CN); $P = 1.0$.
(−)-*trans*-**6m**: $k = 1.7$; $[\alpha]_{365} = -670 \pm 60$, $[\alpha]_{436} = -340 \pm 40$, $[\alpha]_{546} = -190 \pm 30$, $[\alpha]_{578} = -170 \pm 30$, $[\alpha]_{589} = -150 \pm 30$ (c = 0.8 mg/ml; CH_3CN); $P = 0.98$.

[a] k is the capacity factor of the *d*- and l-enantiomers; $k = (t_i - t_0)/t_0$, where t_i = retention time and t_0 = void volume elution time.
[b] Optical purity, calculated from a plot of rotation angle α versus UV absorbance according to Mannschreck *et al.* (1982).

Binding affinities for steroid hormone receptors

Affinities for estrogen (von Angerer *et al.*, 1984) and progesterone receptors (Theofan & Notides, 1984) were determined with calf uterine cytosol as receptor source. Fresh calf uteri, stored in ice-cold saline, were freed of adherent fat and connective tissue at 4°C. After addition of the relevant buffer (10 mM Tris, 1.5 mM EDTA, 3 mM NaN_3, pH 7.4, for ER, 10 mM Tris, 1.5 mM EDTA, 0.25 M sucrose, pH 7.4, for PR) the uteri were

Table IV Diacetoxy-7,11b,12,13-tetrahydro-6*H*-dibenzo[*a,f*]quinolizines **6**



Compound	R^1	R^2	R^3	R^4	*Formula*[a]	*m.p. (°C)*	*RBA*[b] *(ER)*
6a	$OCOCH_3$	H	H	H	$C_{21}H_{21}NO_4$	153	0.3
6b	H	$OCOCH_3$	H	H	$C_{21}H_{21}NO_4$	120	0.4
6c	$OCOCH_3$	H	CH_3	H	$C_{22}H_{23}NO$	135.5	5.4
6d	H	$OCOCH_3$	CH_3	H	$C_{22}H_{23}NO_4$	129	5.8
6e	$OCOCH_3$	H	C_2H_5	H	$C_{23}H_{25}NO_4$	134.5	6.0
6f	H	$OCOCH_3$	C_2H_5	H	$C_{23}H_{25}NO_4$	99.5	8.7
6g	$OCOCH_3$	H	C_3H_7	H	$C_{24}H_{27}NO_4$	81	10.5
6h	H	$OCOCH_3$	C_3H_7	H	$C_{24}H_{27}NO_4$	92	6.8
trans-**6i**	$OCOCH_3$	H	H	CH_3	$C_{22}H_{23}NO_4$	141.5	7.8
cis-**6i**	$OCOCH_3$	H	H	CH_3	$C_{22}H_{23}NO_4$	134.5	0.3
trans-**6k**	H	$OCOCH_3$	H	CH_3	$C_{22}H_{23}NO_4$	92	5.8
cis-**6k**	H	$OCOCH_3$	H	CH_3	$C_{22}H_{23}NO_4$	118	0.2
trans-**6l**	$OCOCH_3$	H	H	C_2H_5	$C_{23}H_{25}NO_4$	129.5	8.3
trans-(+)-**6l**	$OCOCH_3$	H	H	C_2H_5	$C_{23}H_{25}NO_4$	57	6.7
trans-(−)-**6l**	$OCOCH_3$	H	H	C_2H_5	$C_{23}H_{25}NO_4$	58	10.8
cis-**6l**	$OCOCH_3$	H	H	C_2H_5	$C_{23}H_{25}NO_4$	104	0.2
trans-**6m**	$OCOCH_3$	H	H	C_3H_7	$C_{24}H_{27}NO_4$	123	2.5
trans-(+)-**6m**	$OCOCH_3$	H	H	C_3H_7	$C_{24}H_{27}NO_4$	45	2.2
trans-(−)-**6m**	$OCOCH_3$	H	H	C_3H_7	$C_{24}H_{27}NO_4$	46	3.0
cis-**6m**	$OCOCH_3$	H	H	C_3H_7	$C_{24}H_{27}NO_4$	92	0.1

[a] Analyzed for C and H within ±0.4% of the calculated values

[b] Relative binding affinity for the calf uterine estrogen receptor = ratio of molar concentration of 17β-estradiol (E2) and inhibitor required to decrease the amount of bound [^{3}H]E2 by 50%, × 100

Table V ^{1}H-NMR Data of 9-acetoxy-7,11b,12,13-tetrahydro-6*H*-dibenzo[*a,f*]quinolizines **6**

Compound	δ *[p.p.m.] (recorded in $CDCl_3$ with TMS as internal standard)*
6a	1.80 (mc; 1H, H-12ax), 2.25 (mc; 1H, H-12eq), 2.25 (s; 3H,-CO-CH_3), 2.30 (s; 3H,-CO-CH_3), 2.88 (mc; 4H,-CH_2-), 3.26 (mc; 1H, H-6ax), 3.88 (mc; 1H, H-6eq), 4.45 (dd, $J_{1/2}$ = 2.5/9Hz; 1H, H-11b), 6.70–7.05 (m; 5H, ArH), 7.30 (d, $J_{1/2}$ = 9Hz; 1H, ArH)
6b	2.23 (mc; 2H, H-12), 2.25 (s; 3H,-CO-CH_3), 2.30 (s; 3H,-CO-CH_3), 2.65–3.40 (m; 5H,-CH_2, H-6ax), 3.93 (mc; 1H, H-6eq), 4.40 (dd, $J_{1/2}$ = 2/9Hz; 1H, H-11b), 6.25 (dd, $J_{1/2}$ = 3/9Hz; 1H, ArH), 6.45 (d, $J_{1/2}$ = 3Hz; 1H, ArH), 6.60–7.10 (m; 3H, ArH), 7.20 (d, J = 9Hz; 1H, ArH)
6c	1.15 (d, J = 6Hz; 3H,-CH_3), 1.90–2.45 (m; 2H, H-12), 2.25 (s; 3H,-CO-CH_3), 2.30 (s; 3H,-CO-CH_3), 2.65 (ABX, $J_{AX/AB}$ = 2/16Hz; 1H, H-7eq), 2.80–3.10 (m; 2H, H-13), 3.25 (ABX, $J_{BX/BA}$ = 5/16Hz; 1H, H-7ax), 4.10–4.50 (m; 2H, H-6, H-11b), 6.65–7.40 (m; 6H, Arh)
6d	1.05 (d, J = 6Hz; 3H, CH_3), 1.65–2.35 (m; 2H, H-12), 2.20 (s; 3H,-CO-CH_3), 2.25 (s; 3H-CO-CH_3), 2.55 (ABX, $J_{AX/AB}$ = 2/16Hz; 1H, H-7eq), 2.70–3.05 (m; 2H, H-13), 3.15 (ABX, $J_{BX/BA}$ = 6/16Hz; 1H, H-7ax), 4.10–4.50 (m; 2H, H-6, H-11b), 6.40 (dd, $J_{1/2}$ = 2/9Hz; 1H, ArH), 6.70–7.00 (m; 3H, ArH), 7.20 (d, J = 9Hz; 1H, ArH)

Table V ***(continued)***

Compound	δ *[p.p.m.] (recorded in $CDCl_3$ with TMS as internal standard)*
6e	0.90 (t, J = 7.5Hz; 3H, CH_3), 1.20–1.60 (m; 2H,-CH_2-CH_3), 1.80–2.40 (m; 2H, H-12), 2.15 (s; 3H,-CO-CH_3), 2.20 (s; 3H,-CO-CH_3), 2.65 (ABX, $J_{AX/AB}$ = 0.5/16Hz; 1H, H-7eq), 2.70–3.05 (m; 2H, H-13), 3.15 (ABX, $J_{BX/BA}$ = 5/16Hz; 1H, H-7ax), 4.10 (ABX, $J_{XA/XB}$ = 0.5/5Hz; 1H, H-6), 4.35 (dd, $J_{1,2}$ = 2/9Hz; 1H, H-11b), 6.50–7.40 (m; 6H, ArH)
6f	0.90 (t, J = 7.5Hz; 3H,-CH_2-CH_3), 1.10–1.65 (m; 2H,-CH_2-CH_3), 1.75–2.40 (m; 2H, H-12), 2.20 (s; 6H,-CO-CH_3), 2.60 (ABX, $J_{AX/AB}$ = 2/16Hz; 1H, H-7eq), 2.75–2.90 (m; 2H, H-13), 3.10 (ABX, $J_{BX/BA}$ = 6/16Hz; 1H, H-7ax), 3.90 (ABX, $J_{XA/XB}$ = 2/6Hz; 1H, H-6), 4.45 (dd, J = 3/9Hz, 1H, H-11b), 6.30 (dd, $J_{1/2}$ = 3/9Hz; 1H, ArH), 6.45 (d, J = 3Hz; 1H, ArH), 6.70–7.00 (m; 3H, ArH), 7.20 (d, J = 9Hz; 1H, ArH)
6g	0.90 (t, J = 7Hz; 3H,-CH_2-CH_3), 1.25–1.75 (m; 4H,-CH_2-CH_2-CH_3), 1.90–2.40 (m; 2H, H-12), 2.20 (s; 3H,-CO-CH_3), 2.25 (s; 3H,-CO-CH_3), 2.65 (ABX, $J_{AX/AB}$ = 0.5/16Hz; 1H, H-7eq), 2.75–3.00 (m; 2H, H-13), 3.10 (ABX, $J_{BX/BA}$ = 5/16Hz; 1H, H-7ax), 4.10 (ABX, $J_{AB/XB}$ = 0.5/5Hz; 1H, H-6), 4.40 (dd, $J_{1/2}$ = 2/7Hz; 1H, H-11b), 6.70–7.40 (m; 6H, ArH)
6h	0.85 (t, J = 6Hz; 3H,-CH_2-CH_3), 1.05–1.75 (m; 4H,-CH_2-CH_2-CH_3), 1.80–2.45 (m; 2H, H-12), 2.25 (s; 6H,-CO-CH_3), 2.65 (ABX, $J_{AX/AB}$ = 2/16Hz; 1H, H-7eq), 2.75–3.00 (m; 2H, H-13), 3.15 (ABX, $J_{BX/BA}$ = 6/16Hz; 1H, H-7ax), 4.10 (ABX, $J_{1XA/X}B$ = 2/6Hz; 1H, H-6), 4.45 (dd, $J_{1,2}$ = 3/9Hz; 1H, H-11b), 6.40 (dd, $J_{1/2}$ = 3/9Hz; 1H, ArH), 6.55 (d, J = 3Hz; 1H, ArH), 6.80–7.15 (m; 3H, ArH), 7.30 (d, J = 9Hz; 1H, ArH)
trans-**6i**	0.70 (d, J = 7Hz; 3H,-CH_3), 2.20 (s; 3H,-CO-CH_3), 2.25 (s; 3H,-CO-CH_3), 2.50 (ABX, $J_{XA/XB}$ = 5/6Hz; 1H, H-12), 2.60 (ABX, $J_{AX/AB}$ = 0.5/16Hz; 1H, H-13eq), 2.70–3.10 (m; 3H, H-6ax, H-7), 3.35 (ABX, $J_{BX/BA}$ = 6/16Hz, 1H, H-13ax), 4.00 (mc; 1H, H-6eq), 4.45 (d, J = 0.5Hz; 1H, H-11b), 6.65–7.35 (m; 6H, ArH)
cis-**6i**	1.15 (d, J = 6Hz; 3H,-CH_3), 2.20 (s; 3H,-CO-CH_3), 2.25 (s; 3H,-CO-CH_3), 2.30 (ABX, $J_{AX/AB}$ = 3/16Hz; 1H,H-13eq), 2.55 (ABX, $J_{XA/XB}$ = 3/5Hz; 1H, H-12), 2.65 (mc; 1H, H-7), 2.80 (ABX, $J_{BX/BA}$ = 5/16Hz; 1H, H-13ax), 3.00–3.60 (m; 2H, H-6ax, H-7), 3.90–4.25 (m; 2H, H-6eq, H-11b), 6.50 (d, J = 3Hz; 1H, ArH), 6.56 (d, J = 3Hz; 1H, ArH), 6.65–7.35 (m; 6H, ArH)
trans-**6k**	0.70 (d, J = 6.5Hz; 3H,-CH_3), 2.25 (s; 6H,-CO-CH_3), 2.30 (ABX, $J_{XA/XB}$ =0.5/6Hz; 1H, H-12), 2.55 (ABX, $J_{AX/AB}$ = 0.5/16Hz; 1H, H-13eq), 2.65–3.10 (m; 3H, H-6ax, H-7), 3.30 (ABX, $J_{BX/BA}$ = 6/16Hz·; 1H,H-13ax), 4.00 (mc; 1H, H-6eq), 4.45 (d, J = 0.5Hz; 1H, H-11b), 6.35 (dd, $J_{1,2}$ = 3/9Hz; 1H, ArH), 6.45 (d, J = 3Hz; 1H, ArH), 6.75–7.10 (m; 3H, ArH), 7.25 (d, J = 9Hz; 1H, ArH)
cis-**6k**	1.15 (d, J = 6.5Hz; 3H, CH_3), 2.25 (s; 6H,-CO-CH_3), 2.35 (ABX, $J_{AX/AB}$ =0.5/16Hz; 1H, H-13eq), 2.45 (ABX, $J_{XA/XB}$ = 0.5/5Hz; 1H, H-12), 2.45–3.30 (m; 3H, H-6ax, H-7), 3.45 (ABX, $J_{BX/BA}$ = 5/16Hz; 1H, H-13ax), 4.00 (mc; 1H, H-6eq), 4.20 (d, J = 3Hz; 1H, H-11b), 6.35 (dd, $J_{1,2}$ = 3/9Hz; 1H, ArH), 6.55 (d, J = 3Hz; 1H, ArH), 6.70–7.00 (m; 3H, ArH), 7.25 (d, J = 9Hz; 1H, ArH)
trans-**6l**	0.75 (t, J = 7.5Hz; 3H,-C_2-CH_3), 1.00–1.20 (m; 2H,-CH_2-CH_3), 2.20 (s; 3H,-CO-CH_3), 2.25 (s; 3H,-CO-CH_3), 2.35 (ABX, $J_{XA/XB}$ = 0.5/6Hz; 1H, H-12), 2.70 (ABX, $J_{AX/AB}$ = 0.5/16Hz; 1H, H-13eq), 2.80–3.05 (m; 3H, H-6ax, H-7), 3.20 (ABX, $J_{BX/BA}$ = 6/16Hz·; 1H, H-13ax), 3.93 (m; 1H, H-6eq), 4.45 (d, J = 0.5Hz; 1H, H-11b), 6.50–7.35 (m; 6H, ArH)
cis-**6l**	1.05 (t, J = 7.5Hz; 3H,-CH_2-CH_3), 1.30–1.70 (m; 2H,-CH_2-CH_3), 2.15 (s; 3H,-CO-CH_3), 2.20 (s; 3H,-CO-CH_3), 2.40 (ABX, $J_{XA/XB}$ = 0.5/6Hz; 1H, H-12), 2.60 (ABX, $J_{AX/AB}$ = 0.5/16Hz; 1H, H-13eq), 2.75–3.35 (m; 3H, H-6ax, H-7), 3.45 (ABX, $J_{BX/BA}$ = 4/16H; 1H, H-13ax), 4.10 (mc; 1H, H-6-eq), 4.30 (d, J = 2Hz; 1H, H-11b), 6.60–7.40 (m; 6H, ArH)

Table V *(continued)*

Compound	δ *[p.p.m.] (recorded in $CDCl_3$ with TMS as internal standard)*
trans-**6m**	0.75 (t, J = 6Hz; 3H,-CH_2-CH_3), 0.85–1.50 (m; 4H,-CH_2-CH_2-CH_3), 2.20 (s; 3H,-CO-CH_3), 2.25 (s; 3H,-CO-CH_3), 2.35 (ABX, $J_{XA/XB}$ = 0.5/6Hz; 1H, H-12), 2.75 (ABX, $J_{AX/AB}$ = 0.5/16Hz; 1H, H-13eq), 2.85–3.15 (m; 3H, H-6ax, H-7), 3.25 (ABX, $J_{BX/BA}$ = 6/16Hz; 1H, H-13ax), 3.95 (mc; 1H, H-6eq), 4.45 (d, J = 0.5Hz; 1H, H-11b), 6.60–7.35 (m; 6H, ArH)
cis-**6m**	1.00 (t, J = 6Hz; 3H,-CH_2-CH_3), 1.20–1.70 (m; 4H,-CH_2-CH_2-CH_3), 2.20 (s; 3H,-CO-CH_3), 2.25 (s; 3H,-CO-CH_3), 2.35 (ABX, $J_{AX/AB}$ = 2/16Hz; 1H, H-13eq), 2.50–3.20 (m; 4H, H-6ax, H-12, H-7), 3.45 (ABX, $J_{BX/BA}$ = 5/16Hz; 1H, H-13ax), 4.03 (mc; 1H, H-6eq), 4.30 (d, J = 3Hz; 1H, H-11b), 6.70–7.40 (m; 6H, ArH)

homogenized by treatment with a ultraturrax mixer (IKA, FRG) and a glass-in-glass homogenizer (Potter S; Braun, Germany) at 4°C. Lipids were separated by centrifugation at 700 *g* and discarded. The homogenate was centrifuged at 105 000 *g* for 100 min (0°C). The supernatant (cytosol) was then used for determining the affinity of compounds for the receptor. The protein concentration of the cytosol was ~15 mg/ml leading to a final concentration of 3 mg/ml in the assay.

Relative binding affinities (RBA) were determined by the dextran-coated charcoal (DCC) method: 100 µl aliquots of the cytosol were incubated with 100 µl (1 nM) of [3*H*]estradiol (ER) or [3*H*]Org 2058 (PR) and different concentrations of the test compounds at 0–4°C for 16 h (ER) or 2 h (PR). Non-specific radioligand binding was determined by a parallel incubation containing 2 µM of estradiol (ER) or progesterone (PR). After incubation, dextran-coated charcoal suspension (0.625% dextran 80.000, 1.25% Norit A in the relevant buffer) was added and the mixture was shaken for 90 min (ER) or 10 min (AR, PR) at 0–4°C. After centrifugation for 10 min at 800 *g*, the radioactivity of a 100 µl supernatant aliquot was counted. The percentage of bound radioligand was plotted against the concentration of unlabeled test compounds. A standard curve of unlabeled estradiol or progesterone was included in each assay. Four to six concentrations of each competitor were tested. They were chosen to provide a linear portion on a semilogarithmic plot crossing the point of 50% competition. The RBA was calculated as the ratio of the molar concentrations of hormone and test compound required to decrease the amount of bound radioactivity by 50%, multiplied by 100.

In vitro determination of cytostatic activity

Hormone-sensitive human MCF-7 breast cancer cells were obtained from American Type Culture Collection (Rockville, MD, USA). Cells were grown in improved Minimal Essential Medium (MEM), as modified by Richter *et al.* (1972) (Biochrom, Berlin, Germany), supplemented with glutamine (0.3 g/l), gentamycin (60 mg/l and 10% newborn calf serum (NCS) (Gibco) or charcoal-treated NCS (CCS). CCS was prepared by incubation of 500 ml NCS with a dextran-coated charcoal pellet (Scholl *et al.*, 1983) for 4 h in a shaker at 0–4°C. The procedure was repeated with a fresh pellet. After each incubation, the charcoal was removed by centrifugation. The serum was sterilized through a 0.20 µm filter (Sartorius, Göttingen, Germany) and stored at −20°C. All of the experiments were performed in the presence of phenol red. Cells were grown in a humidified incubator in 5% CO_2 at 37°C. Two weeks before the start of the experiment,

cells were switched from NCS to CCS and received two additional media changes before they were harvested with 0.05% trypsin–0.02% EDTA in 0.15 M NaCl.

At the start of the experiment, the cell suspension was transferred to 96-well microtiter plates (100 µl/well). After growing them for 2–3 days in a humidified incubator with 5% CO_2 at 37°C, medium was replaced by one containing the drug. Control wells (16/plate) contained 0.1% of ethanol that was used for the preparation of the stock solution. The initial cell density was determined by addition of vinblastin (10^{-7} M). After incubation for 7 days, the medium was removed and 100 µl of glutaric aldehyde in PBS (1%) were added for fixation. After 15 min, the solution of aldehyde was decanted. Cells were stained by treating them for 25 min with 100 µl of an aqueous solution of crystal violet (0.02%). After decanting, cells were washed several times with water to remove adherent dye. After addition of 100 µl of ethanol (70%), plates were gently shaken for 1 h. Optical density of each well was measured in a microplate autoreader EL 309 (Bio-tek) at 578 nm. Data calculation and analysis were performed with a PC (Reile *et al.*, 1990).

Hormone-independent MDA-MB 231 human mammary tumor cells were obtained from American Type Culture Collection. Cells were grown in McCoy 5a medium (Boehringer Mannheim, Germany) supplemented with 10% NCS and gentamycin (40 µg/ml). Cytostatic activity was determined in a microtiter plate assay as described for MCF-7 cells with one exception: the incubation period was 2–3 days.

Immature mice uterine weight tests

Immature female mice (20 days old, NMRI strain) from Charles River Wiga (Sulzfeld, Germany) were randomly divided into groups of 6–10 animals. To determine estrogenic activity, compounds were dissolved in polyethylene glycol/0.9% saline (7:3; 100 µl/animal) and injected subcutaneously on three consecutive days. Control animals received the vehicle alone. Twenty-four hours after the last injection, the animals were killed by cervical dislocation and weighed. Uteri were dissected free of fat and fixed to Bouin solution (saturated aqueous picric acid, 34%; formaldehyde, glacial acetic acid 15:5:1 by volume) for 2 h. Uteri were freed from connective tissue, washed with ethanol, dried at 100°C for 24 h, and weighed. The relative uterus weight was calculated by the formula: uterine dry weight (mg)/body weight (g), multiplied by 100.

To determine the antiestrogenic activity, the same protocol was used with one modification: Increasing doses of the compounds were injected together with a standard dose (0.4 µg) of estrone. The inhibition (%) of the estrone-stimulated uterine growth was estimated by the formula: $100 - [(W_{S,T} - W_V)/(W_S - W_V) \times 100]$ ($W_{S,T}$ = rel. uterus weight of animals treated with estrone standard (0.4 µg) + text compound; W_V = rel. uterus weight of control animals; W_S = rel. uterus weight of animals treated with estrone standard).

Transplanted MXT-mammary tumors of the mouse

The MXT-M 3.2 mammary tumors were generously provided by Dr A.E. Bogden, EG & G Mason Research Institute, Worcester, MA, USA. Hormone-sensitive tumors grew for 4–5 weeks in the host animals before transplantation. Tumor pieces of 1 mm^2 were serially transplanted into 8- to 10-week-old female $B_6D_2F_1$ mice, obtained from Charles River Wiga (Sulzfeld, Germany). Animals were assigned randomly in groups of 10 and treatment was started 24 h after transplantation. Drugs were dissolved in olive oil and administered subcutaneously on Monday, Wednesday and Friday. After a 5-week period of treatment, animals were killed and autopsied. Tumors were removed and weighed. The uterine dry weight was determined as described above. The change of body weight

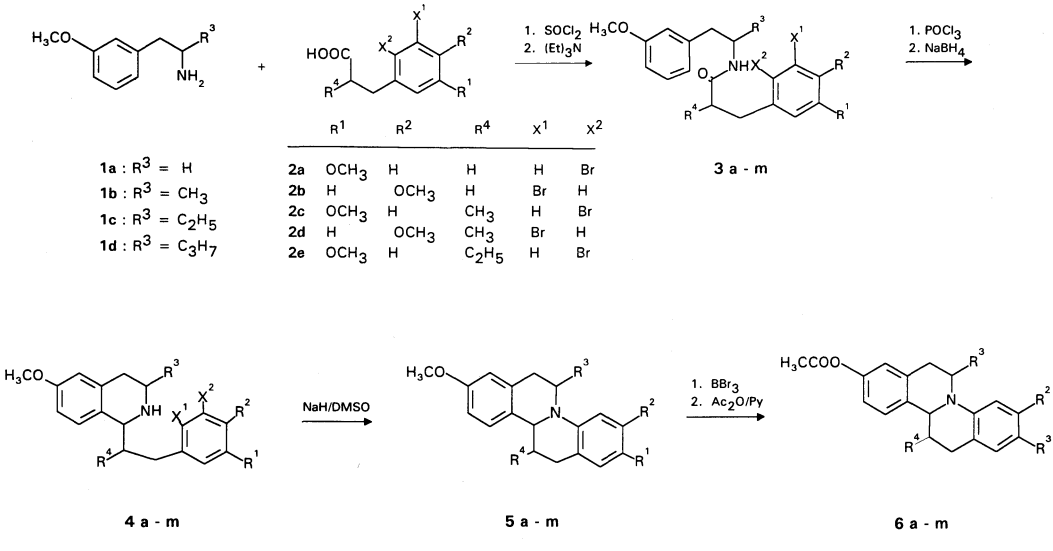
between start and end of therapy was recorded in order to detect obvious toxicity. Significance of differences was determined by the U test according to Wilcoxon, modified by Mann and Whitney.

Results

Chemistry

The synthesis of the tetracyclic dibenzo[*a,f*]quinolizine system involved two ring closure reactions (Scheme 1): The starting amides **3** for the first cyclization were obtained by reacting 1-alkyl-2-(3-methoxyphenyl)ethylamines **1** with the respective (bromomethoxyphenyl)propionic acid chloride. The isoquinoline ring was formed by the Bischler–Napieralski reaction using $POCl_3$ in MeCN. The dihydroisoquinolines were isolated as hydrochlorides. The free bases were reduced with sodium boronhydride to yield the 1-phenethyl-tetrahydroisoquinolines **4**. Derivatives with an alkyl group in α-position to the heterocycle were obtained as mixtures of diastereomers which were not resolved. An alkyl group next to the nitrogen can give rise to the formation of diastereomeric *cis*/*trans*-isomers. The reduction, however, was highly stereoselective leading predominantly to the *cis*-isomers

The second ring closure reaction was accomplished by treating the bromo-substituted phenethyl-tetrahydroisoquinolines **4a–m** with sodium (methylsulfinyl)methanide (Corey & Chaykorsky, 1965) as strong base. The cyclization occurred via a benzyne intermediate as demonstrated by converting both *o*- and *m*-bromo derivatives to the same tetracycle (Kano *et al.*, 1976). In the case of diastereomeric tetrahydroisoquinolines the respective isomers of the tetrahydro-dibenzo[*a,f*]quinolizines **5i–m** were formed which could be separated by column chromatography. Due to the nitrogen as bridge head atom the tetrahydroquinolizine ring connection can easily adopt two different orientations which correspond to *cis*- and *trans*-decalin respectively. When a substituent had been introduced into position 12, the orientation in which this substituent holds an axial position is energetically favoured. Thus, these diastereomers were described as *cis*/*trans*-isomers referring to the steric arrangement of the ring connection. Structural



Scheme 1

assignments of the separated isomers were made mainly by ^{1}H NMR spectroscopy using the NOE technique.

In the case of the 6-alkyl derivatives **5c–h** only one isomer was isolated which has be assumed to be *cis*-configured in respect to the substituents at C-6 and C-11b of the tetracycle. The substituent at C-6 adopts an axial position which forces the quinolizine system into a *trans* orientation of the central ring connection. The minimum energy of the structure with equatorial substituents is about 1.5 kcal/mol higher than the one with axial groups and leads to a nearly perpendicular arrangement of the two aromatic rings.

After ether cleavage of **5**, the free hydroxy derivatives were converted into the corresponding acetates **6** because the phenolic compounds are sensitive to autoxidation. In order to study the interaction of the 12-alkyl derivatives with biological targets in detail we separated the enantiomers of both diastereomeric forms of **6l** and **6m** by liquid chromatography on triacetyl cellulose (Koller *et al.*, 1983) and characterized them. The assignment (+) and (−) for the enantiomers refers to the sign of optical rotation at 578 nm.

Biochemical and biological properties

A prerequisite of a selective action is the ability of a drug to bind to a target protein. Thus, we determined the binding affinities of all of the acetoxy derivatives for the calf uterine estrogen receptor using the DCC method as described (von Angerer *et al.*, 1984). The relative binding affinities (%RBA) are given as the ratio of the molar concentrations of 17β-estradiol and tetrahydro-benzo[α,*f*]quinolizine required to decrease the amount of receptor-bound [^{3}H]17β-estradiol by 50%, multiplied by 100.

All derivatives showed RBA values > 1 except those lacking alkyl groups in both positions 6 and 12 or having a *cis*-structure in relation to the central ring connection (Table IV). The importance of short alkyl groups in the central part of the molecule has been recognized in all non-steroidal structures which bind to the estrogen receptor. Like in other classes of compounds (von Angerer *et al.*, 1984) a chain length of two or three carbon atoms appears to be the optimum. No difference in receptor binding was noticed between derivatives with the second oxygen function at C-2 and the 3-acetoxy series. A striking difference in binding affinity was observed between the *trans*- and the *cis*-isomers. The *cis*-orientation of central ring connection leads to the right-angled bend of the molecule which can no longer mimic the steroidal structure of estradiol. When the racemic mixtures of the *trans*-quinolizines **6l** and **m** had been separated, the enantiomers displayed rather similar binding affinities. This observation is in accord with previous findings in the indoloisoquinoline series in which the enantiomers of the 6-alkyl derivatives did not differ very much in their RBA values (Ambros *et al.*, 1989).

A number of compounds with high binding affinity for the estrogen receptor were also tested for affinity for the progesterone receptor in order to evaluate their molecular selectivity. With most of these derivatives, the RBA values for the progesterone receptor were lower by more than one order of magnitude than those for the estrogen receptor. However, dibenzoquinolizines with the acetoxy function in position 2 and the alkyl group at C-6 (**6d, f, g**) exhibited binding affinities greater then 1 which are close to those for the estrogen receptor (Table VI).

Preliminary studies with the tetrahydro-dibenzo[*a,f*]quinolizines **6a–m** in estrogen-sensitive MCF-7 breast cancer cells revealed four derivatives with significant cytostatic activities at 10^{-5} M (**6f, h,** *trans*-**6l** and *trans*-**6m**). These compounds were also tested at lower concentrations together with the (+) and (−)-enantiomers of both active *trans*-isomers (Table VII). The most potent agent was *trans*-**6l** with an IC_{50}-value of about 5×10^{-7} M. The enantiomers of this racemate did not differ significantly in their cytostatic activity. There was only a slight general superiority of the dextro rotatory form.

Table VI Binding affinity of compounds **6** for the progesterone receptor

Compound	R^1	R^2	R^3	R^4	*RBA*[a] *(PR)*
6c	$OCOCH_3$	H	CH_3	H	<0.01
6d	H	$OCOCH_3$	CH_3	H	3.1
6e	$OCOCH_3$	H	C_2H_5	H	0.1
6f	H	$OCOCH_3$	C_3H_7	H	1.8
6g	$OCOCH_3$	H	C_3H_7	H	0.1
6h	H	$OCOCH_3$	C_3H_7	H	1.2
trans-**6i**	$OCOCH_3$	H	H	CH_3	0.1
trans-**6l**	$OCOCH_3$	H	H	C_2H_5	0.1
trans-**6m**	$OCOCH_3$	H	H	C_3H_7	0.2

[a] Relative binding affinity for the calf uterine progesterone receptor = ratio of molar concentrations of progesterone and inhibitor, required to decrease the amount of bound [^{3}H]Org 2058 by 50%, × 100

Table VII Effect of compounds **6g**, **6h**, *trans*-**6l** and *trans*-**6m** on the growth of MCF-7 and MDA-MB-231 mammary tumor cells

	MCF-7				*MDA-MB-231*[a]
Compound	5×10^{-7} % *T/C*	1×10^{-6} % *T/C*	5×10^{-6} % *T/C*	1×10^{-5} % *T/C*	1×10^{-5} % *T/C*
6f		70 ± 12	40 ± 10[b]	18 ± 5	77 ± 11[b]
6h		80 ± 9	44 ± 8[b]	11 ± 7[b]	70 ± 11[b]
trans-**6l**	42 ± 14[b]	41 ± 14[b]	28 ± 12[b]	2 ± 5[b]	85 ± 11[c]
trans-(+)-**6l**	35 ± 17[b]	29 ± 13[b]	16 ± 10[b]	−3 ± 2[b]	
trans-(−)-**6l**	49 ± 19[b]	46 ± 15[a]	30 ± 11[b]	13 ± 10[b]	
trans-**6m**	73 ± 24[b]	52 ± 17[b]	30 ± 13[b]	7 ± 7[b]	80 ± 9[c]
trans-(+)-**6m**	81 ± 14	44 ± 14	21 ± 14[b]	−2 ± 3[b]	
trans-(−)-**6m**	76 ± 19	70 ± 27	42 ± 19[b]	11 ± 5[b]	
Tamoxifen	83 ± 10	52 ± 13[b]	31 ± 11[b]	7 ± 10[b]	

[a] Estrogen receptor negative cells
[b] Significant inhibition; $P < 0.01$
[c] Significant inhibition; $P < 0.05$

In order to exclude non-specific cytostatic or cytotoxic effects we tested the active compounds in estrogen receptor negative MDA-MB 231 human breast cancer cells. Only at the highest concentration (10 μM) was a weak inhibitory effect observed.

The high binding affinities of **6f**, *trans*-**6l** and *trans*-**6m** and their cytostatic activity in MCF-7 cells prompted us to study them *in vivo* using the MXT mouse mammary tumor model. At a dose of 3 × 10 mg/kg body weight per week, the inhibitory effect was not significant. When the dose had been doubled *trans*-**6l** inhibited tumor growth by 78% (Table VIII). Since the uterus weights of the treated animals were somewhat higher than those of control animals we determined estrogenic and antiestrogenic activity of these drugs in the immature mouse uterine weight test. Compounds **6f** and **6h** displayed dose response curves typical for partial estrogen antagonists (Table IX). The maximum inhibition of estrone-stimulated uterine growth was about 60%. Derivatives *trans*-**6m** and, especially, *trans*-**6l** showed strong but transient antagonistic activity. With low doses (10–50 μg/animal) of *trans*-**6l**, the effect of estrone was nearly completely overcome. At high doses, however, the estrogenic component became dominant.

Table VIII Effect of ***trans*-6l** and ***trans*-6m**, tamoxifen, and ovariectomy on the growth of hormone dependent MXT mammary tumors and uteri of BDF_1 mice

Compound	*Dose*[a] *(mg)*	*Median (mg)*	*Tumor weight*[b] *(range) (mg)*	*T/C %*	*Uterotrophic effect*[c] *(%)*	*Change of body weight*[d] *(g)*
Control		927	(111–2230)	100	100	1.5
Ovariectomy		5	(0–10)	0.5[e]	17[e]	1.2
Tamoxifen	8.8	140	(50–610)	15[e]	79	1.4
trans-**6l**	10	760	(12–1890)	82	96	1.8
trans-**6m**	10	597	(50–1080)	64	132	2.0
Control		443	(176–1886)	100	100	0.6
Ovariectomy		8	(5–42)	2[e]	25[e]	−0.14
Tamoxifen	8.8	107	(41–216)	24[e]	100	−0.15
trans-**6l**	20	96	(20–872)	22[f]	126[f]	0.6
6f	20	235	(83–868)	56	115	0.6

[a] Dose/kg body weight, dissolved in olive oil and administered three times per week subcutaneously
[b] Determined after 5 weeks of treatment; 6 animals per group
[c] Ratio of uterine dry weights of treated and control animals
[d] Change of body weight between days 1 and 6 to detect acute toxic effects
[e] Significant inhibition; $P < 0.01$ (U-test)
[f] Significant inhibition; $P < 0.05$ (U-test)

Discussion

Various tetracyclic alkaloids such as berbine derivatives have been shown to possess cytostatic activity (Zee-Cheng & Cheng, 1973). In order to achieve a more selective action of these substances we modified them in such a way that they bind to specific biochemical structures present in malignant cells. Receptors for steroid hormones such as estrogens are found in the majority of advanced mammary carcinomas and can be used as targets. A prerequisite for high binding affinity for estrogen receptors is the presence of two oxygen functions such as hydroxy or acetoxy groups in appropriate positions of the tetracycle.

Previous studies in this series of tetrahydro-dibenzo[*a,f*]quinolizines and in related classes of compounds (von Angerer & Prekajac, 1986; Ambros *et al.*, 1989; Polossek *et al.*, 1992; von Angerer *et al.*, 1992) (Structures 2) have shown that position 9 for the first oxygen function and either position 2 or 3 for the second one are most favorable for receptor binding. The differences between the latter two positions are not very pronounced. More important was the introduction of a short alkyl group in position 6 or 12 in order to enhance the lipophilicity in the central part of the molecule. Unexpectedly, the basic nitrogen atom which belongs to a tertiary aniline structure did not lower the binding affinity of this system as shown by comparison with analogous dibenzo[*a,g*]-quinolizinones and indolo[*2,1-a*]isoquinolines (Polossek *et al.*, 1992; von Angerer *et al.*, 1992).

An important feature of this partially saturated heterocyclic system is the presence of chiral centers. Since the interactions of drugs with their biological targets are usually characterized by a high degree of stereospecificity, we isolated the stereoisomers and studied them separately. The introduction of an alkyl substituent in position 12 gave rise to the formation of diastereomeric mixtures due to the chiral bridge head atom. Since the 12-alkyl group in both isomers adopts the energetically favored axial position the arrangement of the ring connection in the quinolizine is either *cis* or *trans*. The *cis*-

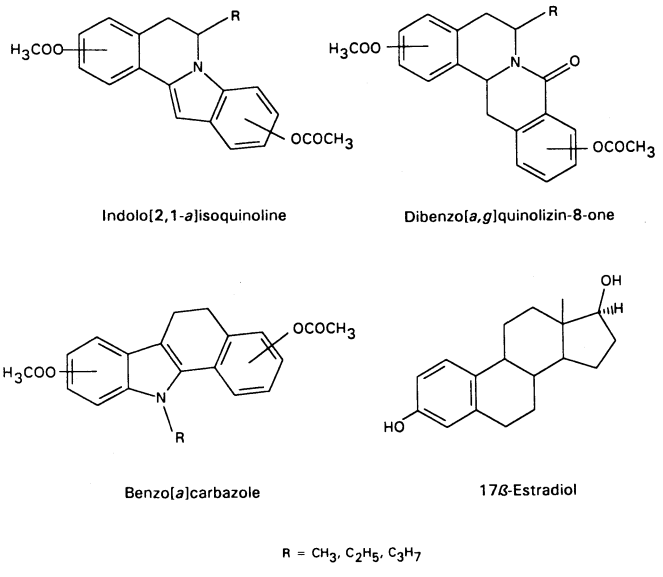
Table IX Estrogenic and antiestrogenic activity of 9-acetoxy-7,11b,12,13-tetrahydro-6*H*-dibenzo[*a,f*]quinolizines **6** in the mouse uterine weight test

Compound	*Dose*[a] *[nmol]*	*Uterotrophic effect*[b,c] *(%)*	*Antiuterotrophic effect*[c,d] *(%)*
Control	—	0	100
Estrone	0.024	100	0
6f	0.12	2	45[e]
	0.6	14[e]	62[e]
	3.0	14[f]	68[e]
	15.0	32[e]	62[e]
6h	0.12	10	31[e]
	0.6	10[f]	58[e]
	3.0	24[e]	58[e]
	15.0	34[e]	50[e]
trans-**6l**	0.12	0	10
	0.6	15[f]	89[e]
	3.0	26[f]	89[e]
	15.0	76[e]	34[f]
trans-**6m**	0.12	4	32[f]
	0.6	13	61[e]
	3.0	76[e]	48[e]
	15.0	100[e]	0

[a] Dose per animal, administered on three consecutive days s.c.
[b] Calculated by the formula $(W_T - W_V)/(W_S - W_V) \times 100$ (W_T = rel. uterus weight of treated animals; W_V = rel. uterus weight of control animals; W_S = rel. uterus weight of animals injected with 0.4 μg estrone/animal)
[c] The U-test according to Wilcoxon, modified by Mann and Whitney, was used to determine significance
[d] Inhibition of estrone-stimulated uterine growth. Animals received the drug and estrone (0.4 μg/animal) simultaneously
[e] Significant at $P < 0.05$
[f] Significant at $P < 0.01$

isomers which are characterized by a bent structure of the molecule showed much lower binding affinities than the corresponding *trans*-isomers. When the enantiomers of the *trans*-derivatives were studied for receptor affinity no distinct differences were noticed. We had made a similar observation when we studied the enantiomers of 6-alkyl-dihydroindolo[*2,1-a*]isoquinolines (Polossek *et al.*, 1992). We were able to rationalize these findings by comparison with steroidal structures bearing a substituent at C-11. Possibly, the position 12 of the dibenzoquinolizines corresponds to the position 11 of 17β-estradiol which is equivalent to carbon 6 in the indoloisoquinoline series (Polossek *et al.*, 1992). This is in accord with results obtained with 6-alkyl-dibenzo[*a,g*]quinolizinones (von Angerer *et al.*, 1992). Enantiomers of this type of compounds differed by one order of magnitude in binding affinity. This difference is expected if the 6-position is equivalent to the C-7 of estradiol.

All of the derivatives with high binding affinities were studied for specific cytostatic activity in human MCF-7 breast cancer cells. A strong inhibitory effect was observed with both the 12-ethyl and 12-propyl derivative with a *trans*-orientation of the central ring connection (*trans*-**6l** and *trans*-**6m**). The effect was equivalent to that of the reference drug tamoxifen. When the respective enantiomers were studied, a somewhat higher activity was observed for the (+)-stereoisomers. But the differences were not so pronounced as in the indoloisoquinoline series where the IC_{50} values differed by one order



Structures 2 Structures of dibenzo[*a,g*]quinolizinones, indolo[2,1-*a*]isoquinolines, benzo[*a*]carbazoles, and 17β-estradiol

of magnitude (Polossek *et al.*, 1992). Despite their similarity in receptor binding, both classes of compounds differed considerably in their general cytostatic properties. Experiments with estrogen receptor negative MDA-MB 231 breast cancer cells revealed that the dibenzoquinolizines require the estrogen receptor for a cytostatic action whereas the antitumor activity of the indoloisoquinolines was obviously not receptor-mediated (Polossek *et al.*, 1992).

Preliminary *in vivo* experiments with transplanted hormone-dependent MXT mouse mammary tumors showed antitumor activity for all of the compounds tested. However, at the applied doses this effect was not yet significant except for *trans*-**6l**, which exhibited an antitumor effect similar to that of the antiestrogen tamoxifen (78% vs. 76%). The inhibition of tumor growth in this model can be a result of either antiestrogenic activity or estrogenic effects. In order to distinguish between these two possible modes of action we determined the endocrine properties of the compounds used in these tumor experiments in the mouse uterine weight test. All of these derivates can be classified as mixed agonists/antagonists with strong antiestrogenic activity at low doses (up to 3 mg/kg body weight) but significant estrogenic effects at higher doses. This endocrine profile does not allow one to attribute the antitumor activity of these compounds to one or the other mechanism. The tetracyclic system of the dibenzo[*a,f*]quinolizines appears to be an appropriate structure for the design of antiestrogens but requires probably the introduction of a functional side chain into position 6 or 12 such as an aminoalkyl group (von Angerer *et al.*, 1990) or a carbamoylalkyl function (Wakeling & Bowler, 1988).

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