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Ultrasound Promoted Synthesis of 2,3-*bis*-(4-Hydroxyphenyl)-indoles as Inherently Fluorescent Ligands for the Estrogen Receptor

Sofia D. Koulocheri and Serkos A. Haroutounian*

Chemistry Laboratory, Agricultural University of Athens, Iera odos 75, Athens 11855, Greece
Tel.: + 30-1-5294247, Fax: + 30-1-5294265, E-mail: sehar@aua.gr

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Abstract: A series of 2,3-*bis*-(4-hydroxyphenyl)-indoles (**4c-f**) was prepared by ultrasound promoted intramolecular cyclodehydration of a -anilinyl (or *m*-anisidyl)-desoxyanisoins (**2c-f**) and their optical spectroscopy and receptor binding properties have been investigated. These compounds showed intense long wavelength fluorescent emission which is sensitive to solvent polarity and pH. However, their binding affinities to ER is modest, except of 2,3-*bis*-(4-hydroxy-phenyl)-1-methyl-1*H*-indol-6-ol (**4e**) which has reasonably good affinity and may be used for further development.

Keywords: indoles; ultrasound; fluorescent probes; estrogen receptor binding.

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-based assay of the estrogen receptors (ER).[3] Such 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] providing useful information for the prediction of responsiveness 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).

Todate, a broad variety of compounds which may be classified in three major classes of fluorescent estrogenic probes have been synthesized: 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 to synthesize and study the photophysical and binding properties of novel 2,3-*bis*-(4-hydroxyphenyl)-indole derivatives.

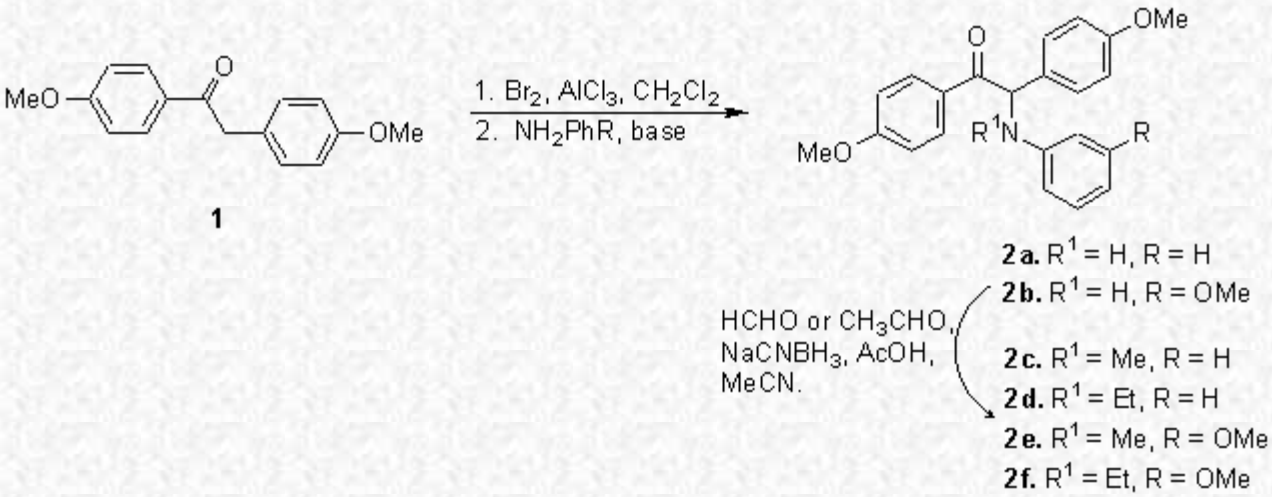
The indole ring belongs to an important class of compounds and natural products[10] with pronounced pharmacological activity,[11] and their donor-acceptor character (provided by the nitrogen and hydroxyl functionalities) makes them potential candidates for use as biological probes. Since 3-alkyl-2-phenyl-indoles

have been found to possess strong estrogenic and tumor inhibition activities,[12] we were envisioned to enhance their absorption and emission properties by replacing the alkyl with a 4-hydroxyphenyl group and study the consequences that this would have on their photophysical properties and binding affinity to ER.

Results and Discussion

Chemistry

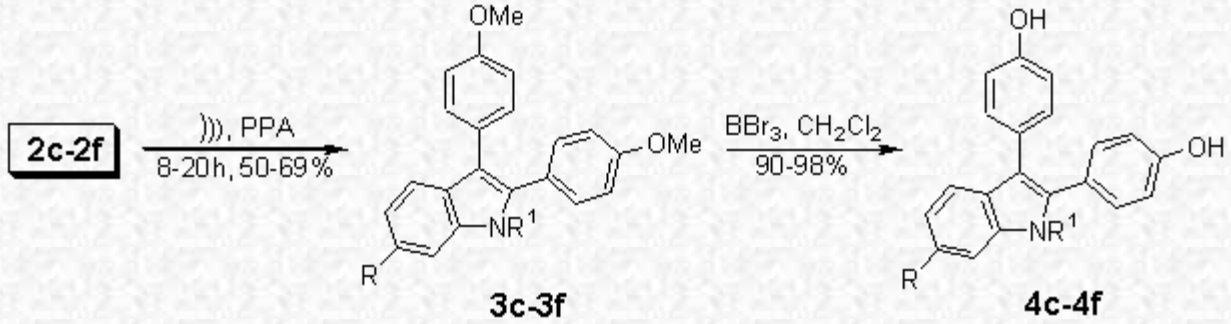
Of the numerous methods developed for the synthesis of indoles the most widely used are the Fischer[13] and Bischler[14] methods. In this report we present a modification of the Bischler method by using ultrasound as promoter for the acid catalyzed intramolecular cyclodehydration of the a -anilinyl (or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



Scheme 1

The synthesis of the a -amino-desoxyanisoin substrates (**2a,b**) is illustrated in Scheme 1. More specifically, a solution of the 2-bromo derivative of desoxyanisoin (prepared by a known procedure)[15] in methylene chloride upon treatment with aniline in the presence of triethylamine produced smoothly the desired a -anilinyl-desoxyanisoin **2a**. In the case of *m*-anisidine however, no product was obtained when triethylamine or pyridine were used as base. The problem was circumvented by the use of 2,6-lutidine along with a catalytic amount of NaI in DMA and the desired a -(*m*-anisidyl)-desoxyanisoin **2b** was obtained in good yield. Finally, reductive alkylation of amines **2a,b** provided effectively the substrates **2c-f**.

A broad variety of acidic conditions have been used for the transformation of α-arylamino ketones to indoles (Bischler method). Typical conditions to effect this cyclodehydration reactions consist of heating an acidic solution of the substrate for several hours or days. The yield of the product (indole) depends strongly on the nature of the substrate. In our case, the use of a variety of acidic environments and temperatures resulted in poor or moderate yields of transformation and very long reaction times (Table 1). Best results were obtained at 60 ° C with PPA as acid, under mechanical stirring and 2-4 days of reaction. This led us to consider the application of ultrasound as low temperature, promoter of the above reaction. Repetition of the reaction using compounds **2c-f** as substrates and PPA as acid in an ultrasound bath and room temperature led to a rapid cyclodehydration (complete in 8-18 h). This ultrasound promoted modification of the classical Bischler method worked satisfactory in all substrates, providing high yields of the indole products (**3c-f**). Furthermore, there is no indication for the formation of resinous side products. In the case of *m*-anisidyl derivatives however, the yield of the reaction was notably higher, presumably because the presence of a methoxy-group activates its ortho-position facilitating thus the cyclodehydration reaction. Finally, deprotection was achieved using BBr_3 in methylene chloride to give the desired products in excellent yields.



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

Table 1

Reactant	Acid	Temperature	Time	Yield (%)
	PPA	60 ° C	4 days	40–45
	CF_3COOH	r.t.	8 h	15–20
2c, 2d	HCl	r.t.	10h	28–35
	H_2SO_4	r.t.	7 h	25–30
	MsOH	40 ° C	3 days	20–30 [a]
	CSA	60 ° C	3 days	5–10[a]
	TosH	60 ° C	3 days	5–10[a]
	PPA,)))	r.t.	18 h	50–55
	PPA	60 ° C	2 days	50–54

	CF_3COOH	r.t.	3 h	20–28
2e, 2f	HCl	r.t.	3.5h	33–37
	H_2SO_4	r.t.	2.5 h	27–35
	MsOH	40 ° C	3 days	25-35[a]
	CSA	60 ° C	3 days	5–10[a]
	TosH	60 ° C	3 days	5–10[a]
	PPA,)))	r.t.	8 h	60–69

a 25-35% of starting material was recovered.

Absorption and Fluorescence Properties

The ultraviolet/visible absorbance spectra of indoles **4c-f** were measured in various aprotic and protic solvents under neutral, acidic and basic conditions. The results of the measurements are summarized in Table 2. Comparison of the absorption bands of indoles in different solvents indicates that they have only limited solvatochromicity in absorbance 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) comparing to the unsubstituted ones. In base however, these compounds showed a new, rather intense, longer wavelength band (*ca* 340 nm).

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

Compd	**Condition**[a]			λ_{abs}^{max} (ε)	
		THF	**CH_3CN**	**EtOH**	**H_2O**
4c	neutral	240 (12,100)	250 (29,300)	255 (25,100)	250 (16,400)
	acid	308 (16,000)	308 (15,800)	302 (15,200)	306 (8,200)
	basic	239 (12,400)	255 (28,100)	255 (21,500)	248 (15,500)
		310 (20,300)	278 (17,700)	302 (11,900)	305 (7,500)
		b	314 (14,700)	267 (25,600)	268 (20,400)
			b	330 (8,200)	308 (13,000)
	neutral	238 (12,600)	245 (24,100)	253 (31,300)	251 (25,200)
	acid	308 (22,500)	310 (12,400)	289 (14,500)	300 (13,900)

4d	basic	235 (12,500) 308 (16,400) b	246 (23,500) 251[c] (21,280) 276 (16,700) 312 (10,800) b	302 (14,500) 250 (51,700) 302 (31,200) 269 (56,100) 304 (35,200)	245 (26,100) 304 (11,200) 269 (32,700) 307 (17,600)
4e	neutral acid basic	241 (10,300) 318 (14,300) 238 (8,500) 321 (8,200) b	250 (11,000) 317 (6,300) 244 (14,500) 317 (7,100) b	253 (24,300) 306 (12,200) 251 (35,500) 310 (19,900) 261 (50,400) 304 (28,900) 339 (22,600)	250 (29,600) 307 (16,300) 254 (29,600) 275 (20,100) 303 (14,200) 268 (23,700) 317 (9,800)
4f	neutral acid basic	242 (10,100) 320 (14,700) 239 (8,900) 322 (10,500) b	242 (11,600) 310 (5,300) 240 (15,200) 310 (6,200) b	258 (33,300) 296 (20,500) 330 (9,600) 251 (35,000) 297 (21,400) 329 (9,500) 267 (69,100)	244 (25,400) 306 (11,200) 245 (23,800) 309 (9,700) 264 (26,600) 302 (16,200) 340 (15,100)

				304 (37,700) 346 (24,300)	

a Acid = 0.1 N HCl, Base = 0.1 N KOH.
[b]Not Soluble
[c]Shoulder

The suitability of fluorescent probes for biological systems, however, often depends on the extend to which their fluorescent properties are sensitive to their environment (solvent and pH).[16] Thus, we have investigated the effect of pH (acidic, basic and neutral) and solvent polarity (tetrahydrofuran, acetonitrile, ethanol and water) on the fluorescence emission maximum and intensity of compounds **4c-f.** Complete results are given in Table 3. It is evident that the fluorescence emission of these compounds is solvent and pH dependent, indicating that there is a large dipole moment in the excited state.[17] Thus, the emission maximum under neutral conditions is shifted to the red in more polar protic solvents. On the other hand, in each solvent there is an additional red shift upon going from neutral to acidic and basic conditions, because the formation of the ionic form of the molecules is facilitated in the excited state.[18]

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

Compd.	**Condition**[a]			λ_{em}^{max} **(relative intensity)**[b]		
		THF		**CH_3CN**	**EtOH**	**H_2O**
4c	neutral acid basic	430 (70) 425 (77) 444[c] (68) d		431 (57) 430 (54) 444[c] (46) d	430 (80) 435 (66) 480 (32)	438 (62) 451 (84) 486 (23)
4d	neutral acid basic	427 (48) 429 (53) 443[c] (49) d		437 (50) 431 (40) 466[c] (36) d	428 (57) 426 (82) 440 (87) 485 (30)	456 (50) 446 (62) 486 (16)
4e	neutral acid basic	428 (36) 400[c] (33) 427 (37) d		428 (36) 400[c] (23) 434 (28) d	427 (37) 443 (38) 449 (46)	448 (38) 466 (44) 450 (7.5)
	neutral acid	435 (29) 450[c] (22)		407 [c] (22) 435 (31)	427 (37) 438 (46) 454[c]	444 (36) 467 (48)

4f	basic	410 (31) 430 (35) 450 (24) d	408 [c] (23) 430 (24) d	(40) 450 (39)	445 (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 (x 10^4 cps) at l_{em}^{max}.
[c] Shoulder
[d] Not Soluble

Estrogen Receptor Binding Affinity (RBA)

The estrogen RBA's 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%.

Even though there are differences in their affinity, most of the new compounds (except **4d,e**) showed limited affinity for ER (i.e., less than 1% that of estradiol). The affinity of 6-hydroxy substituted derivative **4e**, however, is sufficient for its use in biological assays.

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

Compd.	R	R^1	R^2	RBA
3c	H	Me	OMe	<0.01%
3b	H	Et	OMe	nd
3e	OMe	Me	OMe	<0.01%
3f	OMe	Et	OMe	0.042
4c	H	Me	OH	0.408
4d	H	Et	OH	1.124
4e	OH	Me	OH	3.396
4f	OH	Et	OH	nd

ndNot determined

Conclusion

In conclusion, we have described an efficient ultrasound promoted synthesis of several 2,3-*bis*-(4-hydroxyphenyl)-indoles (**4c-f**) and presented an investigation of their photophysical and receptor binding properties. Their absorbance and fluorescence properties show strong dependence on solvent polarity and pH, showing potentials for use as fluorescent probes. On the other hand, their receptor binding affinities are limited. However, 2,3-*bis*-(4-hydroxyphenyl)-1-methyl-1*H*-indol-6-ol (**4e**) is a moderate affinity ER ligand, with good photophysical properties and its synthesis does not require stereospecific methods or complicated isomer separations. Thus, is an attractive candidate for further development and research towards the design and synthesis of novel derivatives containing a second heterocyclic ring is currently underway.

Experimental

Chemistry

Melting points were determined on a Buchi melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 400 or 200 MHz using Bruker DRX-400 or Bruker AC 200 spectrometers respectively. IR spectra were obtained on a Nicolet Magna 750, series II spectrometer. Ultrasound reactions were performed using a Bandelin Sonorex Super RK 100 SH ultrasound bath. HPLC separations were performed using a Hewlett Packard 1100 series instrument with a variable wavelength UV detector and coupled to HP Chem. Station utilizing the manufactorer's 5.01 software package. TLC was conducted on Merck glass plates coated with silica gel 60 F_{254}. Flash column chromatography was performed using Merck silica gel 60 (230-400 mesh ASTM). When analyses

were indicated by symbols of the elements, analytical results obtained for those elements were ±0.37% of the theoretical values.

All solvents were dried by distillation prior to use. Starting materials were purchased from Aldrich (analytical reagent grades) and used without further purification. 2-Bromo-1,2-*bis*-(4-methoxy-phenyl)-ethanone was prepared in 78% yield according to a literature procedure.[15] All compounds were purified by semi-preparative HPLC before the photophysical experiments and RBA assays [Column: Kromasil 10-5C18 (25 cm x 10 mm); Mobile phase: CH_3CN; Detector: UV l 300 nm; Flow: 1mL/min; Load: 3 mg/100 m L solution in mobile phase].

1,2-*bis*-(4-Methoxy-phenyl)-2-phenylamino-ethanone (2a). To a stirred solution of 2-bromo-1,2-*bis*-(4-methoxy-phenyl)-ethanone (2.5 g, 7.46 mmol) and triethylamine (1.3 mL, 9 mmol) in anhydrous CH_2Cl_2 (10 mL) and argon atmosphere, was added aniline (0.8 mL, 9 mmol). The reaction was run at room temperature for 1h, 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 over $MgSO_4$ and concentrated under reduced pressure to give a yellowish solid. Flash chromatographic purification (EtOAc/hexane 1:4, R_f 0.32) and recrystallization from diethylether furnished 2.1g of the yellow title product (80%); mp 113 – 114 ° C; IR (KBr) = 3406 cm^{-1}(N-H), 1672 (C=O); ^{1}H NMR (200 MHz, $CDCl_3$) d 8.01 (d, *J* = 8.9 Hz, 2H, ArH), 7.37 (d, *J* = 8.7 Hz, 2H, ArH), 7.13 (m, 2H, ArH), 6.91 (d, *J* = 8.9 Hz, 2H, ArH), 6.82 (d, *J* = 8.9 Hz, 2H, ArH), (m, 3H, ArH), 5.94 (s, 1H, H-2), 5.38 (s, 1H, NH), 3.84 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3); Anal. ($C_{22}H_{21}NO_3$) C,H,N.

1,2-*bis*-(4-Methoxy-phenyl)-2-(3-methoxy-phenylamino)-ethanone (2b). A solution of 2-bromo-1,2-*bis*-(4-methoxy-phenyl)-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) and argon atmosphere was heated to 50 ° C and stirred for 4h. 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 over $MgSO_4$ and concentrated. Flash chromatographic purification (EtOAc/hexane 1:4, R_f 0.21) and recrystallization from diethylether furnished 2.4g of **2b** (85%) as a yellow solid; mp 112 – 113 ° C; IR (KBr) = 3415 cm^{-1} (N-H), 1680 (C=O); ^{1}H NMR (200 MHz $CDCl_3$) d 8.01 (d, *J* = 8.8 Hz, 2H, ArH), 7.37 (d, *J* = 8.6 Hz, 2H, ArH), 7.03 (m, 2H, ArH), 6.91 (d, *J* = 9.1 Hz, 2H, ArH), 6.82 (d, *J* = 8.8 Hz, 2H, ArH), 6.26 (m, 3H, ArH), 5.93 (d, *J* = 6.2 Hz, 1H, H-2), 5.40 (d, *J* = 6.2 Hz, 1H, NH), 3.85 (s, 3H, OCH_3), 3.73 (s, 6H, OCH_3); Anal. ($C_{23}H_{23}NO_4$) C,H,N.

General procedure for the reductive alkylation of amines.

To a stirred solution of amine (14.8 mmol) and corresponding aldehyde (29.6 mmol) in MeCN (10 mL) were added $NaCNBH_3$ (29.6 mmol) and acetic acid (0.12 mL). The reaction was run for 2–4 h (monitored by TLC) and then concentrated under reduced pressure. The residue was dissolved in diethylether (15mL) and extracted with a saturated solution of $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$, concentrated and purified by flash chromatography.

1,2-*bis*-(4-Methoxy-phenyl)-2-(methyl-phenyl-amino)-ethanone (2c). This compound was obtained as a pale yellow solid (87 %); R_f 0.46 (EtOAc/hexane 1:4); mp 102 – 103 ° C (diethylether/hexane); IR (KBr) = 3323 cm^{-1} (N-H), 1673 (C=O); ^{1}H NMR (200 MHz $CDCl_3$) d 7.91 (d, *J* = 9.2 Hz, 2H, ArH), 7.15 – 7.27 (m, 4H, ArH), 6.86 (d, *J* = 8.7 Hz, 4H, ArH), 6.71 (m, 3H, ArH), 6.35 (s, 1H, H-2), 3.84 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 2.87 (s, 3H, CH_3); Anal. ($C_{23}H_{23}NO_3$) C,H,N.

2-(Ethyl-phenyl-amino)-1,2-*bis*-(4-methoxy-phenyl)-ethanone (2d). This compound was obtained as a yellow solid (83 %); R_f 0.46 (EtOAc/hexane 1:4); mp 125 – 127 ° C (diethylether/hexane); IR (KBr) = 3327 cm^{-1} (N-H), 1680 (C=O); ^{1}H NMR (400 MHz $CDCl_3$) d 7.90 (d, *J* = 9.1 Hz, 2H, ArH), 7.19 (m, 4H, ArH), 6.86 (d, *J* = 8,7 Hz, 4H, ArH), 6.71 (m, 3H, ArH), 6.34 (s, 1H, H-2), 3.82 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.40 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 0.85 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); Anal. ($C_{24}H_{25}NO_3$) C,H,N.

1,2-*bis*-(4-Methoxy-phenyl)-2-[(3-methoxy-phenyl)-methyl-amino]-ethanone (2e). This compound was obtained as a yellowish solid (85%); R_f 0.35 (EtOAc/hexane 1:4); mp 130 – 131 ° C (diethylether/hexane); IR (KBr) = 3320 cm^{-1} (N-H), 1679 (C=O); ^{1}H NMR (400 MHz $CDCl_3$) d 7.90 (d, *J* = 9.1 Hz, 2H, ArH), 7.12 (m, 3H, ArH), 6.86 (dd, *J* = 8.7, 3.7 Hz, 4H, ArH), 6.38 (dd, *J* = 8.3, 2.1 Hz, 1H, ArH), 6.32 (m, 3H, ArH, H-2), 3.82 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3) 2.82 (s, 3H, CH_3); Anal. ($C_{24}H_{25}NO_4$) C,H,N.

2-[Ethyl-(3-methoxy-phenyl)-amino]-1,2-*bis*-(4-methoxy-phenyl)-ethanone (2f). This compound was obtained as off-white crystals (77 %); R_f 0.37 (EtOAc/hexane 1:4); mp 141 – 142 ° C (diethylether/hexane); IR (KBr) = 3340 cm^{-1} (N-H), 1682 (C=O); ^{1}H NMR (400 MHz $CDCl_3$) d 7.89 (d, *J* = 8.7 Hz, 2H, ArH), 7.17 (d, *J* = 8.7 Hz, 2H, ArH), 7.07 (m, 1H, ArH), 6.85 (d, *J* = 8.3 Hz, 2H, ArH), 6.85 (d, *J* = 8.3 Hz, 2H, ArH), 6.32 (s, 1H, H-2), 6.27 – 6.34 (m, 3H, ArH), 3.81 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.38 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 0.84 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); Anal. ($C_{25}H_{27}NO_4$) C,H,N.

General procedure for the cyclization of ketones. A mixture of a-anilinyl (or m-anisidyl)-desoxyanisoins **2c**-**2f** (0.5 g) and PPA (10g) was sonicated at room temperature for 8-20h (monitored by TLC). Then the reaction was quenched with an ice-cold solution of Na_2CO_3 and extracted repetitively with EtOAc (3 x 25mL). The combined organic layers were dried over $MgSO_4$, concentrated *in vacuo* and purified by flash chromatography.

2,3-*bis*-(4-Methoxy-phenyl)-1-methyl-1*H*-indole (3c). This compound was obtained as a white solid (55%); R_f 0.56 (EtOAc/hexane 1:4); mp 127 – 128 ° C (diethylether/hexane); ^{1}H NMR (200 MHz $CDCl_3$) d 7.76 (d, *J* = 7.7 *Hz*, 1H, H-4), 7.39 (d, *J* = 8.3 Hz, 1H, H-7), 7.25 (m, 6H, 4ArH, H-5, H-6), 6.93 (d, *J* = 8.8 Hz, 2H, ArH), 6.85 (d, *J* = 8.8 Hz, 2H, ArH), 3.85 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.67 (s, 3H, CH_3); Anal. ($C_{23}H_{21}NO_2$) C,H,N.

1-Ethyl-2,3-*bis*-(4-methoxy-phenyl)-1*H*-indole (3d). This compound was obtained as a white solid (50%); R_f 0.56 (EtOAc/hexane 1:4); mp 98 – 99 ° C (diethylether/hexane); ^{1}H NMR (400 MHz $CDCl_3$) d 7.79 (d, *J* = 7.9 Hz, 1H, H-4), 7.44 (d, *J* = 8.3 Hz, 1H, H-7), 7.15-7.32 (m, 6H, 4ArH, H-5, H-6), 6.95 (d, *J* = 8.7 Hz, 2H, ArH), 6.85 (d, *J* = 8.7 Hz, 2H, ArH), 4.14 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 3.87 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.67 (s, 3H, CH_3), 1.35 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); Anal. ($C_{24}H_{23}NO_2$) C,H,N.

6-Methoxy-2,3-*bis*-(4-methoxy-phenyl)-1-methyl-1*H*-indole (3e). This compound was obtained as a white solid (69%); R_f 0.31 (EtOAc/hexane 1:4);

mp 139 – 140 ° C (diethylether/hexane); H NMR (400 MHz $CDCl_3$) d 7.59 (dd, *J* = 8.2, 0.9 Hz, 1H, H-4), 7.21 (d, *J* = 8.7 Hz, 2H, ArH), 7.19 (d, *J* = 8.7 Hz, 2H, ArH), 6.89 (d, *J* = 8.8 Hz, 2H, ArH), 6.82 (m, 4H, 2ArH, H-7, H-5), 3.91 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.63 (s, 3H, CH_3); Anal. ($C_{24}H_{23}NO_3$) C,H,N.

1-Ethyl-6-methoxy-2,3-*bis*-(4-methoxy-phenyl)-1*H*-indole (3f). This compound was obtained as a white solid (60 %); R_f 0.31 (EtOAc/hexane 1:4); mp 111 – 112 ° C (diethylether/hexane); ^{1}H NMR (200 MHz $CDCl_3$) d 7.60 (dd, *J* = 8.2, 1.0 Hz, 1H, H-4), 7.24 (d, *J* = 8.7 Hz, 2H, ArH), 7.20 (d, *J* = 8.7 Hz, 2H, ArH), 6.91 (d, *J* = 8.7 Hz, 2H, ArH), 6.85 (m, 4H, 2ArH, H-7, H-5), 4.14 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 3.93 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 1.35 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); Anal. ($C_{25}H_{25}NO_3$) C,H,N.

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

2,3-*bis*-(4-Hydroxy-phenyl)-1-methyl-1*H*-indole (4c). This compound was obtained as a yellow solid (98%); R_f 0,68 (EtOAc/hexane 1:1); mp 230 – 232 ° C (diethylether); IR (KBr) = 3343 cm^{-1} (OH); ^{1}H NMR (200 MHz Acetone-d_6) d 8.4 (s, 2H, OH), 7.63 (d, *J* = 7.7 Hz, 1H, H-4), 7.44 (d, *J* = 8.1 Hz, 1H, H-7), 7.07-7.28 (m, 6H, 4ArH, H-5, H-6), 6.90 (d, *J* = 8.8 Hz, 2H, ArH), 6.77 (d, *J* = 8.8 Hz, 2H, ArH), 3.66 (s, 3H, CH_3); Anal. ($C_{21}H_{17}NO_2$) C,H,N.

1-Ethyl-2,3-*bis*-(4-hydroxy-phenyl)-1*H*-indole (4d). This compound was obtained as a yellowish solid (95 %); R_f 0.69 (EtOAc/hexane 1:1); mp 198 – 199 ° C (diethylether); IR (KBr) = 3343 cm^{-1} (OH); ^{1}H NMR (200 MHz, Acetone-d_6) d 8.5 (s, 2H, OH), 7.65 (d, *J* = 7.7 Hz, 1H, H-4), 7.45 (d, *J* = 8.1 Hz, 1H, H-7), 7.05-7.30 (m, 6H, 4ArH, H-5, H-6), 6.92 (d, *J* = 8.8 Hz, 2H, ArH), 6.80 (d, *J* = 8.8 Hz, 2H, ArH), 4.19 (q, *J* = 7.1 Hz, 2H, CH_2CH_3), 1.38 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); Anal. ($C_{22}H_{19}NO_2$) C,H,N.

2,3-*bis*-(4-Hydroxy-phenyl)-1-methyl-1*H*-indol-6-ol (4e). This compound was obtained as a orange solid (92 %); R_f 0.40 (EtOAc/hexane 1:1); mp 129 – 130 ° C (diethylether); IR (KBr) = 3346.5 cm^{-1} (OH); ^{1}H NMR (400 MHz, Acetone-d_6) d 8.62 (s, 1H, OH), 8.21 (s, 1H, OH), 8.08 (s, 1H, OH), 7.45 (d, *J* = 8.5 Hz, 1H, H-4), 7.17 (d, *J* = 8.6 Hz, 2H, ArH), 7.11 (d, *J* = 8.6 Hz, 2H, ArH), 6.89 (d, *J* = 8.6 Hz, 2H, ArH), 6.85 (d, *J* = 2.1 Hz, 1H, H-7), 6.77 (d, *J* = 8.7 Hz, 2H, ArH), 6.72 (dd, *J* = 8.5, 2.2 Hz, 1H, H-5), 3.75 (s, 3H, CH_3); Anal. ($C_{21}H_{17}NO_3$) C,H,N.

1-Ethyl-2,3-*bis*-(4-hydroxy-phenyl)-1*H*-indol-6-ol (4f). This compound was obtained as pale orange crystals (90 %); R_f 0.41 (EtOAc/hexane 1:1); mp 124 – 125 ° C (diethylether); IR (KBr) = 3347 cm^{-1} (OH); ^{1}H NMR (400 MHz, Acetone-d_6) d 8.60 (s, 1H, OH), 8.20 (s, 1H, OH), 8.07 (s, 1H, OH), 7.43 (d, *J* = 8.5 Hz, 1H, H-4), 7.15 (d, *J* = 8.6 Hz, 2H, ArH), 7.10 (d, *J* = 8.6 Hz, 2H, ArH), 6.85 (d, *J* = 8.6 Hz, 2H, ArH), 6.83 (d, *J* = 2.1 Hz, 1H, H-7), 6.76 (d, *J* = 8.7 Hz, 2H, ArH), 6.70 (dd, *J* = 8.5, 2.2 Hz, 1H, H-5), 4.17 (q, *J* = 7.2 Hz, 2H, CH_2CH_3), 1.38 (t, *J* = 7.2 Hz, 3H, CH_2CH_3); Anal. ($C_{22}H_{19}NO_3$) C,H,N.

Determination of the estrogen receptor binding affinity (RBA).

Relative binding measurments were performed as previously reported,[19] using lamb uterine cytosol, diluted to ~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 18–24h. The unlabeled competitor was diluted in 1:1(v/v) dimethylformamide/TEA buffer (10 mM Tris, 1.5mM EDTA, 3mM sodium azide, pH 7.4 at 25 ° C) to ensure solubility. All data are reported relative to estradiol = 100%.

UV-Vis and fluorescence spectra.

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

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