CHEMMEDCHEM

DOI: 10.1002/cmdc.201000537

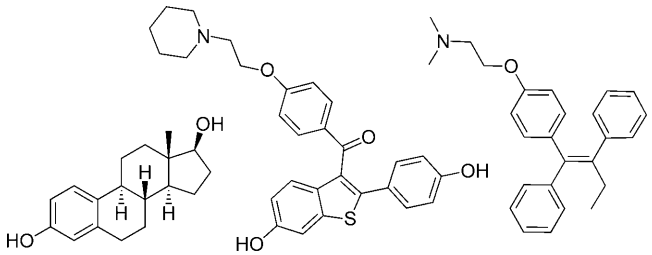
Synthesis and Investigations on the Oxidative Degradation of C3/C5-Alkyl-1,2,4-triarylpyrroles as Ligands for the Estrogen Receptor

Anja Schäfer,[b] Anja Wellner,[b] and Ronald Gust*[a]

In this study, we synthesized 1,2,4-triarylpyrroles as ligands for the estrogen receptor (ER). Two pyrrole series were prepared with either C3-alkyl or C3/C5-dialkyl residues. Compounds from both series were susceptible to oxidative degradation—dialkylated compounds ($t_{1/2}$ = 33–66 h) to a higher extent than their monoalkylated congeners ($t_{1/2}$ = 140–211 h). Nevertheless, stability was sufficient for determination of in vitro ER binding affinity. The most active agonist in hormone-dependent, ERα-positive MCF-7/2a and U2-OS/α cells was 1,2,4-tris(4-hydroxyphenyl)-3-propyl-1*H*-pyrrole (**6d**) (MCF-7/2a: EC_{50} = 70 nM; U2-OS/α: EC_{50} = 1.6 nM). A corresponding inactivity in U2-OS/β cells demonstrated the high ERα selectivity. This trend was confirmed in a competition experiment using estradiol (E2) and purified hERα and hERβ proteins (relative binding affinity (RBA) calculated for **6d**: RBA(ERα) = 1.85%; RBA(ERβ) < 0.01%). Generally, C3/C5-dialkyl substitution led to reduction of activity, possibly due to lower stability.

Introduction

The estrogen receptors ERα[1] and ERβ[2] are ligand-inducible transcription factors that belong to the nuclear hormone receptor superfamily.[3] ER activation or inhibition influences the growth, differentiation, and physiological function of several organs, such as the reproductive tract,[4] bone,[5] liver,[6] and cardiovascular system.[7,8] Selective ER modulators (SERM) like raloxifene (RAL) or tamoxifen (TAM) are used as partial ERα antagonists in the therapy of osteoporosis and hormone-dependent ERα-positive breast cancer, respectively.



E2 RAL TAM

Despite their unquestionable benefits, SERMs may cause serious side effects, including thromboembolisms and endometrial cancer,[9] emphasizing the importance of the search for new derivatives. Over the past years, several substituted heterocycles have been synthesized with the aim of developing ERα/ERβ-selective compounds or substances with tissue-selective activity. Another approach was the design of drugs (type II estrogens) with an ER binding mode different from that of estradiol (E2, type I estrogen),[10,11] resulting in the recruitment of other co-activators or co-repressors.

The binding mode of type I estrogens at ERα is well known from X-ray studies of estradiol (E2) and diethylstilbestrol (DES), for example, enabling the determination of a structure–activity relationship (SAR) for gene activation. In addition to a flat hydrophobic skeleton, two hydroxy groups should be present within an O–O distance of 10.9–12.5 Å. These moieties interact with the amino acids Arg 394/Glu 353, and His 524 within the ligand binding domain (LBD) of ERα. Type II estrogens, such as 4,5-bis(4-hydroxypheny)-2-imidazolines,[11] (Scheme 1 a) are angular compounds, preventing the possibility of contact with His 524. Instead, hydrogen bridges to Tyr 347 or Asp 351 in the LBD side channel are proposed.[10–12]

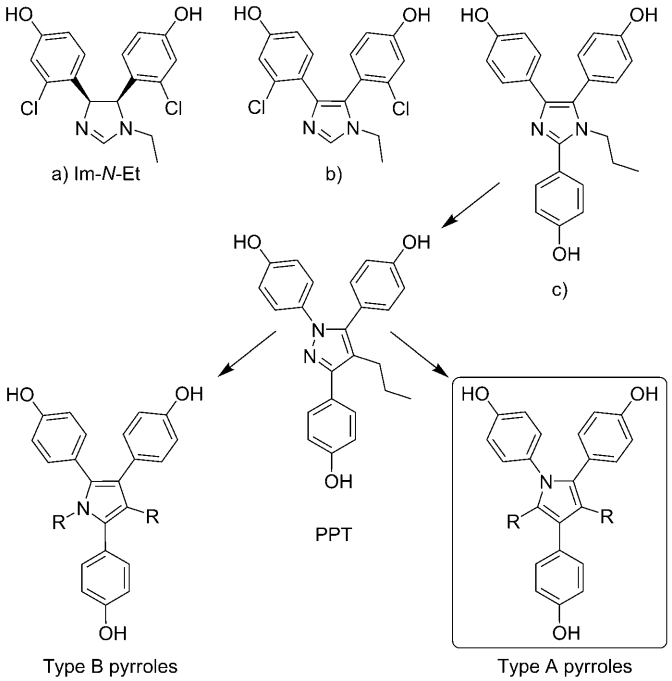
Conversion of the 4,5-bis(4-hydroxypheny)-2-imidazolines to 4,5-bis(4-hydroxyphenyl)imidazoles[13] (Scheme 1 b) increased the distance between the hydroxy groups from 5.1 Å to 8.8 Å and reduced the hormonal activity. Chlorine substituents on the aromatic rings, as well as N-alkylation, counteract these effects.[14] However, an attempt to increase the ER activation by introducing a third phenol ring (Scheme 1 c) to enable an E2-like type I binding mode[15,16] was unsuccessful.

The related 2,4,5-tris(4-hydroxyphenyl)imidazoles were only weak ER agonists, which may be the consequence of nitrogen atom arrangement in the heterocycle in relation to the phenol

[a] *Prof. Dr. R. Gust*
Institut für Pharmazie, Universität Innsbruck
Innrain 52, 6020 Innsbruck (Austria)
Fax: (+43) 512-507-2940
E-mail: ronald.gust@uibk.ac.at

[b] *Dr. A. Schäfer, A. Wellner*
Institut für Pharmazie, Freie Universität Berlin
Königin-Luise-Str. 2+4, 14195 Berlin (Germany)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201000537.



Scheme 1. Structural formulae for substituted heterocycles as ER ligands.

substituents. Analogously substituted pyrazoles exhibited high affinity for the ER.[16] 1,3,5-Tris(4-hydroxyphenyl)-4-propyl-1*H*-pyrazole PPT (Scheme 1), the most hormonally active compound of this class, exhibited a 410-fold ERα selectivity.[17]

These findings led us to synthesize 1*H*-pyrroles and investigate their stability in aqueous solution. The N-position, in relation to the aryl ring substituents on the heterocycle, was selected from the position in PPT resulting in type A or type B pyrroles (Scheme 1). In this paper, we focused our attention on type A pyrroles and attempted to optimize their ER binding affinity using C3- or C3/C5-dialkyl chains.

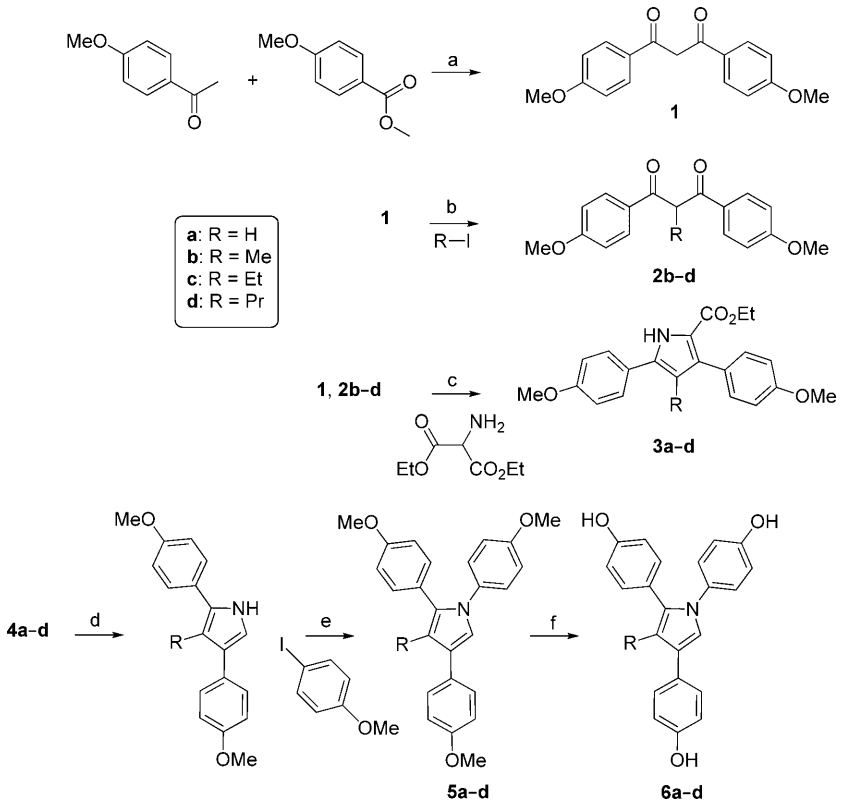
Results and Discussion

Chemistry

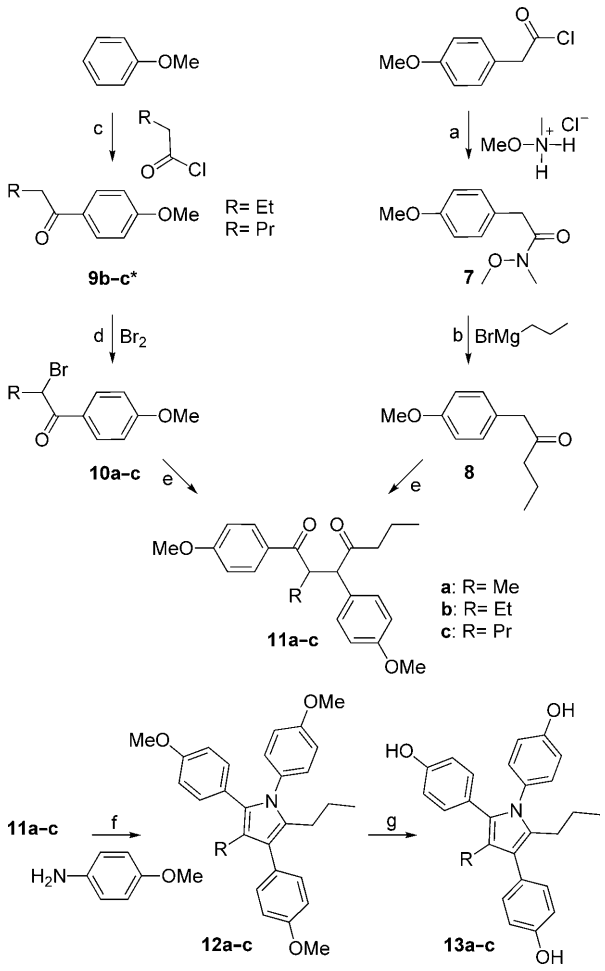
The synthesis of type A pyrroles with a C3-alkyl chain started from the reaction of 4-methoxymethylbenzoate and 4-methoxyphenylethanone in DMSO.[18] The resulting diketone **1** was C-alkylated (**2b**–**d**) with 1.1 equiv of the appropriate iodoalkane and potassium *tert*-butanolate in THF.[19] Reaction of **1** or **2b**–**d** with 2-aminodiethylmalonate in boiling acetic acid led to pyrrole ring closure (**3a**–**d**) in a modified Fischer–Fink synthesis.[20,21] Ester hydrolysis and decarboxylation in boiling ethylene glycol with KOH as base yielded pyrroles **4a**–**d** which were N-arylated (**5a**–**d**) in an Ullman-like reaction in DMF employing cuprous iodide and L-proline.[22,23] The final ether cleavage (**6a**–**d**) was performed with BBr_3 in CH_2Cl_2 at 0 °C (Scheme 2).

1-(4-Methoxyphenyl)pentan-2-one (**8**), a synthon for type A pyrroles with various alkyl chains at C3 and a propyl moiety at C5, was obtained from the reaction of 4-methoxyphenyl acetyl chloride with *N*-methyl-*N*-methoxyammonium hydrochloride (**7**) and subsequent reaction with propylmagnesium bromide. This reaction sequence enables the production of ketones by Grignard reaction (Scheme 3).[24] It was proposed that a stable metal chelate is formed which keeps the ketone from reacting to the corresponding tertiary alcohol. This makes it possible to synthesize ketones from carboxylic acids.[25]

The α-bromoketones (**10a**–**c**) were obtained by Friedel–Crafts acylation of anisole with the respective acyl chloride and subsequent bromination. Reaction of **8** with the appropriate α-bromoketone[26] (**10a**–**c**) at −78 °C resulted in the formation of 1,4-diketones **11a**–**c**,[27] which underwent ring closure in acetic acid using *p*-anisidine (Scheme 3). Ether cleavage was carried out using boron tribromide.



Scheme 2. Synthesis of type A pyrroles with a C3-alkyl chain. *Reagents and conditions*: a) dry DMSO, approx. 8 °C, NaH; b) reflux, dry THF, *tert*-BuOK; c) reflux, acetic acid; d) ethylene glycol, KOH, 185 °C, 1 h; e) CuI, dry DMF, 140 °C, 3 days; f) dry CH_2Cl_2, BBr_3, 0 °C.



Scheme 3. Synthesis of type A pyrroles with C3/C5-alkyl chains. *Reagents and conditions*: a) dry CH_2Cl_2, 0 °C, pyridine, 1 h; b) dry THF, 0 °C, 3 h; c) dry CH_2Cl_2, 0 °C, $AlCl_3$; d) dry diethyl ether, 0 °C, 1 h; e) dry THF, −78 °C, KHMDS solution; f) acetic acid, reflux, *p*-anisidine, 3 h; g) dry CH_2Cl_2, BBr_3, 0 °C. ***9a** (4-methoxypropiophenone) was commercially available.

Stability studies

Some pyrroles are known to be sensitive towards oxidation, so we determined the stability of selected compounds in phosphate buffered saline (PBS) using HPLC methodology. Stock solutions in methanol (10^{-2} M) were diluted with PBS to a final concentration of 5×10^{-5} M, which were incubated at 37 °C in the dark to imitate cell culture conditions.

The stability of **6b–d** and **13a–c** (followed over ~200 h; see Experimental Section for details) was dependent on substituents of the heterocycle. Degradation by Diels–Alder reactions with oxygen or photochemical reactions, for example, is very complex and follows pseudo first-order kinetics.[28,29,30,31] The half-life of all compounds (Table 1) was calculated from the peak area over incubation time correlations as depicted in Figure 1 for **6b** and **13c**. Alkyl chains influenced the stability as well, with dialkylated compounds degraded to a higher degree than their monoalkylated congeners. Furthermore, ethyl- and propyl-substituted pyrroles were more stable than their methyl derivatives (see Table 1).

Table 1. Half life ($t_{1/2}$) of compounds **6b–d** and **13a–c**.

Residue	Compd	$t_{1/2}$ [h]	Compd	$t_{1/2}$ [h]
Methyl	**6b**	140	**13a**	33
Ethyl	**6c**	211	**13b**	66
Propyl	**6d**	169	**13c**	56

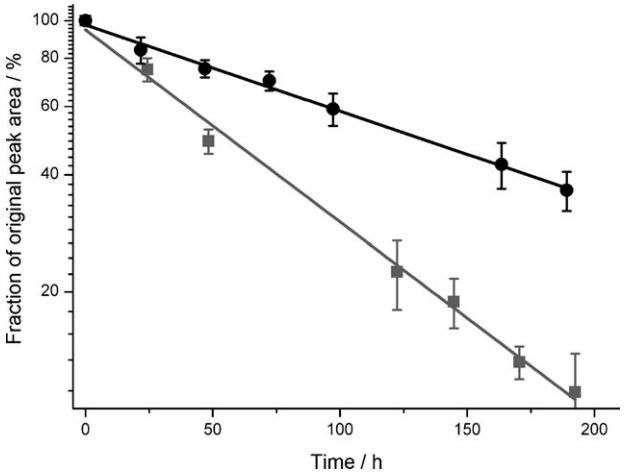


Figure 1. Diagram of the percentage of original peak area (± SD of three separate experiments) over time for compounds **6b** (●) and **13c** (■); see Experimental Section for HPLC method.

The degradation products **6b-1** (t_R = 3.10 min) and **6b-2** (t_R = 4.54 min) were able to be isolated using semipreparative HPLC for characterization by NMR, IR, and mass spectrometry. Compound **6b-1** showed a molecular peak in the ESI-TOF spectra at *m/z* = 374.1447, indicating the addition of one oxygen to **6b** (*m/z* = 358.1483). In the IR spectra, a strong band at 1660 cm^{-1} appeared, characteristic of an amide bond carbonyl. ^{1}H NMR spectra indicated the presence of all AA′BB′ systems of the phenolic rings (Figure 2). The pyrrole proton C5-H (H1) located at δ = 6.92 (**6b**) was shifted to the region of an alkene bond H (δ = 5.63). The influence on the CH_3 group was relatively low (shift from 2.06 to 1.83 ppm).

The ESI-TOF mass chromatogram showed the peak for **6b-2** at *m/z* = 404.1544, signifying an additional *m/z* of 46 as compared to **6b**. In the ^{1}H NMR spectra of **6b-2**, the AA′BB′ systems were arranged similarly to those found for **6b-1**. The C5-H was not detected, while a new singlet (3H) appeared at δ = 3.22.

To the best of our knowledge, only one previous publication details the decomposition of a pyrrole with both alkyl and aryl residues.[32] Wasserman and Miller analyzed the stability of 1-methyl-2,3,5-triphenylpyrrole and identified the cis-dibenzoylstyrenoxide as an oxidation product (Scheme 4). When the nitrogen was phenyl-substituted, the oxidation product did not decompose to the 1,4-diketone but rather remained as the phenylimine intermediate (Scheme 4). Such decomposition products were not identified in the LC-MS spectra of **6b**. Furthermore, NMR and IR spectra of **6b-1** and **6b-2** indicated the absence of an aldehyde or epoxide functionality, respectively. Therefore, another mechanism of decomposition is likely.

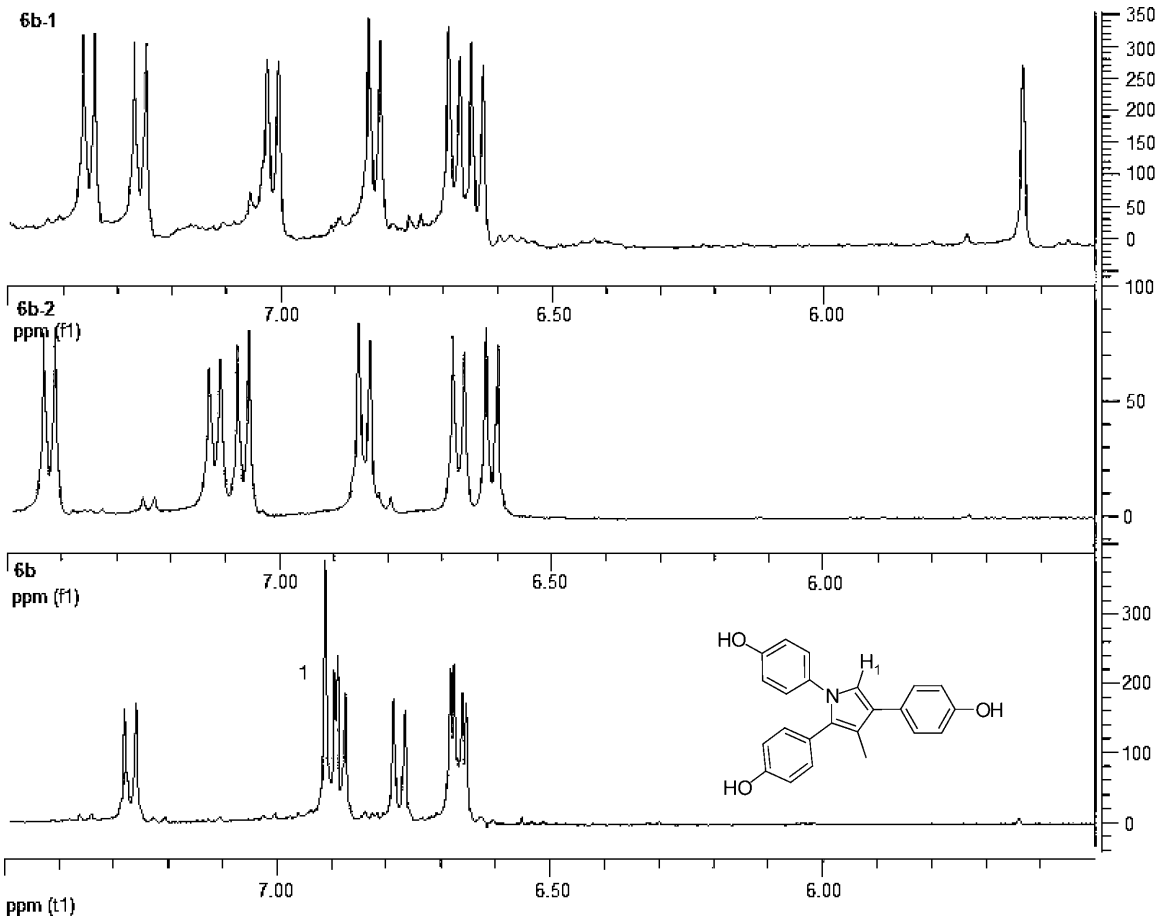
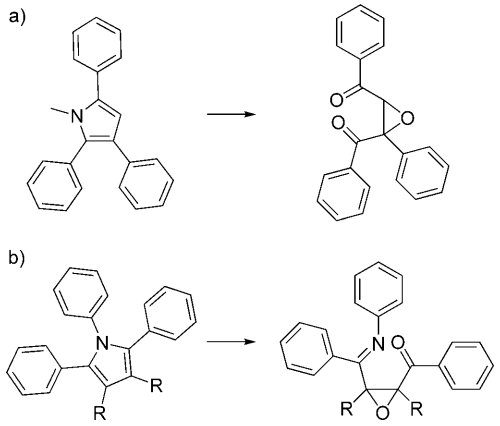


Figure 2. ^{1}H NMR spectra of **6b** and its decomposition products **6b-1** and **6b-2** (in [D_6]DMSO).



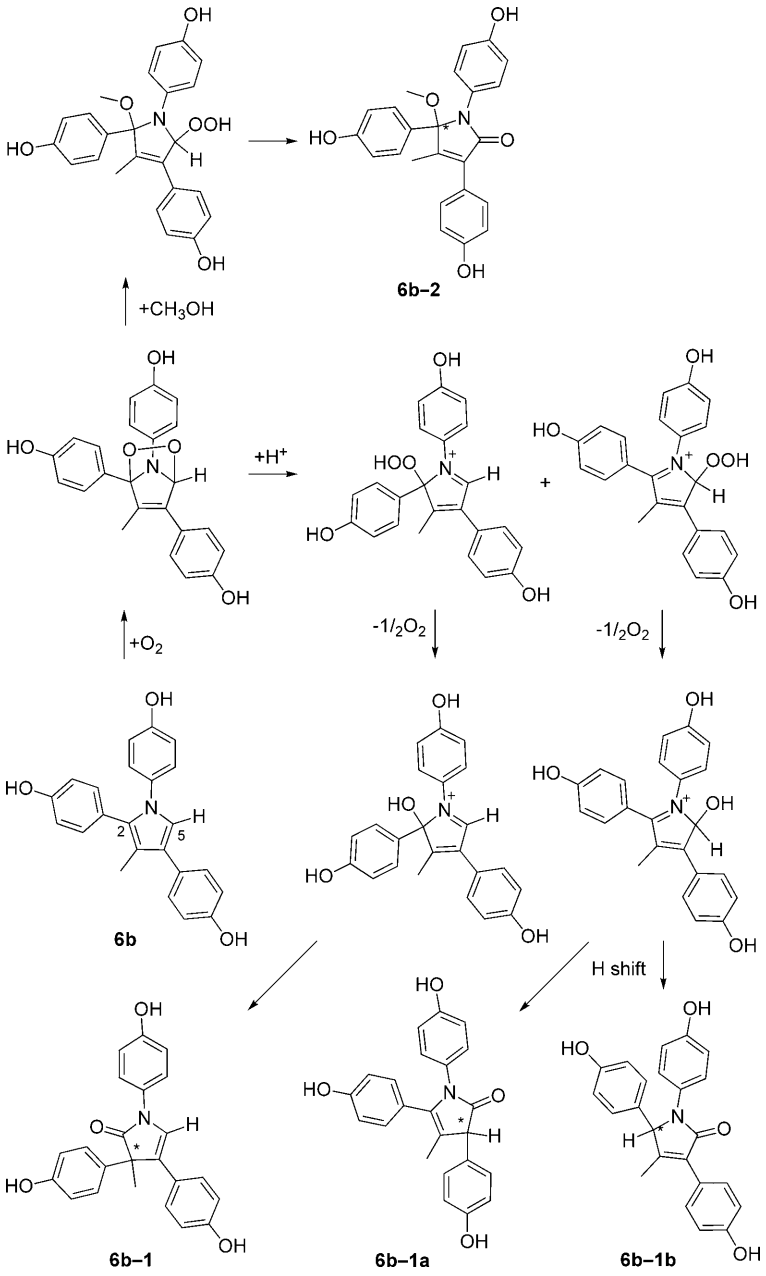
Scheme 4. a) Decomposition of 1-methyl-2,3,5-triphenylpyrrole.[28] b) Decomposition of 1,2,3,5-tetraphenylpyrrole, both isomers are formed.

Pyrroles are electron-rich heterocycles that can react with oxygen in a Diels–Alder reaction to form labile endoperoxides,[33,34] which can be transformed back to pyrroles and molecular oxygen or undergo ring opening by solvent molecules or intramolecular rearrangement.[35] Under the conditions used to analyze **6b**, methanol and water were shown to be present. Indeed, compound **6b-2** appears to be a methanol adduct (OCH_3 group at $\delta = 3.22$). After nucleophilic attack at C5, the endoperoxide was transformed into a hydroperoxide, which subsequently eliminated water to form **6b-2** (Scheme 5). The related C5-OH derivative of **6b-2** ($m/z = 390.1400$) was detected in LC-MS studies as well, but isolation of this compound was unsuccessful.

Another potential mechanism for the building of **6b-1** involves ring opening of the endoperoxide by an intramolecular electron pair shift, resulting in C2- or C5-hydroperoxides that are transformed in the following step to the corresponding alcohols.[36,37] The resulting aminals are stabilized by a hydride or aryl shift, accompanied by oxidation of the hydroxy group to a carbonyl. Of the possible products **6b-1**, **6b-1a**, and **6b-1b**, only **6b-1** was able to be isolated and spectroscopically characterized.

LC–MS analysis (ESI-TOF) of the decomposition products (peak a–f) of **13c** indicated three peaks (a–d) with $m/z = 460.2103$ and two peaks (e and f) with $m/z = 426.2050$ and 428.2215, respectively. Following the studies of Wasserman,[32,38] the decomposition products depicted in Figure 3 could be generated, with m/z values in correlation with those found in LC–MS experiments. Peak e corresponds to the original compound **13c**.

The proposed degradation modes discussed above, beginning in each case with a Diels–Alder reaction of the pyrrole



Scheme 5. Proposed decomposition of **6b**.

with singlet oxygen ($^1O_2$), generated from triplet oxygen ($^3O_2$) by photo- or heat-activation, was confirmed by incubation of **6b** under O_2-free conditions. HPLC analysis demonstrated no degradation over an incubation time of 200 h (data not shown) after degassing the aqueous solutions in an ultrasound bath and purging with dry argon for 10 min. Further support is shown by the lower stability of C3/C5-dialkylated type A pyrroles (**13a–c**). The additional alkyl chain raised the electron density at the diene and enhanced the kinetic reaction with $^1O_2$. This finding is the subject of a current SAR study using electron withdrawing groups at pyrrole carbons to stabilize the pyrrole core. In this context, it should be mentioned that type A pyrroles fulfill the requirements for photosensitizers (they have conjugated π-electron systems and are, to a certain degree, rigid) and thus facilitate their own decomposition.

Biological activity

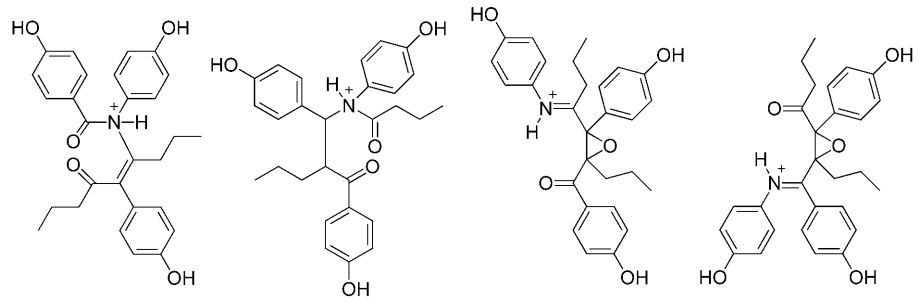
Transactivation assays: MCF-7/2a cells

Hormone-dependent, ERα-positive MCF-7 breast cancer cells were stably transfected with the reporter plasmid ERE_{wtc}luc (MCF-7/2a cells)[39] to study ERα-associated transcription activation in hormone-competent cells. U2-OS cells transiently transfected with the plasmid pSG5-ERα (U2-OS/α), or pSG5-ERβ FL (U2-OS/β) and the reporter plasmid $p(ERE)_2$-luc$^+$, were used to evaluate ER subtype selectivity.[40] The transactivation and intrinsic activity (IA) of type A pyrroles with only a C3-alkyl chain (**6a–d**) at 10 μM in MCF-7/2a cells increased in the order R=H (**6a**; EC_{50}> 10000 nM; IA=27%) <methyl (**6b**; EC_{50}=440 nM; IA=68%) <ethyl (**6c**; EC_{50}=140 nM; IA=96%) < propyl (**6d**; EC_{50}=70 nM; IA=99%) (Figure 4 and Table 2). Introduction of a second alkyl chain at C5 (**13a–c**) led to compounds which exhibited maximum transcription at 1 μM. This transcription decreased at higher concentrations (Figure 4), which is likely due to reduced cell mass at 10 μM, as these compounds more strongly inhibited the growth of MCF-7 cells (see below, "inhibition of cell growth") than did their monoalkylated congeners.

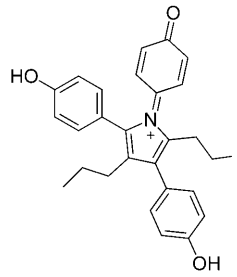
Transactivation assays: U2-OS cells

Performing the same experiments with U2-OS/α cells indicated a higher sensitivity towards hormones. E2 was 25-fold more active in this assay (EC_{50} (ERα)= 0.004 nM). Again, maximum effect was achieved with ethyl and propyl derivatives **6c** and **6d** (EC_{50}=1.1 and 1.6 nM, respectively). Interestingly, and contrary to results obtained in MCF-7/2a cells, dialkylated compounds **13a–c** induced gene activation in a chain length-dependent manner (ERα, Table 2): methyl (**13a**; EC_{50}=45 nM) <ethyl (**13b**; EC_{50}= 27 nM) <propyl (**13c;** EC_{50}=20 nM). Compounds **6a–d** were inactive against U2-OS/β cells and did not induce ERβ activation, indicating high ERα selectivity. An additional alkyl chain at C5 led to a complete loss of ER subtype selectivity (Table 2).

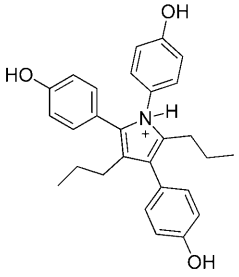
To explore the potential participation of oxidation products **6b-1** and **6b-2** on the transcriptional activity of **6b**, they were also tested in MCF-7/2a cells. As depicted in Figure 5, **6b-2** was completely inactive, and **6b-1** possessed estrogenic activity but to a lower extent (EC_{50}=4700 nM) than **6b**. Therefore, the effect of degradation products on transcriptional activity cannot be excluded. However, based on the stability studies mentioned above, application of the compounds was carried out in such a way that degradation was minimized (freshly pre-



Proposed structures for peaks a, b, c and d with *m/z* $[M+O+O+H]^+$ = 460.2119; found: 460.2103



Proposed structure for peak e with *m/z* [*M* oxidation to quinone imine]$^+$ = 426.2064; found: 426.2050



Proposed structure for peak f (**13c**) with *m/z* $[M+H]^+$ = 428.2220; found: 428.2215

Figure 3. Possible decomposition products of **13c**.

Table 2. Transactivation of compounds **6a–d** and **13a–c** as compared to estradiol (E2) in luciferase reporter assays with MCF-7/2a cells and U2-OSα/β cells.

	MCF-7/2a		U2-OS/α		U2-OS/β	
Compd	IA [%][a]	EC_{50} [nM]	IA [%][c]	EC_{50} [nM]	IA [%]	EC_{50} [nM]
E2	100	0.1	100	0.004	100	0.01
6a	27±2	>10000	42	n.a.	5	–
6b	68±1	440	107	16	5	–
6c	96±8	140	88	1.1	3	–
6d	99±18	70	111	1.6	0	–
13a	34±9[b]	n.a.	85	45	53	n.a.
13b	58±6[b]	n.a.	76	27	88	37
13c	61±3[b]	n.a.	125	20	103	20
PPT	97±6	3	119	0.1	5	–
6b-1	68±15	4700	n.d.	n.d.	n.d.	n.d.

[a] at 10 μM. [b] at 1 μM. [c] at 1 μM. Estradiol is defined as 100%. –: no estrogenic activity; IA: intrinsic activity; n.a.: not applicable due to growth inhibitory effects; n.d.: not determined. For MCF-7/2a cells, the mean values are given ±SD of three independent determinations performed in quadruplicate. For U2-OS cells transiently transfected with ERα or Erβ, values represent the mean of three independent determinations (SD is <20%).

domain (LBD) of both subtypes. In contrast to the C3-methyl (**6b**) and C3-ethyl (**6c**) derivatives, C3-H (**6a**) and C3-propyl (**6d**) analogues did not compete with [^{3}H]E2 for the binding site in ERβ at the concentrations used. An additional C5-alkyl residue at **6d** reduced the relative binding affinity (RBA) at ERα to about 0.15–0.30% without increasing affinity for ERβ (**13a–c**). The higher ERα activity of PPT compared to **6d**, as determined in cellular assays, was confirmed in the HAP assay, however with only a fivefold difference in the RBA values [RBA(PPT) = 10.14%; RBA(**6d**) = 1.85%].

As the compounds strongly activated ERα transcription but only weakly competed with [^{3}H]E2, it is unclear at this stage whether the compounds exert their effects at the receptor protein. It is possible that the pyrroles interact with ERα/β heterodimers, as these are not tested in our binding assay. Other possibilities include interaction with one of the ER coregulators, or binding at an ER domain other than the LBD. Additionally, the compounds may be type II estrogens, in which case their different binding mode would explain the very weak binding affinities in the [^{3}H]E2 displacement assay. Currently, docking studies are under way to explore the reasons for the difference in binding at ERα/β.

pared oxygen-free stock solutions and concentrations lower than 10 μM).

Binding affinity

To gain more insight into the mode of action, competition of the pyrroles for the [^{3}H]E2 binding site was evaluated in a hydroxylapatite (HAP) assay using hERα and hERβ (Table 3). Subtype selectivity observed in the cellular systems could not be confirmed with purified ER proteins for **6b** and **6c**. Both compounds weakly displaced [^{3}H]E2 from the ligand binding

Inhibition of cell growth

Because of the results in MCF-7/2a cells, all compounds were tested for growth inhibitory effects against hormone-dependent MCF-7 and hormone-independent MDA-MB-231 breast cancer cells. As expected, dialkylated pyrroles (**13a–c**, IC_{50} = 6–10 μM) influenced the growth of MCF-7 cells more effectively than their monoalkylated congeners (**6b–d**, IC_{50} = 28–33 μM) (Table 4). Nearly identical results in hormone-insensitive MDA-MB-231 cells suggested an ER-independent interference in cell growth. Interestingly, **6b** was nearly three times more toxic in MDA-MB-231 cells than in MCF-7 cells.

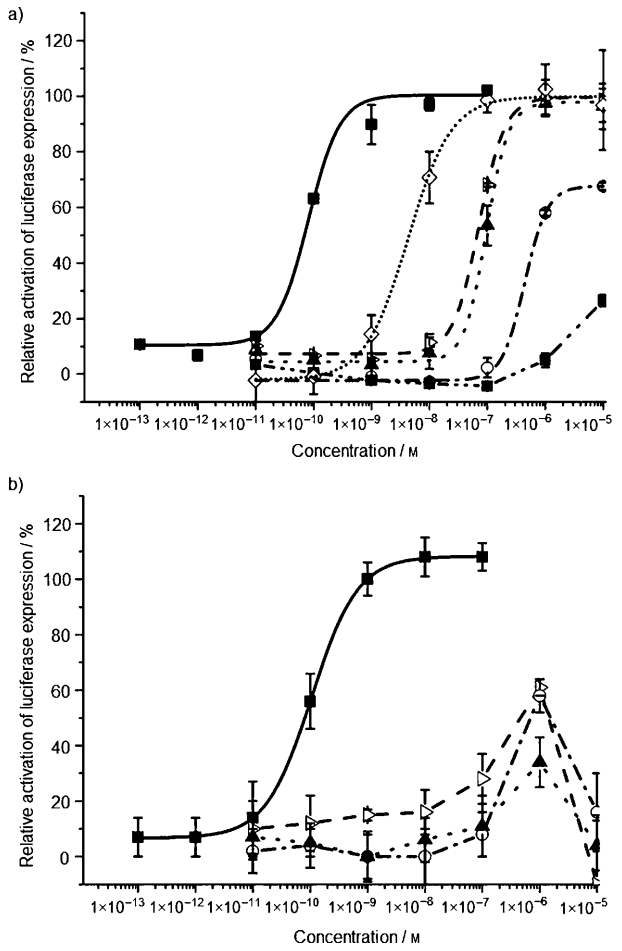


Figure 4. Activation–concentration curves for type A pyrroles. a) Type A pyrroles with a C3-alkyl residue, with PPT for comparison. Estradiol, ■; **6a**, ——■——; **6b**, ○; **6c**, ▲; **6d**, ▷; PPT, ◇; b) Type A pyrroles with C3/C5-alkyl residues. Estradiol, ■; **13a** ○; **13b**, ▲; **13c**, ▷; All pyrroles were tested in a luciferase activation assay using MCF-7/2a cells stably transfected with plasmid ERE_{wtc}luc. The curves are representative examples of one determination performed in quadruplicate (see Experimental Section for details).

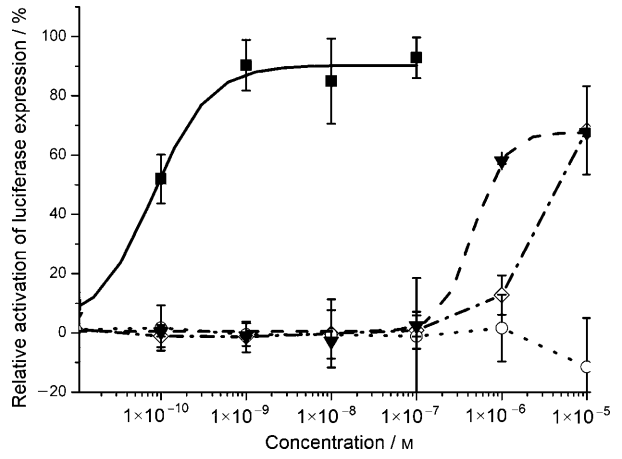


Figure 5. Estrogenic activity of **6b-1** (▼) and **6b-2** (◆) as compared to **6b** (○), estradiol as control: ■.

Table 3. Relative binding affinities (RBA) at hERα and hERβ as determined by HAP assay.[a]

Compd	RBA [%]	
	ERα	ERβ
E2	100	100
6a	0.56	< 0.01
6b	1.67	0.19
6c	0.87	0.38
6d	1.85	< 0.01
13a	0.29	< 0.01
13b	0.15	< 0.01
13c	0.14	< 0.01
PPT	10.14	0.53

[a] Values represent the mean of two independent determinations in duplicate; results are reproducible within ±20% (see Experimental Section for conditions).

Table 4. Growth inhibitory effects of type A pyrroles against MCF-7 and MDA-MB-231 cells.[a]

Residue	Cell line	IC_{50} [μM]	
		6b–d	**13a—c**
Methyl	MCF-7	33.1 ± 2.0	7.9 ± 2.0
	MDA-MB-231	12.0 ± 1.5	12.3 ± 0.3
Ethyl	MCF-7	28.3 ± 1.9	10.0 ± 2.8
	MDA-MB-231	24.8 ± 1.6	16.4 ± 2.9
Propyl	MCF-7	29.4 ± 2.6	6.3 ± 1.9
	MDA-MB-231	26.4 ± 2.6	6.1 ± 0.3

[a] Values represent the mean ± SD of three independent determinations (see Experimental Section for details).

Conclusions

Type A pyrroles were synthesized and analyzed for stability in aqueous solution (comparable to cell culture conditions) as well as for gene activation in ERα-positive MCF-7/2a cells and in U2-OSα/β cells. Alkyl chains at C3/C5 determined the stability and the biological effects. C3-alkyl derivatives were more stable than C3/C5-disubstituted type A pyrroles and activated the ER protein to a higher extent. Therefore, it is necessary to increase stability and to exclude possible degradation in cell culture experiments. This can be attained by introduction of electron-withdrawing substituents at the phenolic rings and changing the N-atom position in relation to the substituents of the heterocycle. These results will be published in forthcoming papers.

Experimental Section

General procedures: Solvents were purified according to literature procedures or purchased in pure form. Syntheses of precursors are described in Supporting Information. IR spectra (KBr pellets) were obtained using an ATI Mattson Genesis and ^{1}H NMR using an Avance DPX 400 spectrometer (Bruker, Karlsruhe) at 400 MHz (internal standard: TMS). All aromatic d are "apparent" doublets. Elemental analysis was performed on a Vario EL (Elementar, Hanau). Purity of compounds **6a–d** and **13a–c** was confirmed by elemental

analysis, with all being >95% (see Supporting Information). Mass spectrometry (EI) was performed on a CH-7A (Varian MAT Bremen, Germany), with ESI–TOF for **13c, 6b-1** and **6b-2** on an Agilent 6210 ESI–TOF (Agilent Technologies, Santa Clara, CA, USA). The solvent flow rate was adjusted to 4 μL min^{-1} with the spray voltage set to 4 kV and the drying gas flow rate set to 15 psi (1 bar). All other parameters were adjusted for a maximum abundance of the relative $[M+H]^+$. A Flashscan S12 Microplate Reader (Analytik Jena, Germany), Wallac Victor^2 1420 multilabel counter (Perkin–Elmer, USA), and Wallac Microbeta TriLux scintillation counter (Perkin–Elmer, USA) were used.

Synthesis

General method for ether cleavage (Method F, Schemes 2 and 3): The respective 1,2,4-tris(4-methoxyphenyl)-1*H*-pyrrole (**5a–d**) was dissolved in 15 mL of dry methylene chloride under inert gas and cooled to 0 °C. Boron tribromide (BBr_3) dissolved in CH_2Cl_2 (5 mL) was added slowly, and the reaction mixture stirred overnight at room temperature. After evaporation of the solvent, redissolving several times in MeOH, purification of the residue was performed on silica gel by column chromatography using CH_2Cl_2/MeOH (9:1).

1,2,4-Tris(4-hydroxyphenyl)-1*H*-pyrrole (6a): BBr_3 (2.2 mmol, 552 mg) was added to **5a** (0.44 mmol, 170 mg) following Method F to produce **6a** as a yellow solid (0.4 mmol, 138 mg, 91%): mp: 119 °C; ^{1}H NMR [(D_6)DMSO]: δ = 9.60 (s, 1H, O*H*), 9.40 (s, 1H, O*H*), 9.21 (s, 1H, O*H*), 7.40 (d, 2H, *J* = 8.6 Hz, Ar*H*), 7.17 (d, 1H, *J* = 2.0 Hz, C*H*), 7.00 (d, 2H, *J* = 8.7 Hz, Ar*H*), 6.92 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.74 (d, 2H, *J* = 8.4 Hz, Ar*H*), 6.73 (d, 2H, *J* = 8.4 Hz, Ar*H*), 6.63 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.51 ppm (d, 1H, *J* = 2.0 Hz, C*H*); MS (EI, 250 °C): *m/z* (%) = 343 $[M]^{+\cdot}$ (100), 293(6), 229(6), 224(15), 172(8), 149(25); IR (KBr): $\tilde{\nu}$ = 3390 (b,s), 1889(w), 1613(m), 1589(m), 1516/1504(s), 1235(b,m), 1171(m), 1102(w), 836(s), 801(m) cm^{-1}; Anal. calcd for $C_{22}H_{17}NO_3 \cdot 0.8\,H_2O$: C, H, N.

1,2,4-Tris(4-hydroxyphenyl)-3-methyl-1*H*-pyrrole (6b): BBr_3 (1.9 mmol, 470 mg) was added to **5b** (0.38 mmol, 150 mg) following Method F to produce **6b** as a yellow solid (0.24 mmol, 85 mg, 63%): mp: 119 °C; ^{1}H NMR [(D_6)DMSO]: δ = 9.44 (s (broad), 3H, O*H*), 7.27 (d, 2H, *J* = 8.5, Ar*H*), 6.89 (m, 5H, 4Ar*H*, 1C*H*), 6.78 (d, 2H, *J* = 8.5 Hz, Ar*H*), 6.67 (m, 4H, Ar*H*), 2.06 ppm (s, 3H, CH_3); MS (EI, 230 °C): *m/z* (%) = 357 $[M]^{+\cdot}$ (100), 238 (8), 223 (18), 179 (6), 107 (4), 65 (5); MS (ESI-TOF): *m/z*: 358.1483; IR (KBr): $\tilde{\nu}$ = 3392(b,s), 1887(w), 1613(w), 1565 (w), 1514(s), 1217(b,m), 1171(m), 1101(w), 834,(s) cm^{-1}; Anal. calcd for $C_{23}H_{19}NO_3 \cdot H_2O$: C, H, N.

3-Ethyl-1,2,4-tris(4-hydroxyphenyl)-1*H*-pyrrole (6c): BBr_3 (2.4 mmol, 606 mg) was added to **5c** (0.48 mmol, 200 mg) following Method F to yield **6c** as a yellow solid (0.35 mmol, 130 mg, 73%): mp: 98 °C; ^{1}H NMR [(D_6)DMSO]: δ = 9.46 (s, 1H, O*H*), 9.42 (s, 1H, O*H*), 9.25 (s, 1H, O*H*), 7.26 (d, 2H, *J* = 8.5 Hz, Ar*H*), 6.90 (m, 4H, Ar*H*), 6.86 (s, 1H, C*H*), 6.77 (d, 2H, *J* = 8.7 Hz, Ar*H*), 6.67 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.64 (d, 2H, *J* = 8.8 Hz, Ar*H*), 2.63 (q, 2H, *J* = 7.5 Hz, CH_2CH_3), 0.87 ppm (t, 3H, *J* = 7.4 Hz, CH_2CH_3); MS (EI, 170 °C): *m/z* (%) = 371 $[M]^{+\cdot}$ (100), 356 (49), 342 (19), 186 (7), 121 (6), 65 (9); IR (KBr): $\tilde{\nu}$ = 3430(b,s), 1889(w), 1613(m), 1512 (m), 1445(m), 1229(b,s), 1172(m), 1101(w), 835(s) cm^{-1}; Anal. calcd for $C_{24}H_{21}NO_3 \cdot 0.5\,H_2O$: C, H, N.

1,2,4-Tris(4-hydroxyphenyl)-3-propyl-1*H*-pyrrole (6d): BBr_3 (1.6 mmol, 410 mg) was added to **5d** (0.33 mmol, 140 mg) following Method F to yield **6d** as a yellow solid (0.14 mmol, 54 mg, 43%): mp: 80 °C; ^{1}H NMR [(D_6)DMSO]: δ = 9.45 (s, 1H, O*H*), 9.41 (s, 1H, O*H*), 9.25 (s, 1H, O*H*), 7.24 (d, 2H, *J* = 8.5 Hz, Ar*H*), 6.89 (m, 4H, Ar*H*), 6.85 (s, 1H, C*H*), 6.76 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.67 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.63 (d, 2H, *J* = 8.8 Hz, Ar*H*), 2.46 (t, 2H, *J* = 8.0 Hz, $CH_2CH_2CH_3$), 1.25 (sextet, 2H, *J* = 7.5 Hz, $CH_2CH_2CH_3$), 0.67 ppm (t, 3H, *J* = 7.3 Hz, $CH_2CH_2CH_3$); MS (EI, 170 °C): *m/z* (%) = 385 $[M]^{+\cdot}$ (83), 356 (100), 330 (16), 193 (7), 131 (7), 77 (3). MS (ESI-TOF): *m/z*: 386.1740; IR (KBr): $\tilde{\nu}$ = 3370(b,s), 2955/2928(m), 1886(w), 1613(m), 1562 (m), 1449(m), 1388(b,m), 1229(b,s), 1170(m), 1098(w), 835(s) cm^{-1}; Anal. calcd for $C_{25}H_{23}NO_3 \cdot 0.75\,H_2O$: C, H, N.

1,2,4-Tris(4-hydroxyphenyl)-3-methyl-5-propyl-1*H*-pyrrole (13a): BBr_3 (3.5 mmol, 888 mg) was added to **12a** (0.71 mmol, 313 mg) following Method F to yield **13a** as a red solid (0.18 mmol, 0.07 g, 25%): mp: 86 °C; ^{1}H NMR [(D_6)DMSO]: δ = 9.55 (s, 1H, O*H*), 9.28 (s, 1H, O*H*), 9.26 (s, 1H, O*H*), 7.09 (d, 2H, *J* = 8.4 Hz, Ar*H*), 6.93 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.85 (d, 2H, *J* = 8.5 Hz, Ar*H*), 6.78 (d, 2H, *J* = 8.5 Hz, Ar*H*), 6.68 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.57 (d, 2H, *J* = 8.5 Hz, Ar*H*), 2.35 (t, 2H, *J* = 7.8 Hz, $CH_2CH_2CH_3$), 1.87 (s, 3H, CH_3), 1.08 (m, 2H, $CH_2CH_2CH_3$), 0.53 ppm (t, 3H, *J* = 7.3 Hz, $CH_2CH_2CH_3$); MS (EI, 50 °C): *m/z* (%) = 399 $[M]^{+\cdot}$ (54), 370 (100), 343 (16), 262 (6); IR (KBr): $\tilde{\nu}$ = 3393(b,s), 2959(m), 1889(w), 1700(s), 1595(m), 1512(s), 1443(s), 1374(s), 1261(b, s), 1171(s), 1101(m), 1044(m), 830(s) cm^{-1}; Anal. calcd for $C_{26}H_{25}NO_3 \cdot 2\,H_2O$: C, H, N.

3-Ethyl-1,2,4-tris(4-hydroxyphenyl)-5-propyl-1*H*-pyrrole (13b): BBr_3 (2.0 mmol, 489 mg) was added to **12b** (0.39 mmol, 178 mg) following Method F to yield **13b** as a reddish orange solid (0.11 mmol, 0.045 g, 28%): mp: 121 °C; ^{1}H NMR [(D_6)DMSO]: δ = 9.52 (s, 1H, O*H*), 9.28 (s, 1H, O*H*), 9.26 (s, 1H, O*H*), 7.09 (d, 2H, *J* = 8.4 Hz, Ar*H*), 6.93 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.87 (d, 2H, *J* = 8.4 Hz, Ar*H*), 6.77 (d, 2H, *J* = 8.4 Hz, Ar*H*), 6.66 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.57 (d, 2H, *J* = 8.5 Hz, Ar*H*), 2.30 (m, 4H, CH_2CH_3 and $CH_2CH_2CH_3$), 1.06 (m, 2H, $CH_2CH_2CH_3$), 0.73 (t, 3H, *J* = 7.3 Hz, $CH_2CH_2CH_3$), 0.53 ppm (t, 3H, *J* = 7.3 Hz, $CH_2CH_2CH_3$); MS (EI, 150 °C): *m/z* (%) = 413 $[M]^{+\cdot}$ (61), 384 (100), 357 (18), 206 (4). IR (KBr): $\tilde{\nu}$ = 3403(b,m), 2960(m), 1891(w), 1610(m), 1512(s), 1443(m), 1374(m), 1342(w), 1230(b,m), 1171(m), 1101(w), 1013(w), 830(m) cm^{-1}; Anal. calcd for $C_{27}H_{27}NO_3 \cdot 2.25\,H_2O$: C, H, N.

1,2,4-Tris(4-hydroxyphenyl)-3,5-dipropyl-1*H*-pyrrole (13c): BBr_3 (1.6 mmol, 400 mg) was added to **12c** (0.32 mmol, 150 mg) following Method F to yield **13c** as a red solid (0.023 mmol, 0.01 g, 7%): mp: 115 °C; ^{1}H NMR [(D_6)DMSO]: δ = 9.52 (s, 1H, O*H*), 9.27 (s, 1H, O*H*), 9.25 (s, 1H, O*H*), 7.07 (d, 2H, *J* = 8.4 Hz, Ar*H*), 6.91 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.84 (d, 2H, *J* = 8.5 Hz, Ar*H*), 6.75 (d, 2H, *J* = 8.4 Hz, Ar*H*), 6.64 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.55 (d, 2H, *J* = 8.5 Hz, Ar*H*), 2.27 (m, 4H, $CH_2CH_2CH_3$ und $CH_2CH_2CH_3$), 1.06 (m, 4H, $CH_2CH_2CH_3$ and $CH_2CH_2CH_3$), 0.57 (t, 3H, *J* = 7.3 Hz, $CH_2CH_2CH_3$), 0.51 ppm (t, 3H, *J* = 7.3 Hz, $CH_2CH_2CH_3$); MS (EI, 170 °C): *m/z* (%) = 427 $[M]^{+\cdot}$ (64), 398 (100), 371 (17), 213 (5); IR (KBr): $\tilde{\nu}$ = 3399(b,m), 2957(m), 1889(w), 1611(m), 1512(s), 1444(m), 1376(m), 1342(w), 1229(b,m), 1170(m), 1100(w), 1012(w), 828(m) cm^{-1}; Anal. calcd for $C_{28}H_{29}NO_3 \cdot 1.5\,H_2O$: C, H, N.

Analytical data for **6b-1** and **6b-2**: **6b-1**: Grey solid; ^{1}H NMR [(D_6)DMSO]: δ = 9.57 (s, 1H, O*H*), 9.43 (s, 1H, O*H*), 9.24 (s, 1H, O*H*), 7.35 (d, 2H, *J* = 8.6 Hz, Ar*H*), 7.25 (d, 2H, *J* = 8.9 Hz, Ar*H*), 7.01 (d, 2H, *J* = 8.4 Hz, Ar*H*), 6.82 (d, 2H, *J* = 8.6 Hz, Ar*H*), 6.68 (d, 2H, *J* = 8.5 Hz, Ar*H*), 6.64 (d, 2H, *J* = 8.9 Hz, Ar*H*), 5.63 (s, 1H, C*H*), 1.83 ppm (s, 3H, CH_3); MS (EI, 225 °C): *m/z* (%) = 373 $[M]^{+\cdot}$ (52), 358 (51), 280 (4), 252 (5), 212 (24), 135 (37), 121 (56); MS (ESI-TOF): *m/z*: 374.1447; IR (KBr): $\tilde{\nu}$ = 3250(b,s), 2880(m), 1870(w), 1720(m), 1640(s), 1590(s), 1500(s), 1430(s), 1390(m), 1360(m), 1210(b, s), 1155(s), 1100(m), 1130(w), 830(s) cm^{-1}. **6b-2**: Grey solid; ^{1}H NMR [(D_6)DMSO]: δ = 9.65 (s, 1H, O*H*), 9.47 (s, 1H, O*H*), 9.30 (s, 1H, O*H*), 7.42 (d, 2H, *J* = 8.5 Hz, Ar*H*), 7.12 (d, 2H, *J* = 8.5 Hz, Ar*H*), 7.07 (d,

2H, $J=8.8$ Hz, Ar*H*), 6.84 (d, 2H, $J=8.6$ Hz, Ar*H*), 6.67 (d, 2H, $J=8.7$ Hz, Ar*H*), 6.61 (d, 2H, $J=8.9$ Hz, Ar*H*), 3.22 (s, 3H, OCH_3), 1.72 ppm (s, 3H, CH_3); MS (EI, 250 °C): m/z (%) = 403 $[M]^{+\cdot}$ (1), 373 (38), 358 (39), 284 (6), 252 (4), 212 (22), 135 (71), 121 (32); MS (ESI-TOF): m/z: 404.1544; IR (KBr): $\tilde{\nu}=3250$(b,s), 2900(m), 1870(w), 1730(m), 1660(s), 1600(s), 1500(s), 1430(s), 1390(m), 1360(m), 1330(m), 1210(b, s), 1190(s), 1150(m), 1030(m), 1010(m), 880(w), 840(s) cm^{-1}.

Stability testing

For HPLC (Bio-Tek Kontron Instruments, Germany) a C_{18} reverse phase column (Eurospher 100–5 C_{18}, 250×4 mm, Knauer GmbH, Germany) was used, with water/MeOH (3:7) in an isocratic elution with a detection wavelength of 265 nm and a flow rate of 0.8 mL min^{-1} over 20 min. Compounds were dissolved in MeOH (10^{-2} M) and diluted with PBS to a final concentration of 5×10^{-5} M. Compounds were kept at 37 °C and were measured once directly after dissolving and six more times during the following 195 h.

Biological activity

Cell lines and growth conditions: MCF-7/2a cells, MCF-7 cells, and MDA-MB-231 cells were maintained as monolayer cultures at 37 °C in a humidified atmosphere (95% air, 5% CO_2) in T-25 flasks. U2-OS cells were maintained as a monolayer culture at 37 °C in humidified atmosphere (92.5% air, 7.5% CO_2) in T-25 flasks. Phenol red-free high glucose DMEM with sodium pyruvate (110 mg L^{-1}), 5% dextran charcoal-treated FCS (ct-FCS), and 0.5% geneticin solution was used as growth media for the MCF-7/2a cells, while L-glutamine and high glucose DMEM containing sodium pyruvate and 5% FCS was used for U2-OS, MCF-7, and MDA-MB-231 cells.

Transactivation assay with MCF-7/2a cells: Four days before beginning the experiment, MCF-7/2a cells were cultivated in DMEM supplemented with ct-FCS (50 mL L^{-1}). Cells from an 80% confluent monolayer were removed by trypsinization and suspended to approximately 10^5 cells mL^{-1} in the growth medium mentioned above. The cell suspension was then cultivated in 96-well flat-bottomed plates at growing conditions (see above). After 24 h, test compounds were added in concentrations ranging from 10^{-5}–10^{-11} M (estradiol 10^{-7}–10^{-13} M), and the plates were incubated for 50 h. Subsequently, 50 μL of cell culture lysis reagent was added to each well. After 30 min of lysis at room temperature, 50 μL of the Promega luciferase assay reagent (Promega, Mannheim, Germany) were added. Luminescence in relative light units (RLU) was measured for 10 s using a luminometer. Estrogenic activity was expressed as the percentage of activation of a 3×10^{-9} M estradiol control (100%).

Transactivation assay with U2-OS cells: U2-OS cells were transferred to DMEM supplemented with ct-FCS 24 h before the start of the experiment. Cells from a nearly confluent monolayer were split and seeded in 10 cm Ø Petri dishes at a concentration of 1×10^6 cells per dish at least 24 h prior to transfection. Transient transfection of the cells with 0.05 μg of receptor plasmid pSG5-ERα or pSG5-ERβ FL and 5 μg of reporter plasmid p$(ERE)_2$-luc^+ was carried out after 6 h using 15 μL Fugene 6 (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. After 18 h, the cells were washed with PBS, harvested by trypsinization, and seeded in white 96-well plates at a concentration of 10^4 cells per well in 100 μL of ct-DMEM. After 3 h, the medium was replaced by medium containing either E2 or test compounds in final concentrations ranging from 0.1 pM–10 nM (E2) or 0.1 nM–10 mM (test compounds). After 18 h incubation, medium was removed and cells were lysed and luciferase activity assayed as described above.

Estrogen receptor binding: recombinant ERα/ERβ (HAP assay): An ethanol solution (250 fmol μL^{-1}) of recombinant human estrogen receptor (hERα or hERβ, Calbiochem) was diluted with Tris-HCl buffer, pH 8.0 (1:100). These hERα or hERβ preparations were adsorbed on hydroxylapatite pellets (prepared in 10 mmol L^{-1} of Tris-HCl buffer, pH 8.0, containing 1 mg mL^{-1} BSA) which were labeled with increasing concentrations of the test compounds (10^{-10}–10^{-5} M) or [^{3}H]E2 (5 nM, Perkin–Elmer) and were incubated for 3 h at room temperature. Bound [^{3}H]E2 was then successively extracted with EtOH and aliquots of 200 μL of the ethanol extracts were transferred to scintillation vials containing 3.5 mL of scintillator Ecoscint H (National Diagnostics, Atlanta) for radioactivity counting. All measurements were performed in duplicate. The results (relative radioactivity) were expressed as the percentage of a solvent treated control sample. Relative concentrations of compounds required to reduce the binding of [^{3}H]E2 by 50% were determined as their IC_{50} values. Relative binding affinities were calculated by dividing the IC_{50} of E2 by the IC_{50} of the respective compound; therefore, binding affinities are expressed relative to estradiol (RBA(E2) = 100%).

Growth inhibition of MCF-7 and MDA-MB-231 human breast cancer cells: Cells from an 80% confluent monolayer were harvested by trypsinization and suspended to approximately 10^4 (MCF-7 cells) or 7.5×10^3 cells mL^{-1} (MDA-MB-231 cells). At the beginning of the experiment, the cell suspension was transferred to 96-well microplates (100 μL per well). After cultivating for 3 days under growth conditions, the test compounds (dissolved in DMEM) were added in final concentrations ranging from 3.13 μM–50 μM. Control wells (16 per plate) contained 0.1% DMSO, which was used for the preparation of stock solutions. Initial cell density was determined by addition of glutaric dialdehyde to one plate (1% in PBS; 100 μL per well). After incubation for three (MDA-MB-231 cells) or four (MCF-7 cells) days, the medium was removed, and glutaric dialdehyde (1% in PBS; 100 μL per well) was added for fixation. After 30 min, the aldehyde solution was decanted and 150 μL PBS per well was added. The plates were stored at 4 °C prior to staining of the cells by treating them for 30 min with 100 μL of an aqueous crystal violet solution (0.02%). After decanting, cells were washed several times with water to remove the adherent dye. After addition of 180 μL EtOH (70%), plates were gently shaken for 3–4 h. Optical density of each well was measured using a microplate autoreader at 590 nm.

Acknowledgements

The technical assistance of Silke Bergemann is gratefully acknowledged.

Keywords: autoxidation · cytotoxicity · drug design · hormones · nitrogen heterocycles

[1] G. L. Greene, M. F. Press, *J. Steroid Biochem.* **1986**, *24*, 1.
[2] E. Enmark, M. Pelto-Huikko, K. Grandien, S. Lagercrantz, J. Lagercrantz, G. Fried, M. Nordenskjold, J.-Å. Gustafsson, *J. Clin. Endocrinol. Metab.* **1997**, *82*, 4258.
[3] M. G. Parker, *Curr. Opin. Cell Biol.* **1993**, *5*, 499.
[4] J. F. Couse, K. S. Korach, *Endocr. Rev.* **1999**, *20*, 358.
[5] Q. Qu, M. Perala-Heape, A. Kapanen, J. Dahllund, J. Salo, H. K. Vaananen, P. Harkonen, *Bone* **1998**, *22*, 201.

[6] C. H. Lee, A. M. Edwards, *Carcinogenesis* **2001**, *22*, 1473.
[7] P. A. Komesaroff, K. Sudhir, *Reprod. Fertil. Dev.* **2001**, *13*, 261.
[8] S. Hulley, D. Grady, T. Bush, C. Furberg, D. Herrington, B. Riggs, E. Vittinghoff, for the Heart and Estrogen/Progestin Replacement Study Research Group, *JAMA* **1998**, *280*, 605.
[9] B. Fisher, J. P. Costantino, D. L. Wickerham, C. K. Redmond, M. Kavanah, W. M. Cronin, V. Vogel, A. Robidoux, N. Dimitrov, J. Atkins, M. Daly, S. Wieand, E. Tan-Chiu, L. Ford, N. Wolmark, *J. Natl. Cancer Inst.* **1998**, *90*, 1371.
[10] V. C. Jordan, J. M. Schafer, A. S. Levenson, H. Liu, K. M. Pease, L. A. Simons, J. W. Zapf, *Cancer Res.* **2001**, *61*, 6619.
[11] R. Gust, R. Keilitz, K. Schmidt, *J. Med. Chem.* **2001**, *44*, 1963.
[12] P. M. Kekenes-Huskey, I. Muegge, M. von Rauch, R. Gust, E.-W. Knapp, *Bioorg. Med. Chem.* **2004**, *12*, 6527.
[13] R. Gust, R. Keilitz, K. Schmidt, M. von Rauch, *Arch. Pharm. Chem. Life Sci.* **2002**, *335*, 463.
[14] M. von Rauch, M. Schlenk, R. Gust, *J. Med. Chem.* **2004**, *47*, 915.
[15] T. Wiglenda, R. Gust, *J. Med. Chem.* **2007**, *50*, 1475.
[16] B. E. Fink, D. S. Mortensen, S. R. Stauffer, Z. D. Aron, J. A. Katzenellenbogen, *Chem. Biol.* **1999**, *6*, 205.
[17] S. R. Stauffer, C. J. Coletta, R. Tedesco, G. Nishiguchi, K. Carlson, J. Sun, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *J. Med. Chem.* **2000**, *43*, 4934.
[18] J. P. Anselme, *J. Org. Chem.* **1967**, *32*, 3716.
[19] D. Gibson, I. Binyamin, M. Haj, I. Ringel, A. Ramu, J. Katzhendler, *Eur. J. Med. Chem.* **1997**, *32*, 823.
[20] J. B. Paine, D. Dolphin, *J. Org. Chem.* **1985**, *50*, 5598.
[21] H. Fischer, E. Fink, *Hoppe-Seyler's Z. Physiol. Chem.* **1944**, *280*, 123.
[22] S. V. Zaitseva, S. A. Zdanovich, A. S. Semeikin, O. A. Golubchikov, *Russ. J. Gen. Chem.* **2003**, *73*, 467.
[23] H. Zhang, Q. Cai, D. Ma, *J. Org. Chem.* **2005**, *70*, 5164.
[24] U. Ghosh, D. Ganessunker, V. J. Sattigeri, K. E. Carlson, D. J. Mortensen, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *Bioorg. Med. Chem.* **2003**, *11*, 629.
[25] S. Nahm, S. M. Weinreb, *Tetrahedron Lett.* **1981**, *22*, 3815.
[26] C. H. Park, R. S. Givens, *J. Am. Chem. Soc.* **1997**, *119*, 2453.
[27] D. S. Mortensen, A. L. Rodriguez, K. E. Carlson, J. Sun, B. S. Katzenellenbogen, J. A. Katzenellenbogen, *J. Med. Chem.* **2001**, *44*, 3838.
[28] R. H. Young, N. Chinh, C. Mallon, *Ann. N. Y. Acad. Sci.* **1970**, *171*, 130.
[29] R. H. Young, R. L. Martin, N. Chinh, C. Mallon, R. H. Kayser, *Can. J. Chem.* **1972**, *50*, 932.
[30] A. Wassermann, *J. Chem. Soc.* **1935**, 828.
[31] L. J. Andrews, R. M. Keefer, *J. Am. Chem. Soc.* **1955**, *77*, 6284.
[32] H. H. Wasserman, A. H. Miller, *J. Chem. Soc. Chem. Commun.* **1969**, 199.
[33] D. A. Lightner, C.-S. Pak, *J. Org. Chem.* **1975**, *40*, 2724.
[34] K. Gollnick, G. O. Schenck in *Organic Chemistry, A series of monographs: 1,4-Cycloaddition reactions Vol. 8* (Ed.: J. Hamer), Academic press, NY and London, **1967**.
[35] G. B. Quistad, D. A. Lightner, *Tetrahedron Lett.* **1971**, *12*, 4417.
[36] G. Rio, A. Ranjon, O. Pouchot, *C. R. Hebd. Seances Acad. Sci.* **1966**, *263*, 634.
[37] D. A. Lightner, L. K. Low, *J. Heterocycl. Chem.* **1972**, *9*, 167.
[38] H. H. Wasserman, *Ann. N. Y. Acad. Sci.* **1970**, *171*, 108.
[39] F. Hafner, E. Holler, E. von Angerer, *J. Steroid Biochem. Mol. Biol.* **1996**, *58*, 385.
[40] I. Laïos, A. Cleeren, G. Leclercq, D. Nonclercq, G. Laurent, M. Schlenk, A. Wellner, R. Gust, *Biochem. Pharmacol.* **2007**, *74*, 1029.

Received: December 13, 2010
Published online on March 17, 2011

