Ultrasound-Promoted Synthesis of 2,3-Bis(4-hydroxyphenyl)indole Derivatives as Inherently Fluorescent Ligands for the Estrogen Receptor

Sophia D. Koulocheri[a] and Serkos A. Haroutounian*[a]

Keywords: Indoles / Cyclization / Ultrasound / Estrogen receptor

A series of 2,3-bis(4-hydroxyphenyl)indole derivatives **4c–f** was prepared by ultrasound-promoted intramolecular cyclodehydration of a polyphosphoric acid solution of α-anilinyl- (or 3-anisidyl)desoxyanisoins **2c–f**, and their optical spectroscopy and estrogen receptor (ER) binding properties were studied. Compounds **4c–f** give intense long-wavelength fluorescent emission, sensitive to solvent polarity and pH. Furthermore, the two indol-6-ols **4e**, **f** display reasonably good binding affinities to ER and appear to be well suited for use as fluorescent probes for the detection of ER in cells.

Introduction

The development and use of fluorescent probes as tools for the assay and characterization of cellular binding sites of steroidal hormone receptors is a subject of considerable research activity.[1,2] Of particular interest is the development of a fluorescence assay for the estrogen receptors (ER).[3] Such a method would permit a cell-by-cell assay of the quantity and distribution of ER in breast cancer cells using flow cytometry[4] or fluorescence microscopy,[5] and would provide useful information on the response to hormonal therapy.[6] A suitable fluorescent probe should exhibit: (i) a relatively high binding affinity (RBA) for the ER, (ii) fluorescence at wavelengths greater than 430 nm (in order to be distinguishable from the background of cell autofluorescence) and (iii) environmental sensitivity, which results in substantial changes in emission intensity and/or wavelength in media of different polarity and/or pH.

Three major classes of fluorescent estrogenic probes have been developed: estrogen−fluorophore conjugates,[7] inherently fluorescent estrogens,[8] and photofluorogenic estrogens.[9] However, most of the agents described do not possess the desired optimal photophysical properties or binding affinity. Thus, the search for lead structures and novel compounds that will prove suitable for use as fluorescent probes for the assay of the ER represents an intriguing research goal. In this context, we were interested in the synthesis and the study of the photophysical and binding properties of novel 2,3-bis(4-hydroxyphenyl)indole derivatives.

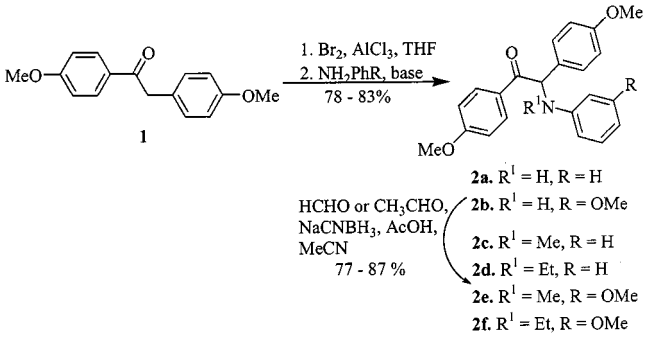
The indole ring belongs to an important class of compounds and natural products[10] with pronounced pharmacological activity.[11] The 1,3-dialkyl-2-phenylindol-6-ols have been found to possess strong estrogenic and tumor inhibition activities,[12] while their donor−acceptor character (provided by the nitrogen and hydroxy functionalities) makes them potential candidates for use as biological probes. This has stimulated our interest to enhance their absorption and emission properties. Thus, we were intrigued by the possible incorporation of a *p*-hydroxyphenyl group in the place of the 3-alkyl moiety and the study of the consequences that this would have on the photophysical properties and binding affinity to ER of the indole derivatives.

Results and Discussion

Chemistry

Of the numerous methods developed for the synthesis of indoles,[13] the most widely used are the Fischer[14] and Bischler[15] methods. In this report we present a modification of the classical Bischler method by using ultrasound as the promoter of the acid-catalyzed intramolecular cyclodehydration of the α-anilinyl- (or 3-anisidyl)desoxyanisoin derivatives (**2c−f**).

The synthesis of the α-aminodesoxyanisoin substrates **2a**, **b** is illustrated in Scheme 1. A solution of the 2-bromo derivative of desoxyanisoin (prepared by a known procedure),[16] in dichloromethane upon treatment with aniline in the presence of triethylamine, produced the desired α-anilinyldesoxyanisoin **2a** smoothly. With 3-anisidine however, no reaction was observed under these experimental conditions or by use of pyridine as base. Thus, the reaction was per-



Scheme 1

[a] Chemistry Laboratory, Agricultural University of Athens, Iera odos 75, Athens 11855, Greece
Fax: (internat.) + 30-1/5294265
E-mail: sehar@aua.gr

  1434−193X/01/0509−1723 $ 17.50+.50/0

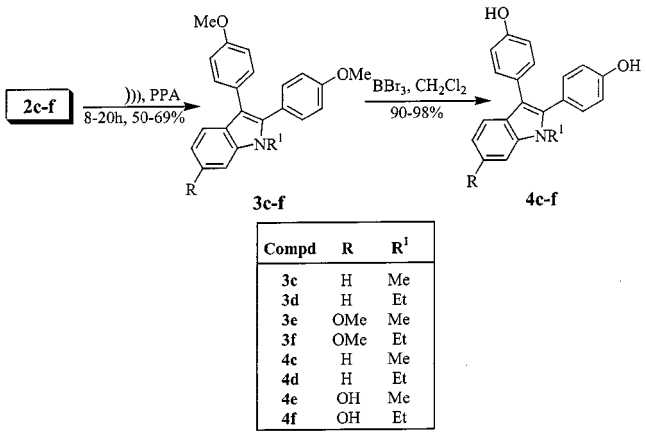
formed in a DMA solution with 2,6-lutidine as base and a catalytic amount of NaI as initiator, and the desired α-(3-anisidyl)desoxyanisoin **2b** was furnished in good yield. Finally, reductive alkylation of amines **2a**, **b** provided the substrates **2c**−**f** efficiently.

A broad variety of acidic conditions have been used for the transformation of α-arylamino ketones to indoles (**3c**−**f**, Bischler method).[15] Typical conditions to promote these cyclodehydration reactions include the prolonged heating of an acidic solution of the substrate, while the yield of the indole product depends strongly on the nature of the substrate. In this context, we have experimented with a broad range of acidic conditions and temperatures, and only poor or moderate yields of transformations were obtained in very long reaction times (Table 1). The best results were achieved when a polyphosphoric acid (PPA) mixture of the corresponding substrate was mechanically stirred at 60 °C for 2−4 d. This led us to consider the application of ultrasound as a low-temperature promoter of the above reaction. Thus, when we performed the same reaction in a thermostated ultrasound bath at 35 °C, a rapid cyclodehydration (complete in 8−18 h) of substrates **2c**−**f** was observed. This ultrasound-promoted modification worked satisfactorily for all substrates, and the indole products **3c**−**f** were obtained in 50−69% yields (Scheme 2). In the case of 3-anisidyl derivatives, however, the reaction yield was notably higher, presumably because the methoxy group activates its *ortho* position, and facilitates the cyclodehydration reaction. For compound **2f**, the course of the ultrasound-promoted cyclodehydration was studied by analytical HPLC (Figure 1), which showed that the reaction was completed within 8 h and the indole **3f** was obtained as product (69%). Furthermore, the gradual formation of a resinous side-product (retention time: 3 min, 20% yield) was also observed. Finally, the corresponding deprotected indoles **4c**−**f** were obtained in excellent yields by reaction with BBr_3 in dichloromethane.

Table 1. Experimental conditions and yields for the acid-catalyzed cyclodehydration of substrates **2c**−**f**

Reactant	Acid	Temperature	Time	Yield (%)
2c, **d**	PPA	60 °C	4 d	40−45
	CF_3COOH	room temp.	8 h	15−20
	HCl	room temp.	10 h	28−35
	H_2SO_4	room temp.	7 h	25−30
	MsOH	40 °C	3 d	20−30[a]
	CSA	60 °C	3 d	5−10[a]
	TosH	60 °C	3 d	5−10[a]
	PPA,)))	35 °C	18 h	50−55
2e, **f**	PPA	60 °C	2 d	50−54
	CF_3COOH	room temp.	3 h	20−28
	HCl	room temp.	3.5h	33−37
	H_2SO_4	room temp.	2.5 h	27−35
	MsOH	40 °C	3 d	25−35[a]
	CSA	60 °C	3 d	5−10[a]
	TosH	60 °C	3 d	5−10[a]
	PPA,)))	35 °C	8 h	60−69

[a] 25−35% of starting material was recovered.



Compd	R	R^1
3c	H	Me
3d	H	Et
3e	OMe	Me
3f	OMe	Et
4c	H	Me
4d	H	Et
4e	OH	Me
4f	OH	Et

Scheme 2

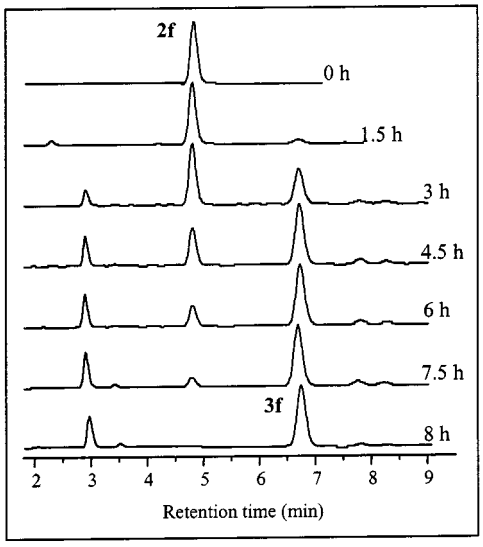


Figure 1. Progress of the ultrasound-promoted cyclodehydration of substrate **2f** monitored by HPLC [t_R(**2f**) = 4.8 min; t_R(**3f**) = 6.7 min; t_R(by-product) = 3 min]

Absorption and Fluorescence Properties

The UV/Vis absorbance spectra of indoles **4c**−**f** were measured in tetrahydrofuran, acetonitrile, ethanol, and water under neutral and acidic (0.1 N HCl) conditions. Spectra in the latter two solvents were also obtained under basic conditions (0.1 N KOH); THF and CH_3CN were excluded from studies in base because of solubility problems. Table 2 summarizes the measurements, and the spectra of compound **4f** in H_2O are shown in Figure 2. Comparison of the absorption bands of indoles in different solvents indicates that their absorbance spectra showed only limited solvatochromicity under neutral and acidic conditions. On the other hand, the absorption maxima of the 6-hydroxy-substituted compounds **4e,f** are at longer wavelength (approx. 10−20 nm) compared with the unsubstituted ones. In base, however, these compounds showed a new, rather intense, longer wavelength band (ca. 340 nm).

The suitability of fluorescent probes for biological systems depends strongly on the extent to which their fluorescent properties are sensitive to their environment (solvent

Table 2. Long wavelength absorbance maxima for 2,3-bis(4-hydroxyphenyl)indoles

Compd.	Cond.[a]	λ_{abs}^{max} (ε)			
		THF	CH_3CN	EtOH	H_2O
4c	neutral	240 (12,100) 313 (16,000)	250 (29,300) 313 (15,800)	255 (25,100) 307 (15,200)	250 (16,400) 311 (8,200)
	acid	239 (12,400) 315 (20,300)	255 (28,100) 278 (17,700) 319 (14,700)	255 (21,500) 307 (11,900)	248 (15,500) 310 (7,500)
	basic	[b]	[b]	267 (25,600) 335 (8,200)	268 (20,400) 313 (13,000)
4d	neutral	238 (12,600) 313 (22,500)	245 (24,100) 315 (12,400)	253 (31,300) 289 (14,500) 307 (14,500)	251 (25,200) 305 (13,900)
	acid	235 (12,500) 313 (16,400)	246 (23,500) 251[c] (21,280) 276 (16,700) 317 (10,800)	250 (51,700) 307 (31,200)	245 (26,100) 309 (11,200)
	basic	[b]	[b]	269 (56,100) 309 (35,200)	269 (32,700) 312 (17,600)
4e	neutral	241 (10,300) 323 (14,300)	250 (11,000) 322 (6,300)	253 (24,300) 311 (12,200)	250 (29,600) 312 (16,300)
	acid	238 (8,500) 326 (8,200)	244 (14,500) 322 (7,100)	251 (35,500) 315 (19,900)	254 (29,600) 275 (20,100) 308 (14,200)
	basic	[b]	[b]	261 (50,400) 309 (28,900) 344 (22,600)	268 (23,700) 322 (9,800)
4f	neutral	242 (10,100) 325 (14,700)	242 (11,600) 315 (5,300)	258 (33,300) 296 (20,500) 330 (9,600)	244 (25,400) 311 (11,200)
	acid	239 (8,900) 327 (10,500)	240 (15,200) 315 (6,200)	251 (35,000) 297 (21,400) 334 (9,500)	245 (23,800) 314 (9,700)
	basic	[b]	[b]	267 (69,100) 309 (37,700) 351 (24,300)	264 (26,600) 307 (16,200) 345 (15,100)

[a] Acid = 0.1 N HCl; Base = 0.1 N KOH. – [b] Not soluble. – [c] Shoulder.

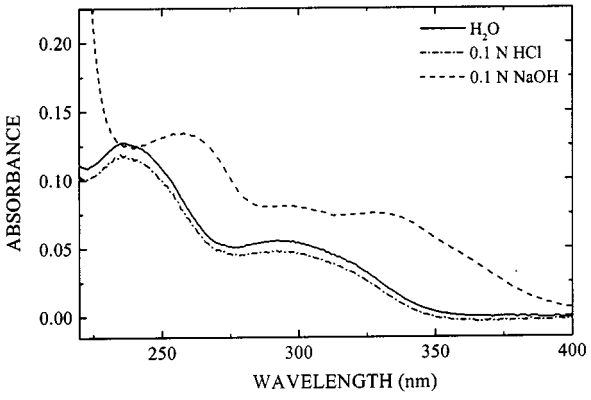


Figure 2. UV absorbance spectra of 1-ethyl-2,3-bis(4-hydroxyphenyl)-1*H*-indol-6-ol (**4f**) in H_2O (5×10^{-6} M) under neutral, acidic (0.1 N HCl) and basic (0.1 N NaOH) conditions

and pH).[17] Thus, we have measured the fluorescence emission of the four indoles **4c**–**f** in each of the aforementioned solvents and pH conditions, using the major long wavelength absorbance maximum as the excitation wavelength. In each case, wavelengths of maximum emission were measured. As expected, the fluorescence emission is relatively weak, because the 2- and 3-aryl rings are twisted out of planarity from one another.

Fluorescence emission spectra of compound **4f** in EtOH are shown in Figure 3 and complete results on fluorescence measurements are given in Table 3, and show that the emission maximum under neutral conditions is shifted to the red in more polar protic solvents. Furthermore, in each solvent there is an additional red shift from neutral to acidic and basic conditions, presumably because in the excited state the formation of the ionic form of the molecules is facilitated.[18] The fluorescence emission of these compounds, however, is solvent- and pH-dependent and indicates that there is a large dipole moment in the excited state.[19]

Estrogen Receptor Binding Affinity (RBA)

The estrogen RBAs of the new compounds were determined by a competitive binding assay and are shown in Table 4. The affinities were obtained by competition with the tracer compound [^{3}H]estradiol and have been expressed on a percent scale, relative to estradiol, whose affinity was considered to be 100%.

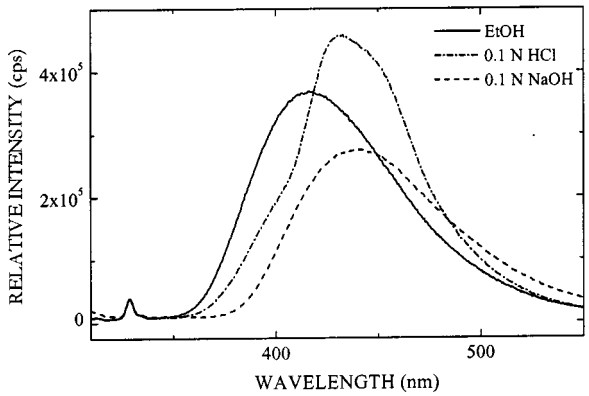


Figure 3. Fluorescence emission spectra of 1-ethyl-2,3-bis(4-hydroxyphenyl)-1*H*-indol-6-ol (**4f**) in ethanol (5×10^{-6} M) under neutral, acidic (0.1 N HCl) and basic (0.1 N NaOH) conditions

Table 3. Long wavelength emission maxima for 2,3-bis(4-hydroxyphenyl)indoles

Cmpd.	Cond.[a]	λ_{em}^{max} (relative intensity)[b]			
		THF	CH_3CN	EtOH	H_2O
4c	neutral	440 (70)	441 (57)	440 (80)	448 (62)
	acid	425 (77)	430 (54)	445 (66)	461 (84)
	basic	454[c] (68) [d]	454[c] (46) [d]	490 (32)	496 (23)
4d	neutral	437 (48)	447 (50)	438 (57)	466 (50)
	acid	429 (53)	431 (40)	426 (82)	456 (62)
	basic	453[c] (49) [d]	476[c] (36) [d]	450 (87) 495 (30)	496 (16)
4e	neutral	438 (36)	438 (36)	437 (37)	458 (38)
	acid	400[c] (33)	400[c] (23)	453 (38)	476 (44)
	basic	437 (37) [d]	444 (28) [d]	459 (46)	460 (7.5)
4f	neutral	435 (29) 460[c] (22)	407[c] (22) 445 (31)	427 (37)	444 (36)
	acid	410 (31)	408[c] (23)	438 (46)	477 (48)
	basic	430 (35) 460 (24) [d]	440 (24) [d]	464[c] (40) 460 (39)	455 (2.5)

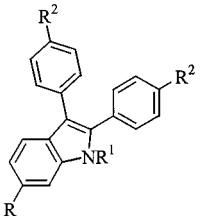
[a] Acid = 0.1 N HCl; Base = 0.1 N KOH. Excitation was always at the major long wavelength band. – [b] Numbers in parentheses represent the relative intensity of emission ($\times10^4$ cps) at λ_{em}^{max}. – [c] Shoulder. – [d] Not soluble.

Most of the compounds showed limited affinity to the ER. In every case, an increase in RBA is observed as the alkyl steric bulk is increased. As expected, the two indol-6-ols (compounds **4e**, **f**) displayed much better affinities to the ER compared with the other indoles. These RBA values may be considered suitable for use in biological assays.

Conclusion

In summary, we have described an efficient ultrasound-promoted synthesis of several 2,3-bis(4-hydroxyphenyl)indoles **4c**–**f** and presented a preliminary evaluation of their photophysical and receptor-binding properties. 2,3-Bis(4-hydroxyphenyl)-1-methyl-1*H*-indol-6-ol (**4e**) and 1-ethyl-2,3-bis(4-hydroxyphenyl)-1*H*-indol-6-ol (**4f**) possess moderate to good affinities for the ER, while their absorbance and fluorescence spectra display strong dependence on solvent polarity and pH. In addition, their syntheses do not require stereospecific methods or complicated isomer separations. Thus, they are attractive candidates for further development as fluorescent probes. Research towards the design and synthesis of novel indole derivatives with a second heterocyclic ring is currently underway.

Table 4. Estrogen receptor binding affinity of 2,3-bis(4-methoxyphenyl)indoles **3c**–**f** and 2,3-bis(4-hydroxyphenyl)indoles **4c**–**f**



Compd.	R	R^1	R^2	RBA
3c	H	Me	OMe	<0.01
3b	H	Et	OMe	0.01
3e	OMe	Me	OMe	<0.01
3f	OMe	Et	OMe	0.04
4c	H	Me	OH	0.41
4d	H	Et	OH	1.12
4e	OH	Me	OH	3.40
4f	OH	Et	OH	10.37

Experimental Section

General: Air- and/or moisture-sensitive reactions were carried out under argon in flame-dried glassware. All starting materials were purchased from Aldrich (analytical reagent grades) and used without further purification. Solvents were distilled from the appropriate drying agents prior to use. 2-Bromo-1,2-bis(4-methoxyphenyl)-ethanone was prepared in 78% yield according to a literature procedure.[16] All reactions were monitored by thin-layer chromatography using TLC sheets coated with silica gel 60 F_{254} (Merck); spots were visualized with UV light or by treatment with an acidic solution of *p*-anisaldehyde. – Products were purified by flash chromatography on Merck silica gel 60 (230–400 mesh ASTM). The course of the cyclodehydration reaction of compound **2f** was monitored by analytical HPLC [column: Kromasil 100-5, C-18, (25 cm × 4 mm); mobile phase: CH_3CN/H_2O (8:2); detector: UV (λ = 254 nm); flow: 1 mL/min]. Before the photophysical experiments and RBA assays, all compounds were purified by semi-preparative HPLC [column: Kromasil 10–5C18 (25 cm × 10 mm); mobile phase: CH_3CN; detector: UV (λ = 300 nm); flow: 1 mL/min; load: 3 mg/100μL of solution in mobile phase]. – Melting points (uncorrected) were measured with a Büchi melting point apparatus. – FT-IR spectra were recorded with a Nicolet Magna 750, series II. Samples were recorded as KBr pellets, unless otherwise stated. – ^{1}H NMR spectra were recorded with Bruker DRX-400 or Bruker AC 200 spectrometers in the indicated solvents. Chemical shifts are referenced to internal TMS. – Ultrasound reactions were carried out in a Bandelin Sonorex Super RK 100 SH ultrasound bath. – HPLC were performed with a Hewlett Packard 1100 series instrument with a variable-wavelength UV detector and coupled to HP Chem.-Station with the manufacturer's 5.01 software package.

1,2-Bis(4-methoxyphenyl)-2-(phenylamino)ethanone (2a): To a stirred solution of 2-bromo-1,2-bis(4-methoxyphenyl)ethanone (2.5 g, 7.46 mmol) and triethylamine (1.3 mL, 9 mmol) in anhydrous CH_2Cl_2 (10 mL), was added aniline (0.8 mL, 9 mmol). The reaction was run at room temp. for 1 h, then the solvent was evaporated under reduced pressure and the remaining slurry was partitioned between EtOAc (50 mL) and H_2O (30 mL). The organic layer was separated, washed with brine, dried with $MgSO_4$, and concentrated under reduced pressure to give a yellowish solid. Flash chromatographic purification (EtOAc/hexane, 1:4; $R_f = 0.32$) and recrystallization from diethyl ether furnished (2.1 g, 80%) of the yellow title product, m.p. 113−114 °C. − IR (neat): $\tilde{\nu}$ = 3406 cm^{-1} (N−H), 1672 (C=O). − ^1H NMR (200 MHz, $CDCl_3$): δ = 3.72 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 5.38 (s, 1 H, NH), 5.94 (s, 1 H, H-2), 6.68 (m, 3 H, ArH), 6.82 (d, J = 8.9 Hz, 2 H, ArH), 6.91 (d, J = 8.9 Hz, 2 H, ArH), 7.13 (m, 2 H, ArH), 7.37 (d, J = 8.7 Hz, 2 H, ArH), 8.01 (d, J = 8.9 Hz, 2 H, ArH). − $C_{22}H_{21}NO_3$ (347.4): calcd. C 76.06, H 6.09, N 4.03; found C 76.16, H 6.01, N 4.11.

1,2-Bis(4-methoxyphenyl)-2-[(3-methoxyphenyl)amino]ethanone (2b): A solution of 2-bromo-1,2-bis(4-methoxyphenyl)ethanone (2.5 g, 7.5 mmol), 2,6-lutidine (1 mL, 8.5 mmol), anisidine (1 mL, 8.5 mmol) and traces of NaI in dry DMA (10 mL) was stirred for 4 h at 50 °C. Then the solvent was removed under reduced pressure, and the residue was partitioned between EtOAc (50 mL) and H_2O (30 mL). The organic layer was washed with brine, dried with $MgSO_4$ and concentrated. Flash chromatographic purification (EtOAc/hexane, 1:4; R_f = 0.21) and recrystallization from diethyl ether furnished **2b** (2.4 g, 85%) as a yellow solid, m.p. 112−113 °C. − IR (neat): $\tilde{\nu}$ = 3415 cm^{-1} (N−H), 1680 (C=O). − ^1H NMR (200 MHz, $CDCl_3$): δ = 3.73 (s, 6 H, OCH_3), 3.85 (s, 3 H, OCH_3), 5.40 (d, J = 6.2 Hz, 1 H, NH), 5.93 (d, J = 6.2 Hz, 1 H, H-2), 6.26 (m, 3 H, ArH), 6.82 (d, J = 8.8 Hz, 2 H, ArH), 6.91 (d, J = 9.1 Hz, 2 H, ArH), 7.03 (m, 2 H, ArH), 7.37 (d, J = 8.6 Hz, 2 H, ArH), 8.01 (d, J = 8.8 Hz, 2 H, ArH). − $C_{23}H_{23}NO_4$ (377.4): calcd. C 73.19, H 6.14, N 3.71; found C 73.31, H 6.17, N 3.60.

General Procedure for the Reductive Alkylation of Amines: To a stirred solution of amine (14.8 mmol) and corresponding aldehyde (29.6 mmol) in acetonitrile (10 mL) were added sodium cyanoborohydride (29.6 mmol) and acetic acid (0.12 mL). The reaction was run for 2−4 h (monitored by TLC), and the solvent was evaporated under reduced pressure. The residue was dissolved in diethyl ether (15 mL) and extracted with a saturated solution of $NaHCO_3$ (15 mL). The organic layer was separated, dried with $MgSO_4$, concentrated in vacuo, and purified by flash chromatography.

1,2-Bis(4-methoxyphenyl)-2-(methylphenylamino)ethanone (2c): This compound was obtained as a pale yellow solid (4.65 g, 87%). − M.p. 102−103 °C (diethyl ether/hexane). − R_f = 0.46 (EtOAc/hexane, 1:4). − IR: $\tilde{\nu}$ = 3323 cm^{-1} (N−H), 1673 (C=O). − ^1H NMR (200 MHz, $CDCl_3$): δ = 2.87 (s, 3 H, CH_3), 3.80 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 6.35 (s, 1 H, H-2), 6.71 (m, 3 H, ArH), 6.86 (d, J = 8.7 Hz, 4 H, ArH), 7.15−7.27 (m, 4 H, ArH), 7.91 (d, J = 9.2 Hz, 2 H, ArH). − $C_{23}H_{23}NO_3$ (361.4): calcd. C 76.43, H 6. 41, N 3.88; found C 76.60, H 6.35, N 3.71.

2-(Ethylphenylamino)-1,2-bis(4-methoxyphenyl)ethanone (2d): This compound was obtained as a yellow solid (4.6 g, 83%). − M.p. 125−127 °C (diethyl ether/hexane). − R_f = 0.46 (EtOAc/hexane, 1:4). − IR (neat): $\tilde{\nu}$ = 3327 cm^{-1} (N−H), 1680 (C=O). − ^1H NMR (400 MHz, $CDCl_3$): δ = 0.85 (t, J = 7.1 Hz, 3 H, CH_2CH_3), 3.40 (q, J = 7.1 Hz, 2 H, CH_2CH_3), 3.78 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 6.34 (s, 1 H, H-2), 6.71 (m, 3 H, ArH), 6.86 (d, J = 8.7 Hz, 4 H, ArH), 7.19 (m, 4 H, ArH), 7.90 (d, J = 9.1 Hz, 2 H, ArH). − $C_{24}H_{25}NO_3$ (375.4): calcd. C 76.77, H 6. 71, N 3.73; found C 76.55, H 6.83, N 3.91.

1,2-Bis(4-methoxyphenyl)-2-[(3-methoxyphenyl)methylamino]-ethanone (2e): This compound was obtained as a yellowish solid (4.9 g, 85%). − M.p. 130−131 °C (diethyl ether/hexane). − R_f = 0.35 (EtOAc/hexane, 1:4). − IR (neat): $\tilde{\nu}$ = 3320 cm^{-1} (N−H), 1679 (C=O). − ^1H NMR (400 MHz, $CDCl_3$): δ = 2.82 (s, 3 H, CH_3), 3.72 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 6.32 (m, 3 H, ArH, H-2), 6.38 (dd, J = 8.3, 2.1 Hz, 1 H, ArH), 6.86 (dd, J = 8.7, 3.7 Hz, 4 H, ArH), 7.12 (m, 3 H, ArH), 7.90 (d, J = 9.1 Hz, 2 H, ArH). − $C_{24}H_{25}NO_4$ (391.4): calcd. C 73.64, H 6.44, N 3.58; found C 73.81, H 6.51, N 3.42.

2-[Ethyl-(3-methoxyphenyl)amino]-1,2-bis(4-methoxyphenyl)-ethanone (2f): This compound was obtained as off-white crystals (4.62 g, 77%). − M.p. 141−142 °C (diethyl ether/hexane). − R_f = 0.37 (EtOAc/hexane, 1:4). − IR (neat): $\tilde{\nu}$ = 3340 cm^{-1} (N−H), 1682 (C=O). − ^1H NMR (400 MHz, $CDCl_3$): δ = 0.84 (t, J = 7.1 Hz, 3 H, CH_2CH_3), 3.38 (q, J = 7.1 Hz, 2 H, CH_2CH_3), 3.70 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 6.27−6.34 (m, 3 H, ArH), 6.32 (s, 1 H, H-2), 6.85 (d, J = 8.3 Hz, 2 H, ArH), 6.85 (d, J = 8.3 Hz, 2 H, ArH), 7.07 (m, 1 H, ArH), 7.17 (d, J = 8.7 Hz, 2 H, ArH), 7.89 (d, J = 8.7 Hz, 2 H, ArH). − $C_{25}H_{27}NO_4$ (405.5): calcd. C 74.05, H 6.71, N 3.45; found C 73.88, H 6.56, N 3.51.

General Procedure for the Cyclization of Ketones: A mixture of α-anilinyl- (or 3-anisidyl)desoxyanisoins **2c**−**f** (0.5 g) and PPA (10 g) was sonicated in a thermostated bath at 35 °C for 8−20 h (monitored by TLC or HPLC). The reaction was quenched with an ice-cold solution of Na_2CO_3 and extracted repetitively with EtOAc (3 × 25 mL). The combined organic layers were dried with $MgSO_4$, concentrated in vacuo and purified by flash chromatography.

2,3-Bis(4-methoxyphenyl)-1-methyl-1*H*-indole (3c): This compound was obtained as a white solid (0.26 g, 55%). − M.p. 127−128 °C (diethyl ether/hexane). − R_f = 0.56 (EtOAc/hexane, 1:4). − ^1H NMR (200 MHz, $CDCl_3$): δ = 3.67 (s, 3 H, CH_3), 3.81 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 6.85 (d, J = 8.8 Hz, 2 H, ArH), 6.93 (d, J = 8.8 Hz, 2 H, ArH), 7.25 (m, 6 H, 4ArH, 5-H, 6-H), 7.39 (d, J = 8.3 Hz, 1 H, 7-H), 7.76 (d, J = 7.7 *Hz*, 1 H, 4-H). − $C_{23}H_{21}NO_2$ (343.4): calcd. C 80.44, H 6.16, N 4.08; found C 80.16, H 6.25, N 3.96.

1-Ethyl-2,3-bis(4-methoxyphenyl)-1*H*-indole (3d): This compound was obtained as a white solid (0.25 g, 50%). − M.p. 98−99 °C (diethyl ether/hexane). − R_f = 0.56 (EtOAc/hexane 1:4). − ^1H NMR (400 MHz, $CDCl_3$) δ = 1.35 (t, J = 7.1 Hz, 3 H, CH_2CH_3), 3.82 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 4.14 (q, J = 7.1 Hz, 2 H, CH_2CH_3), 6.85 (d, J = 8.7 Hz, 2 H, ArH), 6.95 (d, J = 8.7 Hz, 2 H, ArH), 7.15−7.32 (m, 6 H, 4 ArH, 5-H, 6-H), 7.44 (d, J = 8.3 Hz, 1 H, 7-H), 7.79 (d, J = 7.9 Hz, 1 H, 4-H). − $C_{24}H_{23}NO_2$ (357.4): calcd. C 80.64, H 6.49, N 3.92; found C 80.51, H 6.55, N 3.81.

6-Methoxy-2,3-bis(4-methoxyphenyl)-1-methyl-1*H*-indole (3e): This compound was obtained as a white solid (0.33 g, 69%). − M.p. 139−140 °C (diethyl ether/hexane). − R_f = 0.31 (EtOAc/hexane, 1:4). − ^1H NMR (400 MHz, $CDCl_3$): δ = 3.63 (s, 3 H, CH_3), 3.76 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 6.82 (m, 4 H, 2 ArH, 7-H, 5-H), 6.89 (d, J = 8.8 Hz, 2 H, ArH), 7.19 (d, J = 8.7 Hz, 2 H, ArH), 7.21 (d, J = 8.7 Hz, 2 H, ArH), 7.59 (dd, J = 8.2, 0.9 Hz, 1 H, 4-H). − $C_{24}H_{23}NO_3$ (373.4): calcd. C 77.19, H 6.21, N 3.75; found C 76.93, H 6.35, N 3.91.

1-Ethyl-6-methoxy-2,3-bis(4-methoxyphenyl)-1*H*-indole (3f): This compound was obtained as a white solid (0.29 g, 60%). − M.p. 111−112 °C (diethyl ether/hexane). − R_f = 0.31 (EtOAc/hexane, 1:4). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.35 (t, *J* = 7.1 Hz, 3 H, CH_2CH_3), 3.78 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 3.93 (s, 3 H, OCH_3), 4.14 (q, *J* = 7.1 Hz, 2 H, CH_2CH_3), 6.85 (m, 4 H, 2 ArH, 7-H, 5-H), 6.91 (d, *J* = 8.7 Hz, 2 H, ArH), 7.20 (d, *J* = 8.7 Hz, 2 H, ArH), 7.24 (d, *J* = 8.7 Hz, 2 H, ArH), 7.60 (dd, *J* = 8.2, 1.0 Hz, 1 H, 4-H). − $C_{25}H_{25}NO_3$ (387.5): calcd. C 77.49, H 6.50, N 3.61; found C 77.43, H 6.66, N 3.53.

General Procedure for the Deprotection of Phenols: To a stirred solution of indoles **3c−f** (0.35 mmoL) in CH_2Cl_2 (5 mL), at −78 °C was added a solution of BBr_3 as a 1 N solution in CH_2Cl_2 (1.5 mL, 1.5 mmoL). The reaction mixture was allowed to warm to room temp. and stirred for 16−24 h. After quenching by addition of H_2O (5 mL), the layers were separated, and the aqueous layer was extracted with EtOAc (3×10 mL). The combined organic layers were dried with $MgSO_4$, concentrated under reduced pressure and purified by flash chromatography.

2,3-Bis(4-hydroxyphenyl)-1-methyl-1*H*-indole (4c): This compound was obtained as a yellow solid (108 mg, 98%). − M.p. 230−232 °C (diethyl ether). − R_f = 0.68 (EtOAc/hexane, 1:1). − IR (neat): $\tilde{\nu}$ = 3343 cm^{-1} (OH). − ^{1}H NMR (200 MHz, [D_6]acetone): δ = 3.66 (s, 3 H, CH_3), 6.77 (d, *J* = 8.8 Hz, 2 H, ArH), 6.90 (d, *J* = 8.8 Hz, 2 H, ArH), 7.07−7.28 (m, 6 H, 4ArH, 5-H, 6-H), 7.44 (d, *J* = 8.1 Hz, 1 H, 7-H), 7.63 (d, *J* = 7.7 Hz, 1 H, 4-H), 8.4 (s, 2 H, OH). − $C_{21}H_{17}NO_2$ (315.4): calcd. C 79.98, H 5.43, N 4.44; found C 80.18, H 5.31, N 4.63.

1-Ethyl-2,3-bis(4-hydroxyphenyl)-1*H*-indole (4d): This compound was obtained as a yellowish solid (110 mg, 95%). − M.p. 198−199 °C (diethyl ether). − R_f = 0.69 (EtOAc/hexane 1:1). − IR: $\tilde{\nu}$ = 3343 cm^{-1} (OH). − ^{1}H NMR (200 MHz, [D_6]acetone): δ = 1.38 (t, *J* = 7.1 Hz, 3 H, CH_2CH_3), 4.19 (q, *J* = 7.1 Hz, 2 H, CH_2CH_3), 6.80 (d, *J* = 8.8 Hz, 2 H, ArH), 6.92 (d, *J* = 8.8 Hz, 2 H, ArH), 7.05−7.30 (m, 6 H, 4ArH, 5-H, 6-H), 7.45 (d, *J* = 8.1 Hz, 1 H, 7-H), 7.65 (d, *J* = 7.7 Hz, 1 H, 4-H), 8.5 (s, 2 H, OH). − $C_{22}H_{19}NO_2$ (329.4): calcd. C 80.22, H 5.81, N 4.25; found C 80.50, H 5.96, N 4.31.

2,3-Bis(4-hydroxyphenyl)-1-methyl-1*H*-indol-6-ol (4e): This compound was obtained as an orange solid (107 mg, 92%). − M.p. 129−130 °C (diethyl ether). − R_f = 0.40 (EtOAc/hexane, 1:1). − IR (neat): $\tilde{\nu}$ = 3346.5 cm^{-1} (OH). − ^{1}H NMR (400 MHz, [D_6]acetone): δ = 3.75 (s, 3 H, CH_3), 6.72 (dd, *J* = 8.5, 2.2 Hz, 1 H, 5-H), 6.77 (d, *J* = 8.7 Hz, 2 H, ArH), 6.85 (d, *J* = 2.1 Hz, 1 H, 7-H), 6.89 (d, *J* = 8.6 Hz, 2 H, ArH), 7.11 (d, *J* = 8.6 Hz, 2 H, ArH), 7.17 (d, *J* = 8.6 Hz, 2 H, ArH), 7.45 (d, *J* = 8.5 Hz, 1 H, 4-H), 8.08 (s, 1 H, OH), 8.21 (s, 1 H, OH), 8.62 (s, 1 H, OH). − $C_{21}H_{17}NO_3$ (331.4): calcd. C 76.12, H 5.17, N 4.23; found C 76.41, H 5.35, N 4.45.

1-Ethyl-2,3-bis(4-hydroxyphenyl)-1*H*-indol-6-ol (4f): This compound was obtained as pale orange crystals (109 mg, 90%). − M.p. 124−125 °C (diethyl ether). − R_f = 0.41 (EtOAc/hexane 1:1). − IR (neat): $\tilde{\nu}$ = 3347 cm^{-1} (OH). − ^{1}H NMR (400 MHz, [D_6]acetone): δ = 1.38 (t, *J* = 7.2 Hz, 3 H, CH_2CH_3), 4.17 (q, *J* = 7.2 Hz, 2 H, CH_2CH_3), 6.70 (dd, *J* = 8.5, 2.2 Hz, 1 H, 5-H), 6.76 (d, *J* = 8.7 Hz, 2 H, ArH), 6.83 (d, *J* = 2.1 Hz, 1 H, 7-H), 6.85 (d, *J* = 8.6 Hz, 2 H, ArH), 7.10 (d, *J* = 8.6 Hz, 2 H, ArH), 7.15 (d, *J* = 8.6 Hz, 2 H, ArH), 7.43 (d, *J* = 8.5 Hz, 1 H, 4-H), 8.07 (s, 1 H, OH), 8.20 (s, 1 H, OH), 8.60 (s, 1 H, OH). − $C_{22}H_{19}NO_3$ (345.4): calcd. C 76.50, H 5.54, N 4.06; found C 76.77, H 5.61, N 4.32.

Determination of the Estrogen Receptor Binding Affinity (RBA): Relative binding measurements were performed as previously reported,[20] using lamb uterine cytosol, diluted to ca. 1.5 nM receptor. The protein solution was incubated with buffer or several concentrations of unlabeled competitor together with 10 nM [^{3}H]estradiol at 0 °C for 18−24 h. The free ligand was removed by adsorption to dextran-coated charcoal. Unlabeled competitors were prepared and serially diluted in dimethylformamide/TEA buffer [1:1 (v/v) 10 mM Tris, 1.5 mM EDTA, 3 mM sodium azide, pH = 7.4 at 25 °C] to ensure solubility. All data are reported relative to estradiol = 100%.

UV/Vis and Fluorescence Spectra: UV/Vis spectra were recorded with a Jasco V-550 spectrophotometer. Fluorescence spectra were acquired by photon counting with a Jobin-Yvon Fluorolog-3 spectrophotometer. All spectra were recorded at room temp. and are corrected for phototube sensitivity and the solvent background was subtracted. Excitation was at the wavelength of maximum absorbance. Samples were prepared from a stock solution (10^{-3} M) of the corresponding compound in EtOH, with a final concentration of 5×10^{-6} M. Acidic or basic solutions were prepared by addition of 6 N HCl or 6 N KOH solution in water, to give a final concentration of 0.1 N.

Acknowledgments

Support of this research through a grant from the Board of Research of the Agricultural University of Athens (gr. No 23.02.306) is gratefully acknowledged. We thank Kathryn E. Carlson for the estrogen receptor binding measurements. We also thank Dr George Mousdis for his help with physical measurements.

[1] A. S Waggoner in *Applications of Fluorescence in the Biomedical Sciences* (Eds.: P. L. Taylor, A. S. Waggoner, R. F. Murphy, F. Lanni, R. R.Birge), Alan Liss, New York, **1986**.
[2] D. L. Rousseau, *Optical techniques in Biological Research*, Academic Press, New York, **1984**, chapter 4.
[3] C. Mazur Bowen, J. A Katzenellenbogen, *J. Org. Chem.* **1997**, *62*, 7650 and references therein.
[4] N. T. Van, M., Raber, G. H. Barrows, B. Barlogie, *Science* **1984**, *224*, 876.
[5] L. P. Pertschuk, K. B. Eisenberg, A. C. Carter, J. G. Feldman, *Breast Cancer Res. Treat.* **1985**, *5*, 137.
[6] C. G. Castles, S. A. W. Fuqua, "Alterations within the estrogen receptor in breast cancers" in *Hormone Dependent Breast Cancer* (Eds.: J. R Pasqualini, B. S. Katzenellenbogen), Marcell Dekker, New York, **1996**, chapter 3, pp. 81−105 and references cited therein.
[7] For examples of estrogen−fluorophore conjugates see: [7a]T. L. Fevig, J. E. Lloyd, J. A. Zablocki, J. A. Katzenellenbogen, *J. Med. Chem.* **1987**, *30*, 156. − [7b] A. N. French, S. R. Wilson, M. J. Welch, J. A. Katzenellenbogen, *Steroids* **1993**, *58*, 157.
[8] For examples of inherently fluorescent estrogens see: [8a] G. M. Anstead, J. L. Ensing, C. S. Peterson, J. A. Katzenellenbogen, *J. Org. Chem.* **1989**, *54*, 1485. − [8b] G. M. Anstead, S. R. Wilson, J. A. Katzenellenbogen, *J. Med. Chem.* **1989**, *32*, 2163. − [8c] G. M. Anstead, C. S. Peterson, J. A. Katzenellenbogen, *J. Steroid. Biochem.* **1989**, *33*, 877. − [8d] K.-J. Hwang, K E. Carlson, G. M. Anstead, J. A Katzenellenbogen, *Biochemistry* **1992**, *31*, 11536. − [8e] R. S. Miksicek, K E. Carlson, K. − J. Hwang, J. A. Katzenellenbogen, *Mol. Endocrinol.* **1995**, *9*, 592.
[9] For examples of photofluorogenic estrogens see: [9a] R. D. Bindal, J. A. Katzenellenbogen, *J. Steroid. Biochem.* **1985**, *23*, 929. − [9b] R. D. Bindal, J. A. Katzenellenbogen, *Photochem. Photobiol.* **1995**, *9*, 592. − [9c] A. W. Scribner, S. A. Haroutounian, K. E. Carlson, J. A. Katzenellenbogen, *J. Org. Chem.* **1997**, *62*, 1043.
[10] G. W. Gribble in *Second Supplements to the 2nd Edition of Rodd's Chemistry of Carbon Compounds*, vol. IV [*Heterocyclic*

Compounds, pt. B (Ed.: M. Sainsbury)], Elsevier Pub.; Amsterdam, **1997**, pp. 69−164.
[11] R. J. Sunberg, *Indoles*, Academic Press, London, **1996**.
[12] E. von Angerer, J. Prekajac, J. Strohmeier, *J. Med. Chem.* **1984**, *27*, 1439.
[13] For a recent review on indole synthesis see: G. W. Gribble, *J. Chem. Soc., Perkin Trans. 1* **2000**, 1045.
[14] D. L. Hughes, *Org. Prep. Proced. Int.* **1993**, *25*, 607.
[15] M. T. Baumgartner, M. A. Nazareno, M. C. Murguia, A. B. Pierini, R. A. Rossi, *Synthesis* **1999**, 2053, and references therein.
[16] B. E. Fink, D. S. Mortensen, S. R. Stauffer, Z. D. Aron, J. A. Katzenellenbogen, *Chem. Biol.* **1999**, *6*, 205.
[17] A. C. Stevens, R. P. Frutos, D. F. Harvey, A. A. Brian, *Bioconj. Chem.* **1993**, *4*, 19.
[18] J. F. Ireland, A. H. Wyatt, *Adv. Phys. Org. Chem.* **1976**, *12*, 131.
[19] J. R. Lakowicz, in *Principles of Fluorescence Spectroscopy*, Plenum Press, New York, **1983**.
[20] J. A. Katzenellenbogen, H. J. Johnson, H. N. Meyers, *Biochemistry* **1973**, *12*, 4085.

Received September 18, 2000
[O00482]