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Dibenzo[*a,g*]quinolizin-8-ones: synthesis, estrogen receptor affinities, and cytostatic activity

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Summary: A number of acetoxy-substituted dibenzo[*a,g*]quinolizin-8-ones were synthesized by the reaction of 1-oxoisoquinolines with substituted homophthalic acid anhydride. All of the derivatives with acetoxy groups in positions 3 and 10 bind to the estrogen receptor. Relative binding affinities (RBA) ranged from 1.8 to 5.6 (estradiol: RBA = 100) when the substituent at C-6 was a short alkyl group. Introduction of additional oxygen functions in the 2- and/or 11-position decreased binding affinities. Analyses of the enantiomers of 6-methyl (**6b**) and 6-ethyl (**6c**) derivatives revealed that the receptor binding is mainly due to one optical isomer (e.g. (−)-**6b**, 9.9; (+)-**6b**, 0.6). In hormone-sensitive human MCF-7 breast cancer cells, compounds with one acetoxy group in each aromatic ring strongly inhibited cellular growth. Despite marked differences in receptor affinity, the enantiomers displayed similar activities in this cell culture. In hormone-independent MDA-MB 231 mammary tumor cells, only a weak cytostatic effect was recorded at 10^{-5} M. In the immature mouse uterine weight test, minimal estrogenic activity was observed. At higher doses, a significant anti-estrogenic effect became evident. It is assumed that the estrogen antagonism is responsible for the specific cytostatic effect in MCF-7 breast cancer cells.

Key words: cytostatic activity/dibenzo[*a,g*]quinolizin-8-ones/estrogen receptor affinity/synthesis

Introduction

Endocrine therapy of hormone-dependent breast cancer has proved to be a valuable alternative to the treatment with cytostatic drugs which usually lack specificity for tumor cells. The drugs presently in use for this indication display only a rather limited spectrum of activity. Among the patients with estrogen receptor-positive mammary carcinoma, ~40% do not respond to the treatment with tamoxifen which is still the antiestrogen of choice (Harmsen & Porsius, 1988). Our approach to improve the activity of drugs acting via the estrogen receptor is based on structural modifications of compounds with inherent cytostatic potency.

In a previous paper (Ambros *et al.*, 1990), we reported on the biological activities of a number of indolo[2,1-*a*]isoquinolines. We extended these studies to other tetracyclic systems containing two fused six-membered rings with a nitrogen as bridge head atom (Weimar *et al.*, 1991a,b). One of these structures is dibenzo[*a,g*]quinolizine which is closely related to the berbine alkaloids (Zee-Cheng & Cheng, 1973). This class of natural

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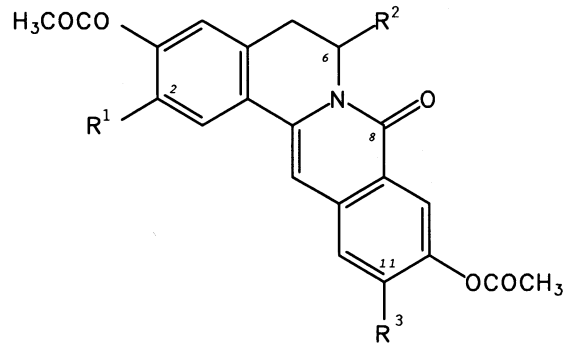
products is known to possess cytostatic activity, especially if the compounds contain a quaternary nitrogen atom (Cushman *et al.*, 1979). For high binding affinity for the estrogen receptor, it is necessary to avoid structural elements with a basic character in the center of the molecule (von Angerer *et al.*, 1982). Therefore, we introduced a carbonyl group next to the nitrogen atom. In this study, a number of dibenzo[*a,g*]quinolizin-8-ones with acetoxy groups in different positions of the aromatic rings and short alkyl chains at C-6 (Chart 1) were synthesized and tested for their estrogen receptor affinities, their cytostatic activities and their endocrine properties.



R^1, R^3 = H, $OCOCH_3$

R^2 = CH_3, C_2H_5, C_3H_7

Chart 1

Materials and methods

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

Reagents

[2,4,6,7-^{3}H(N)]Estradiol (110 Ci/mmol) was obtained from New England Nuclear (Dreieich, FRG). Hormones and biochemicals were purchased from Sigma (München, FRG). TEA [Tris buffer (10 mM, pH 7.4) supplemented with EDTA (1.5 mM) and NaN_3 (3 mM)] was used as buffer.

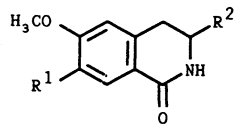
*General procedure for the synthesis of the 1,2,3,4-tetrahydro-methoxy-1-oxo-isoquinolines **3b–f***

The respective β-phenylethylamine **1** (100 mmol) was dissolved in dry CH_2Cl_2 (50 ml) and $N(C_2H_5)_3$ (10.0 g). Ethyl chloroformate (10.8 g) in 50 ml of dry CH_2Cl_2 was added

and the mixture was stirred for 1 h. The precipitate was dissolved in 2 N HCl, stirred again and extracted with CH_2Cl_2. After washing of the organic layer with water and drying (Na_2SO_4), the solvent was evaporated. All compounds **2** were obtained as colorless oils and were used without further purification. Yields were nearly quantitative.

Polyphosphoric acid (100 g) was preheated to 140°C (oil bath temperature). Compound **2** was poured directly into the polyphosphoric acid and the mixture was kept at 140°C for 1 h. After cooling, the mixture was suspended in ice water (100 ml). The solution was made alkaline and extracted with CH_2Cl_2 (6 × 150 ml). The organic layer was dried (Na_2SO_4). After evaporation of the solvent, the residue was purified by chromatography (SiO_2; EtOAc) to give colorless crystals. Yields and m.p. values are reported in Table I.

Table I 1,2,3,4-Tetrahydro-methoxy-1-oxoisoquinolines **3**



Compound	R^1	R^2	*% Yield*	*Formula*[a]	*m.p.*[b] *(°C)*
3a[c]	H	H	65	$C_{10}H_{10}NO_2$	137–138
3b	H	CH_3	50	$C_{11}H_{13}NO_2$	147
3c	H	C_2H_5	50	$C_{12}H_{15}NO_2$	116
3d	H	C_3H_7	45	$C_{13}H_{17}NO_2$	73
3e	OCH_3	CH_3	50	$C_{12}H_{15}NO_3$	171
3f	OCH_3	C_2H_5	50	$C_{13}H_{17}NO_3$	151

[a] Analyzed for C and H within ±0.4% of the calculated values
[b] Recrystallized from Et_2O
[c] Umezawa *et al.* (1980)

General procedure for the synthesis of the 5,6-dihydro-methoxy-8H-dibenzo[a,g]*quinolizine-8-ones* ***5b–i***

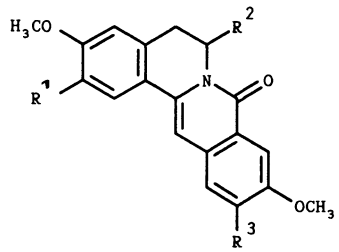
1-Oxoisoquinoline **3** (5 mmol) was suspended in chlorobenzene (15 ml), pyridine (0.4 ml) and $POCl_3$ (0.2 ml dissolved in 5 ml of chlorobenzene) were added. The suspension was stirred for 15 min at room temperature, then homophthalic acid anhydride **4a** (Horeau & Jacques, 1948) or **4b** (Potts & Robinson, 1955) was added. The mixture was stirred for 15 min, then refluxed for 1 h. After cooling, CH_2Cl_2 (50 ml) was added. The organic layer was washed with 10% NaOH, dried (Na_2SO_4) and the solvent was evaporated. The residue was chromatographed (SiO_2; EtOAc) to afford colorless crystals. Yields and m.p. are reported in Table II.

Compound **5a** has been described previously (Weimar *et al.*, 1991a).

General procedure for the synthesis of acetoxy-5,6-dihydro-8H-dibenzo[a,g] *quinolizine-8-ones* ***6b–i***

Methoxy-8-oxoberbine **5** (100 mg dissolved in 10 ml of dry CH_2Cl_2) was added dropwise to BBr_3 (0.1 ml) in dry CH_2Cl_2 (10 ml). The mixture was refluxed for 1 h and stirred at

Table II 5,6-Dihydro-methoxy-8*H*-dibenzo[*a*,*g*]quinolizin-8-ones **5**



Compound	R^1	R^2	R^3	*% Yield*	*Formula*[a]	*m.p.*[b] *(°C)*
5a[c]	H	H	H	60	$C_{19}H_{17}NO_3$	176–177
5b	H	CH_3	H	55	$C_{20}H_{19}NO_3$	138
5c	H	C_2H_5	H	55	$C_{21}H_{21}NO_3$	140
5d	H	C_3H_7	H	40	$C_{22}H_{23}NO_3$	134
5e	H	CH_3	OCH_3	50	$C_{21}H_{21}NO_4$	192–194
5f	H	C_2H_5	OCH_3	50	$C_{22}H_{23}NO_4$	186–187
5g	OCH_3	CH_3	H	55	$C_{21}H_{21}NO_4$	141
5h	OCH_3	C_2H_5	H	55	$C_{22}H_{23}NO_4$	154
5i	OCH_3	C_2H_5	OCH_3	55	$C_{23}H_{25}NO_5$	191

[a] Analyzed for C and H within ±0.4% of the calculated values
[b] Recrystallization from EtOH
[c] Weimar *et al.* (1991a)

room temperature for 12 h. With cooling, 10 ml of an aqueous solution of $NaHCO_3$ was added. Then, EtOAc (25 ml) was added and the mixture was stirred for 15 min. The organic layer was separated and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with saline and dried (Na_2SO_4). After the solvent was removed, the residue was dried *in vacuo*. Ac_2O (6 ml) and pyridine (1 ml) were added, and the mixture was refluxed for 1 h. Excess of reagents was evaporated *in vacuo*. The remaining residue was washed with ether and chromatographed (SiO_2; EtOAc). Recrystallization from MeOH yielded **6** as colorless crystals. Yields and m.p. are reported in Table III, and NMR data are given in Table IV.

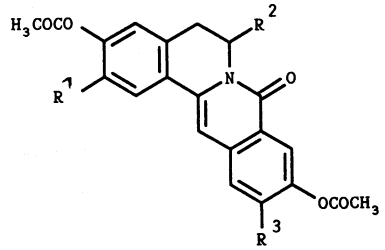
Compound **6a** has been described previously (Weimar *et al.*, 1991a).

*Chromatographic separation of the enantiomers of **6b** and **6c***

The enantiomers of **6b** and **6c** were separated semipreparatively by liquid chromatography on triacetylcellulose (20–30 μm) (Koller *et al.*, 1983) with EtOH as eluent at 22°C (2–3 bar, flow rate 3–4 ml/min). Both racemates showed baseline separations with enantioselectivities of 2.2 and 2.0, respectively. The enantiomeric purities were close to 100%.

(−)-**6b:** k_- = 2.9, m.p. 113–115°C, $[\alpha]_{546} = -54° \pm 6°$, $[\alpha]_{436} = +16° \pm 4°$ in EtOH.
(+)-**6b:** k_+ = 1.3, m.p. 96–98°C, $[\alpha]_{546} = +50° \pm 6°$, $[\alpha]_{436} = -16° \pm 4°$ in EtOH.
(−)-**6c:** k_- = 2.3, m.p. 112–114°C, $[\alpha]_{546} = -33° \pm 6°$, $[\alpha]_{436} = +80° \pm 9°$ in EtOH.
(+)-**6c:** k_+ = 1.2, m.p. 108–110°C, $[\alpha]_{546} = +24° \pm 5°$, $[\alpha]_{436} = -80° \pm 8°$ in EtOH.

Table III Acetoxy-5,6-dihydro-8*H*-dibenzo[*a*,*g*]quinolizine-8-ones **6**



Compound	R^1	R^2	R^3	*% Yield*	*Formula*[a]	*m.p.*[b] *(°C)*	*RBA*[c]
6a[d]	H	H	H	75	$C_{21}H_{17}NO_5$	206–208	0.3
6b	H	CH_3	H	65	$C_{22}H_{19}NO_5$	179–181	5.6
(+)-6b	H	CH_3	H	–	$C_{22}H_{19}NO_5$	96–98	0.6
(−)-6b	H	CH_3	H	–	$C_{22}H_{19}NO_5$	113–115	9.9
6c	H	C_2H_5	H	65	$C_{23}H_{21}NO_5$	164–166	2.8
(+)-6c	H	C_2H_5	H	–	$C_{23}H_{21}NO_5$	108–110	0.7
(−)-6c	H	C_2H_5	H	–	$C_{23}H_{21}NO_5$	112–114	5.8
6d	H	C_3H_7	H	55	$C_{24}H_{23}NO_5$	124–125	1.8
6e	H	CH_3	$OCOCH_3$	65	$C_{24}H_{21}NO_7$	214–216	0.8
6f	H	C_2H_5	$OCOCH_3$	65	$C_{25}H_{23}NO_7$	133–135	0.4
6g	$OCOCH_3$	CH_3	H	70	$C_{24}H_{21}NO_7$	215–216	0.6
6h	$OCOCH_3$	C_2H_5	H	70	$C_{25}H_{23}NO_7$	159–160	0.5
6i	$OCOCH_3$	C_2H_5	$OCOCH_3$	60	$C_{27}H_{25}NO_9$	154–155	0.1

[a] Analyzed for C and H within ±0.4% of the calculated values
[b] Recrystallization of the racemates from MeOH
[c] Relative binding affinity for the calf uterine estrogen receptor is the ratio of the molar concentration of 17β-estradiol (E2) and inhibitor required to decrease the amount of bound [^{3}H]E2 by 50%, ×100
[d] Weimar *et al.* (1991a)

Estradiol receptor binding assay

Fresh calf uteri, stored in ice-cold saline, were freed of adherent fat and connective tissue at 4°C. After the addition of TEA buffer (1 ml/g), the uteri were homogenized by treatment with an ultraturrax mixer (IKA, FRG) and a glass-in-glass homogenizer (Potter S; Braun, FRG) 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 estrogen receptor. The protein concentration of the cytosol was ~15 mg/ml, leading to a final concentration of 3 mg/ml in the assay.

For the determination of the relative binding affinity (RBA), the described procedure was applied with modifications (von Angerer *et al.*, 1984). The 500 μl incubation mixture comprised 5 nM [^{3}H]17β-estradiol (added in 100 μl TEA), 10^{-9}–10^{-5} M competing ligand (in 100 μl TEA), 100 μl of uterine cytosol and TEA. The mixture was incubated for 18 h at 4°C, 0.5 ml of dextran-coated charcoal (DCC) slurry (0.8% charcoal Norit A and 0.008% dextran in TEA) was added to the tubes and the contents were mixed. The tubes were incubated for 90 min at 4°C and then centrifuged at 700 *g* for 10 min to pellet the charcoal. An aliquot (100 μl) of the supernatant was removed and the radioactivity was

Table IV ^{1}H-NMR data of acetoxy-6-alkyl-5,6-dihydro-8*H*-dibenzo[*a,g*]quinolizin-8-ones **6b–i**

Compound	δ *[p.p.m.] (recorded in $CDCl_3$ with TMS as internal standard)*
6b	1.13 (d,J = 7Hz;3H,-CH_3), 2.30 (s;6H,-CO-CH_3), 2.63–3.38 (m;2H,-CH_2-), 5.45–5.77 (m;1H,-CH-), 6.87–7.90 (m;6H,ArH,VinylH), 8.12 (d,J = 2.5Hz; 1H,ArH)
6c	0.88 (t,J = 7.5Hz;3H,-CH_3), 1.20–1.75 (m;2H,-CH_2-CH_3), 2.33 (s;6H,-CO-CH_3), 2.93–3.52 (m;2H,-CH_2-), 5.18–5.63 (m;1H,-CH-), 6.92–7.93 (m;6H,ArH,VinylH), 8.10 (d,J = 2.5Hz;1H,ArH)
6d	0.85 (t,J = 7.25Hz;3H,-CH_3), 1.26–1.55 (m;4H,-CH_2-CH_2-CH_3), 2.33 (s;3H,-CO-CH_3), 2.35 (s;3H,-CO-CH_3), 2.96 (ABX,$^2J_{AB}$ = 16Hz, $^3J_{BX}$ = 5.5Hz;1H,-CH-*H*), 3.15 (ABX, $^2J_{AB}$ = 16Hz,$^3J_{AX}$ = 2Hz;1H,-C*H*-H), 5.46–5.55 (m;1H,-CH-), 6.98 (s;1H,VinylH), 7.02 (d,J = 2.5Hz;1H,ArH), 7.11 (dd,$J_{1,2}$ = 9/2.5Hz; 1H,ArH), 7.39 (dd,$J_{1/2}$ = 9/2.5Hz;1H,ArH), 7.58 (d,J = 9Hz;1H,ArH), 7.82 (d,J = 9Hz;1H,ArH), 8.11 (d,J = 2.5Hz;1H,ArH)
6e	1.18 (d,J = 7Hz;3H,-CH_3), 2.35 (s;9H,-CO-CH_3), 2.61–3.62 (m;2H,-CH_2-), 5.48–5.79 (m;1H,-CH-), 6.93–7.94 (m;5H,ArH,VinylH), 8.23 (s;1H,ArH)
6f	0.88 (t,J = 7.5Hz;3H,-CH_3), 1.13–1.75 (m;2H,-CH_2-CH_3), 2.30 (s;9H,-CO-CH_3), 3.03 (t,J = 3Hz;2H,-CH_2-), 5.22–5.53 (m;1H,-CH-), 6.82–7.93 (m;5H,ArH,VinylH), 8.23 (s;1H,ArH)
6g	1.18 (d,J = 6Hz;3H,-CH_3), 2.34 (s;9H,-CO-CH_3), 2.80 (ABX,$^2J_{AB}$ = 16Hz,$^3J_{BX}$ = 2Hz;1H,-CH-*H*), 3.07 (ABX,$^2J_{AB}$ = 16Hz,$^3J_{AX}$ = 6Hz;1H,-C*H*-H), 5.40–5.80 (m;1H,-CH-), 6.93 (s;1H,VinylH), 7.12 (s;1H,ArH), 7.39–7.62 (m;3H,ArH), 8.10 (d,J = 2Hz;1H,ArH)
6h	0.88 (t,J = 7.5Hz;3H,-CH_3), 1.20–1.73 (m;2H,-CH_2-CH_3), 2.31 (s;9H,-CO-CH_3), 3.05 (m;2H,-CH_2-), 5.22–5.56 (m;1H,-CH-), 6.93 (s;1H,VinylH), 7.13 (s;1H,ArH), 7.45 (dd,$J_{1/2}$ = 9/2Hz;1H,ArH), 7.56 (s;1H,ArH), 7.68 (s;1H,ArH), 8.12 (d,J = 2Hz;1H,ArH)
6i	0.89 (t,J = 7.5Hz;3H,-CH_3), 1.34–1.64 (m;2H,-CH_2-CH_3), 2.32 (s;3H,-CO-CH_3), 2.34 (s;9H,-CO-CH_3), 2.95–3.15 (m;2H,-CH_2-), 5.35–5.42 (m;1H,-CH-), 6.87,7.12, 7.41,7.62,8.20 (5 × s;5H,ArH,VinylH)

determined by liquid scintillation measurement after the addition of 2 ml of Quickszint 212 (Zinsser). Non-specific binding was calculated using 4 μM 17β-estradiol as competing ligand. Six concentrations of competitor (1, 2, 5 and 10 × 10^{-9} to 10^{-6}) were chosen to provide values between 10 and 90% of specifically bound radioactivity. Radioactivity was plotted as a function of log concentration of competing ligand in the assay. The RBA was calculated as the ratio of the molar concentrations of estradiol 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 the American Type Culture Collection (Rockville, MD). Cells were grown in improved Minimal Essential Medium (MEM), as modified by Richter *et al.* (1972) (Biochrom, Berlin, FRG), supplemented with glutamine (0.3 g/l), gentamycin (60 mg/l) and 5% newborn calf serum (NCS) (Gibco) or charcoal-treated NCC (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, FRG) 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, the 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 the addition of vinblastin (10^{-7} M). After incubation for 3 days, medium was removed and 100 μl of glutaric aldehyde in PBS (1%) was 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 the addition of 100 μl of ethanol (70%), plates were gently shaken for 1 h. The optical density of each well was measured in a microplate autoreader EL 309 (Bio-tek) at 578 nm. Data calculation and analysis were performed on an Olivetti M24 PC (Reile *et al.*, 1990).

Hormone-independent MDA-MB 231 human mammary tumor cells were obtained from the American Type Culture Collection. Cells were grown in McCoy 5a medium (Boehringer Mannheim, FRG) 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 6 days.

Immature mice uterine weight tests

Immature female mice (20 days old, of the NMRI strain) from Charles River Wiga (Sulzfeld, FRG) 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 in Bouin solution (saturated aqueous picric acid–40% formaldehyde–glacial acetic acid, 15:5:1 by vol.) for 2 h. Uteri were freed from connective tissue, washed with a saturated alcoholic solution of LiCl, 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, injections contained a standard dose (0.4 μg) of estrone and increasing doses of the compounds. The inhibition (%) of the estrone-stimulated uterine growth was estimated by the formula:

$$100 - [(W_{S,T} - W_V)/(W_S - W_V) \times 100]$$

where $W_{S,T}$ = relative uterus weight of animals treated with estrone standard (0.4 μg) + test compound, W_V = relative uterus weight of control animals and W_S = relative uterus weight of animals treated with estrone standard.

Results

Chemistry

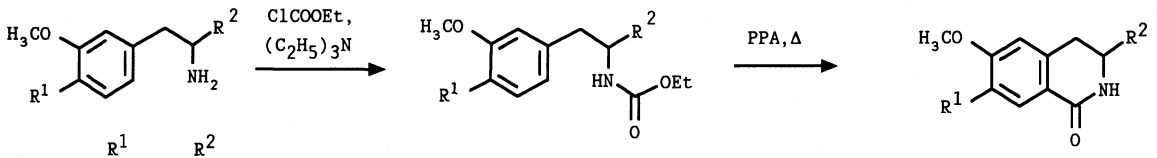
For the synthesis of the methoxy-substituted 8*H*-dibenzo[*a,g*]quinolizin-8-ones **5b–i**, the corresponding 1,2,3,4-tetrahydro-1-oxo-isoquinolines **3b–f** were used as starting materials. They were obtained by the reaction of the respective β-phenylethylamine **1** with ethyl chloroformate, followed by cyclization with polyphosphoric acid (Scheme 1). The 1-oxoisoquinolines **3** were reacted with the substituted homophthalic acid anhydride **4a** or **4b**, according to the method described by Haimova *et al.* (1980), to afford the dibenzo[*a,g*]quinolizinones **5**. After ether cleavage of **5**, the free hydroxy derivatives were converted into the corresponding acetates **6** because the phenolic compounds are sensitive to autoxidation when exposed to air. All derivatives except **6a** possess a chiral center in position 6. In order to study the influence of the steric arrangement at this particular carbon atom, we separated the enantiomers of **6b** and **6c** by liquid chromatography on triacetyl cellulose (Koller *et al.*, 1983) and characterized them (Table III). The assignment (+) and (−) for the enantiomers refers to the sign of optical rotation at 546 nm.

Biochemical and biological properties

This study was primarily designed to develop agents for treating hormone-dependent mammary tumors. Since hormonal activity of an agent requires binding to the respective receptor, the binding affinities of acetoxy-substituted compounds for the estrogen receptor were determined. The affinities were measured in a competitive binding assay using [^{3}H]17β-estradiol as tracer and calf uterine cytosol as receptor source (von Angerer *et al.*, 1984). The DCC method was applied to remove unbound components of low molecular weight. The relative binding affinities (%RBA) are given as the ratio of the molar concentrations of 17β-estradiol and dibenzoquinolizinone required to decrease the receptor-bound radioactivity by 50%, multiplied by 100. As in previous studies (Ambros *et al.*, 1990), the acetates were used for determination of the relative binding affinities for the estrogen receptors because the free hydroxy derivatives are unstable in solution and undergo oxidation by air.

From preliminary studies, we knew that oxygen functions in positions 3 and 10 provide the best conditions for receptor binding (Weimar, 1990). Therefore, we kept this structural arrangement constant, varied the alkyl substituent at C-6, and introduced additional acetoxy groups into positions 2 and 11 because vicinal oxygen functions are known to enhance cytostatic activity in nitrogen-containing tetracycles (Ambros *et al.*, 1988). As shown in Table III, a methyl or ethyl group at C-6 increased the binding affinity for the estrogen receptor, whereas catechol structures in one or both aromatic rings showed an opposite effect. The racemates with the highest binding affinities were separated into the enantiomers. There was a marked difference in binding affinity between (+)- and (−)-isomers. The RBA values of the (−)-forms exceeded that of the (+)-enantiomers by one order of magnitude.

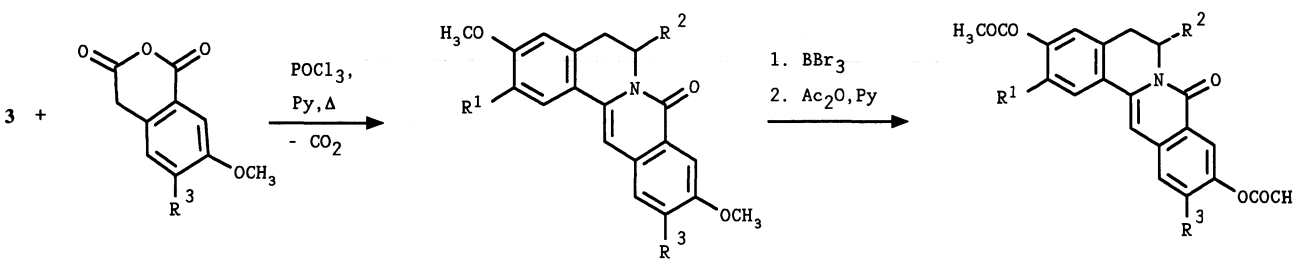
All of the acetoxy-substituted derivatives were tested for cytostatic activity against hormone-sensitive human MCF-7 breast cancer cells. At 10^{-5} M, all compounds except **6i** with two catechol structures strongly inhibited cellular growth. At 1 μM, only derivatives with one oxygen function in each aromatic ring were very active. We also tested the enantiomers of **6b** and **6c** together with the racemates. Cytostatic activities of both stereoisomers and the racemic mixture of **6c** were the same within the experimental error, despite the different binding affinities of (+)- and (−)-**6c**. With derivative **6b**, a



	R^1	R^2
1a	H	H
1b	H	CH_3
1c	H	C_2H_5
1d	H	C_3H_7
1e	OCH_3	CH_3
1f	OCH_3	C_2H_5

2a–f

3a–f



4a : R^3 = H

4b : R^3 = OCH_3

5a–i

6a–i

R^1, R^3 = H, $OCOCH_3$

R^2 = alkyl

Scheme 1

stronger cytostatic effect was observed for the (−)-enantiomer than for the (+)-isomer. This is in accord with the higher RBA value of the laevorotary form.

In order to find out whether the cytostatic effect is receptor mediated or due to a general cytotoxic activity of this class of compounds, we determined the growth inhibitory effect of the acetoxy-substituted dibenzoquinolizinones in hormone-independent human MDA-MB 231 mammary tumor cells (Table V). Only at the highest concentration (10^{-5} M) was cytostatic activity recorded. Derivatives with catechol structures showed rather similar activities in both cell lines, making an unspecific mode of action likely. However, derivatives **6b**, **6c** and **6d**, which possess high binding affinities for the estrogen receptor, already exerted a strong inhibitory effect in hormone-sensitive cells at a concentration of 10^{-6} M. Since 10-fold higher concentrations were required for an equivalent inhibition in hormone-independent cells, a mechanism mainly mediated by the estrogen receptor has to be assumed for these agents.

For further studies, it was of interest whether these compounds act *in vivo* as estrogens or as antiestrogens. Therefore, we determined their endocrine profile in the mouse uterine weight test (Table VI). Estrogenic activity was generally very low. A weak uterotrophic effect was only observed at doses 1000-fold higher than that of estrone. At these doses, however, most compounds gave rise to a significant antiestrogenic effect. With compounds **6c** and **6d**, the stimulation of uterine growth by estrone was inhibited by 80%. In this assay, the (+)- and (−)-stereoisomers of **6b** and **6c** were tested too. At a dose of 100 nmol/animal, only minor differences were found between the enantiomers concerning the uterotrophic effect. In the antiuterotrophic test, the (−)-isomers were more active than the (+)-enantiomers. Since the racemic mixtures were tested in experiments different to those for the enantiomers, the values do not match exactly those of the enantiomers.

Table V Effect of compounds **6a–i** on the growth of MCF-7 and MDA-MB-231 mammary tumor cells

Compound	*MCF-7*			*MDA-MB-231*[a]
	1 × 10⁻⁶ M % *T/C*	*5 × 10⁻⁶ M* % *T/C*	*1 × 10⁻⁵ M* % *T/C*	*1 × 10⁻⁵ M* % *T/C*
6a	57 ± 8[b]	34 ± 7[b]	21 ± 4[b]	76 ± 5[b]
6b	54 ± 15[b]	35 ± 13[b]	10 ± 12[b]	52 ± 5[b]
(+)-6b	75 ± 20	52 ± 13	5 ± 10[b]	
(−)-6b	37 ± 16[b]	28 ± 12[c]	1 ± 10[b]	
6c	50 ± 22[b]	37 ± 17[b]	13 ± 10[b]	65 ± 6[b]
(+)-6c	44 ± 14[c]	41 ± 19[b]	7 ± 12[b]	
(−)-6c	61 ± 14	39 ± 17[b]	3 ± 3[b]	
6d	54 ± 9[b]	37 ± 8[b]	25 ± 4[b]	90 ± 4[b]
6e	80 ± 13[b]	85 ± 15[c]	70 ± 11[b]	41 ± 4[b]
6f	73 ± 11[b]	74 ± 12[b]	58 ± 10[b]	78 ± 9[b]
6g	87 ± 18	66 ± 13[b]	43 ± 9[b]	37 ± 4[b]
6h	72 ± 13[b]	66 ± 10[b]	38 ± 8[b]	55 ± 6[b]
6i	96 ± 12	94 ± 17	87 ± 13[c]	43 ± 4[b]
Tamoxifen	52 ± 14[b]	31 ± 12[b]	7 ± 11[b]	

[a] Estrogen receptor-negative cells
[b] Significant at $P < 0.01$
[c] Significant at $P < 0.05$

Table VI Estrogenic and antiestrogenic activity of 5,6-dihydroquinolizin-8*H*-dibenzo[*a,g*]-8-ones **6** in the mouse uterine weight test

Compound	*Dose*[a] *[nmol]*	*Uterotrophic test* *Relative uterus weight*[b]	*Antiuterotrophic test* *Relative uterus weight*[b,c]	*Inhibition*[d] *(%)*
Control		15.5 ± 2.3	15.5 ± 2.3	
Estrone	1.5	48.6 ± 10.4		
6b	100	17.2 ± 4.8	38.8 ± 8.8	29.7[e]
	1000	23.5 ± 3.5	33.6 ± 12.4	45.3[e]
6d	100	17.2 ± 4.3	44.7 ± 19.7	11.7
	1000	32.7 ± 6.8	22.1 ± 7.2	80.0[f]
6f	100	16.3 ± 2.1	48.7 ± 13.1	
	1000	16.7 ± 1.0	40.5 ± 9.6	24.4
Control		14.9 ± 5.5	14.9 ± 5.5	
Estrone	1.5	43.5 ± 7.9		
6a	100	17.6 ± 1.9	43.5 ± 3.7	
	1000	15.1 ± 2.0	45.3 ± 6.3	
6c	100	19.3 ± 3.0	37.9 ± 14.4	19.4
	1000	21.8 ± 6.2	19.1 ± 6.5	85.3[f]
Control		14.1 ± 3.7	14.1 ± 3.7	
Estrone	1.5	52.5 ± 6.6		
(−)-6b	100	16.5 ± 2.9	41.6 ± 7.8	28.0
(+)-6b	100	13.6 ± 3.8	44.0 ± 5.3	21.7
(−)-6c	100	17.5 ± 3.0	33.6 ± 2.9	49.0[e]
(+)-6c	100	16.2 ± 2.2	40.5 ± 8.2	30.9

[a] Dose per animal, administered on three consecutive days s.c.
[b] Uterus dry weight (mg)/body weight (g) × 100, determined 24 h after the last injection; mean of 10 animals ±SD
[c] Simultaneous administration of 1.5 nmol (0.4 μg) of estrone/animal and day
[d] The *t*-test according to Student was used
[e] Significant at $P < 0.05$
[f] Significant at $P < 0.01$

Discussion

A number of dibenzo[*a,g*]quinolizin-8-ones were synthesized by reacting substituted 1-oxoisoquinolines with the respective homophthalic acid anhydride. The methoxy functions were cleaved and converted into acetoxy groups. Essential structural elements for high binding affinity for the estrogen receptor are a single acetoxy group in each aromatic ring and a short alkyl chain in position 6 of the tetracycle. It could be demonstrated that mainly the (−)-enantiomers contribute to the binding affinity. The stereospecificity of receptor binding was not unexpected since the binding of steroids is also dependent on their steric structure (Wakeling & Bowler, 1988a). Molecular modeling studies showed that the substituent in position 6 adopts an axial orientation, such as the methyl group in estradiol does. When we studied other tetracyclic systems, we observed that differences in receptor binding between enantiomers are not a general feature of this class of heterocycles. In the 6-alkyl-indolo[2,1-α]isoquinoline series, both optical isomers displayed similar RBA values (Polossek *et al.*, 1992).

Since the objective of this work has been the development of drugs with a specific cytostatic action on estrogen receptor-positive tumors, we compared the effects of the dibenzoquinolizinones in two different cell lines. One line (MCF-7) contains estrogen

receptors and therefore can be considered hormone dependent, the other one (MDA-MB 231) lacks them. Derivatives with one acetoxy group in each benzene ring (**6a–d**) strongly inhibited the growth of MCF-7 cells. At 1 μM, the inhibitory effect was still 50%. Comparison of the cytostatic effects in MCF-7 and MDA-MB 231 breast cancer cells at a concentration of 1×10^{-5} M showed that these compounds are preferably active in estrogen receptor-positive cells. These data are in accord with the high binding affinity for the estrogen receptor. Concerning the specific cytostatic activity of the stereoisomers of derivatives **6b** and **6c**, it was shown that (−)-**6b** is more active than its (+)-analogue due to its higher affinity. For **6c**, we were not able to demonstrate a similar difference in activity.

Concerning the endocrine profile of these compounds, we determined their estrogenic and antiestrogenic properties in the uterine weight test. Only a very weak stimulation of uterine growth was recorded. All derivatives with activity in MCF-7 cells, except **6a**, exhibited a significant antiestrogenic effect in immature mice at a dose of 1 μmol/animal. Surprisingly, the enantiomers of **6b** and **6c** did not differ very much in their estrogenic activities, despite the marked differences in receptor binding. This is probably due to the rather flat course of the uterotrophic dose–response curves observed for this class of compounds. In direct comparison, a trend towards higher endocrine activity of the stereoisomers which are preferably bound by the estrogen receptor can be recognized. The antiuterotrophic effect showed a much stronger dose dependence, therefore differences in activity are more pronounced. It also has to be taken into account that the RBA values had been determined with the acetates, whereas the *in vivo* activities of the dibenzoquinolizinones are probably due to the free phenolic compounds which are readily formed by esterase-catalyzed hydrolysis (Birnböck *et al.*, 1987).

Derivatives with antiestrogenic properties in this assay strongly inhibited the growth of hormone-sensitive breast cancer cells. This relationship of estrogen antagonism *in vivo* and antiproliferative activity in MCF-7 breast cancer cells has been well established (Wakeling & Bowler, 1988b). The specific mode of action of derivatives **6a–d** on estrogen receptor-positive mammary tumor cells warrants further experiments to evaluate their antineoplastic activity *in vivo*.

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