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1,2-DIARYL-3,4-DIHYDRONAPHTHALENES: PHOTOFLUOROGENIC LIGANDS FOR THE ESTROGEN RECEPTOR

RAJESHWAR D. BINDAL and JOHN A. KATZENELLENBOGEN*
Department of Chemistry, University of Illinois, Urbana, IL 61801, U.S.A.

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Summary—1,2-Diaryl-3,4-dihydronaphthalene systems related to the Upjohn antiestrogen nafoxidine (U11, 110A) (**1**) have high affinity for the estrogen receptor, but are essentially non-fluorescent. Upon u.v. irradiation, however, the *cis*-stilbene system within their structure undergo a photocyclization (**2**) followed by a spontaneous oxidation, forming a dihydrobenzochrysene system (**3**) that is highly fluorescent, but is only weakly bound by the estrogen receptor. This system can be oxidized further to the benzochrysene system, which exhibits similar fluorescence. The 1,2-diarylnaphthalene systems, obtained by direct oxidation of the dihydronaphthalenes, however, do not undergo photocyclization. The binding affinity of these derivatives for the estrogen receptor appears to be related to their degree of non-planarity and can be correlated with their molecular surface area. This facile photochemical conversion of a high-affinity ligand to a highly fluorescent species offers a new approach to the design of fluorescent estrogens.

INTRODUCTION

There has been much interest in the preparation of fluorescent estrogens to measure the kinetics of estrogen receptor–ligand interactions and to assay the concentration of estrogen receptors in individual cells and in cell and tissue preparations [1–9]. In the latter applications, it is hoped that fluorescent estrogens will be useful in expanding our understanding of the relationship between the estrogen receptor content of breast tumor cells and the hormone responsiveness of the cancer [10, 11]. There have, however, been many problems concerning the purity, stability and receptor affinity of various fluorescent estrogens that have been described and the conditions under which they have been studied; so, there is currently serious debate over the validity of the results that have been thus far obtained [12, 13].

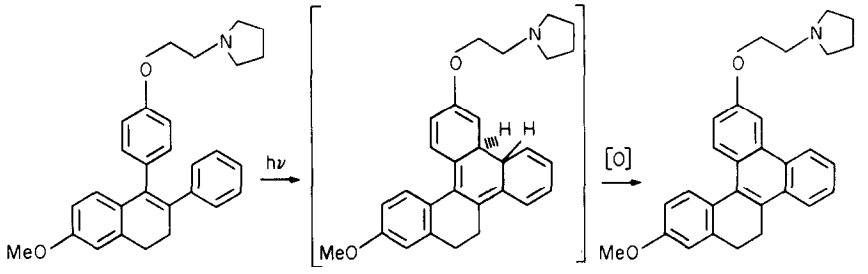
In earlier publications [14, 15], we presented preliminary data on the receptor binding and the fluorescence characteristics of 9(11)-dehydro-12-oxoestradiol, an estrogen derivative having a coumaryl fluorophore contained nearly completely within the steroidal structure. However, despite the reasonably favorable characteristics of this compound, it still represents a compromise between the structural characteristics required for high receptor binding affinity and those required for strong fluorescence.

In this report, we describe the preparation and properties of a 1,2-diaryl-3,4-dihydronaphthalene system that we have termed a "photofluorogenic" ligand for the estrogen receptor. In its parent form, this molecule is non-planar, has high affinity for the estrogen receptor and is essentially non-fluorescent. However, upon u.v. irradiation, the *cis*-stilbene unit within its structure undergoes a photocyclization [16], followed by a spontaneous oxidation, to furnish a 5,6-dihydrobenzo[*p*]chrysene system that is highly fluorescent but has diminished receptor binding affinity (Fig. 1). This facile photochemical conversion of a high-affinity ligand to a highly fluorescent species offers a new approach to the development of fluorescent ligands for the estrogen receptor.

EXPERIMENTAL

[^{1}H]-NMR spectra were recorded on Varian Associates spectrometers, Models EM-390 and HR-220. Chemical shifts are reported in parts per million (ppm) down-field from a tetramethylsilane internal standard (δ-scale). Data are reported as δ-values of protons (multiplicity of signal, number of protons, identity of protons). Mass spectra were obtained from a Varian MAT CH-5 spectrometer by electron impact at 10 eV unless otherwise indicated. Data are presented in the form m/z (intensity relative to base peak). Exact mass determinations by high-resolution mass spectrometry were obtained on a Varian MAT 731 spectrometer. u.v. Spectra were taken on Beckman DU-8 and Hewlett-Packard 8451-Diode Array spectrophotometer coupled with a microcomputer. Fluorescence spectra were recorded by photon counting on a Spex Fluorolog Spectrophotometer 111C with a Datamate microprocessor. Melting points were determined on a Thomas Hoover capillary melting-point apparatus; the temperatures reported

*Address correspondence to: Dr J. A. Katzenellenbogen, 461 Roger Adams Laboratory, Box 37, University of Illinois, 1209 W. California St, Urbana, IL 61801, U.S.A.



1 2 3

Fig. 1. Photocyclization and spontaneous oxidation of nafoxidine (**1**), representative of the 1,2-diaryl-3,4-dihydronaphthalene systems, into the 5,6-dihydrobenzo[*p*]chrysene system (**3**).

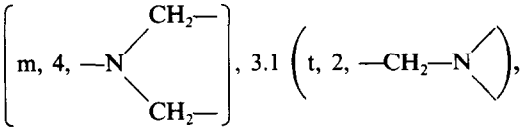
are uncorrected. Flash column chromatography [17] was carried out using Woelm (32–63 μm) silica gel. Elemental analyses were provided by the microanalytical service laboratory of the University of Illinois.

The chemicals and reagents used in this study were obtained from the following sources: Nafoxidine, Upjohn Pharmaceuticals, Kalamazoo, Mich., DDQ, Aldrich Chemical Co., Milwaukee, Wis.; and aluminum bromide from Alfa Chemicals, St Louis, Mo.

Binding affinity measurements to rat and lamb uterine estrogen receptor preparations were performed as described previously [18].

*2-Methoxy-7-(2-*N*-pyrrolidinoethoxy)benzo*[p]-*13,14-dihydrochrysene* (**3**)

A stirred solution of 1-[4-(2-*N*-pyrrolidinoethoxy)-phenyl]-2-phenyl-6-methoxy-3,4-dihydronaphthalene (**1**) (213 mg, 0.5 mmol) in benzene (250 ml) was irradiated for 20 min in a Rayonet Reactor (Model RPR-100) using four 8 W 254 nm bulbs. The solvent was removed under reduced pressure, and the residue was chromatographed over silica gel (15 g; 3% triethylamine/benzene), furnishing compound **3** (180 mg, 84%), m.p. 152–158°C (as citrate). [^{1}H]-NMR, δ: 1.72 (m, 4, C—CH_2CH_2—C), 2.0 (m, 4, Ar—CH_2—CH_2—Ar), 2.8



4.0 (s, 3, $ArOCH_3$), 4.4 (t, 2, Ar—O—CH_2), 6.9–8.7 (m, 10, Ar—H). Mass spectrum (10 eV) *m/e* (rel. intensity): 423 (88%, M^+), 419 (7%), 325 (10%), 98 (61%), 84 (100%). Analysis:

$C_{29}H_{29}NO_2$

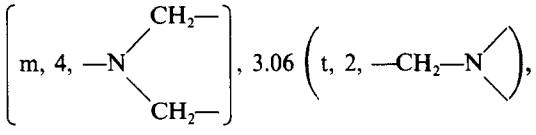
Calculated: mol. wt = 432.2198
Found: mol. wt = 432.2196.

*1-[4-(2-*N*-pyrrolidinoethoxy)phenyl]-2-phenyl-6-hydroxy-3,4-dihydronaphthalene* (**4**)

A mixture of compound **1** (213 mg, 0.5 mmol) and aluminum bromide (267 mg, 1.0 mmol) in dry benzene (15 ml) was stirred at room temperature for 2 h. The reaction was then cooled to 10°C and quenched by the dropwise addition of aq. HCl (10 ml, 5%). After being neutralized with saturated sodium bicarbonate solution, the aqueous layer was extracted with ethyl acetate (3 × 25 ml). The combined organic layer was washed with water (2 × 20 ml) and dried over anhydrous sodium sulfate; solvent was then removed by distillation *in vacuo*. The resulting oil was chromatographed over 20 g of silica gel (10% triethylamine in benzene), giving compound **4** as a colorless oil (134 mg, 65%), which upon crystallization from methanol furnished a white solid, m.p. 175–177°C ([19], m.p. 165°C). Mass spectrum (10 eV) *m/e* (rel. intensity): 411 (27%, M^+), 407 (6.6%), 98 (10.4%), 84 (100%)].

*2-Hydroxy-7-(2-*N*-pyrrolidinoethoxy)benzo*[p]-*13,14-dihydrochrysene* (**5**)

A stirred solution of compound **4** (82.2 mg, 0.2 mmol) in dioxane (100 ml) was irradiated at 254 nm for 20 min, and solvent was removed under reduced pressure. The residual oil was chromatographed over silica gel (20 g; 8% triethylamine/benzene) to furnish compound **5** (70 mg, 85%), m.p. 140–145°C (as citrate). [^{1}H]-NMR ($CDCl_3$), δ: 1.83 (m, 4, C—CH_2—CH_2—C), 2.0 (4, m, Ar—CH_2—CH_2—Ar), 2.76



4.35 (t, 2, —Ar—O—CH_2—), 7.0–8.85 (m, 10, Ar—H). Analysis:

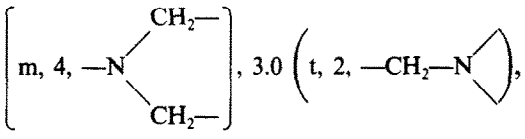
$C_{28}H_{27}NO_2$

Calculated: mol. wt = 409.2041
Found: mol. wt = 409.2043.

*2-Methoxy-7-(2-*N*-pyrrolidinoethoxy)benzo*[p]-*chrysene* (**6**)

To a stirred solution of compound **3** (59 mg, 0.14 mmol) in dry benzene (3 ml) was added a solution of 2,3-dichloro-5,6-dicyano-benzoquinone

(48 mg, 0.21 mmol) in benzene (2 ml). The dark solution was stirred for 30 min, and then the solvent was removed under reduced pressure. The residual mass was dissolved in 1 ml of (3% triethylamine/benzene) and percolated through 2 g of silica to furnish compound **6** (53 mg, 91%), m.p. 172–174°C (as citrate). [^{1}H]-NMR ($CDCl_3$), δ: 1.85 (m, 4, —CH_2—CH_2—), 2.68



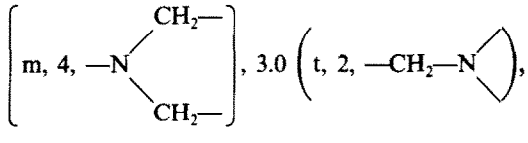
4.0 (s, 3, $ArOCH_3$), 4.35 (t, 2, —Ar—O—CH_2—), 7.05–8.85 (m, 12, Ar—H). Mass spectrum (10 eV) *m/e* (rel. intensity): 421 (43.8%, M^+), 417 (1.5%), 324 (3.0%), 98 (34.9%), 84 (100%). Analysis:

$C_{29}H_{27}NO_2 . 1/3\ C_6H_8O_7$

Calculated: C 76.70, H 6.12
Found: C 76.84, H 6.02.

2-Hydroxy-7-(2-N-pyrrolidinoethoxy)-benzo[p]-*chrysene* (**7**)

To a stirred solution of diethylene glycol (1 ml) and solid potassium hydroxide was added compound **6** (42.1 mg, 0.1 mmol). The reaction mixture was stirred at 220°C for 30 min, cooled and then mixed with 100 ml water. After the reaction mixture was neutralized with acetic acid, the aqueous phase was extracted with ethyl acetate (3 × 15 ml). The combined organic phase was washed with water and dried over anhydrous sodium sulfate. After the solvent was removed by distillation under reduced pressure, the residual oil was chromatographed over 2 g of silica (5% triethylamine/benzene) to afford compound **7** (18 mg, 45%). [^{1}H]-NMR ($CDCl_3$), δ: 1.84 (m, 4,—CH_2—CH_2—), 2.68



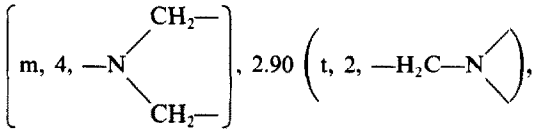
4.35 (t, 2, —Ar—O—CH_2—), 7.02–8.85 (m, 12, Ar—H). Mass spectrum (10 eV) *m/e* (rel. intensity): 407 (45%, M^+), 98 (36%), 84 (100%). Analysis:

$C_{28}H_{25}NO_2$

Calculated: mol. wt = 407.1885
Found: mol. wt = 407.1884.

1-[4-(2-N-pyrrolidinoethoxy)phenyl]-2-phenyl-6-methoxynaphthalene (**8**)

To a refluxing solution of compound **1** (42.5 mg, 0.1 mmol) in dry benzene (2 ml) was added dropwise a solution of 2,3-dichloro-5,6-dicyano-benzoquinone (3.40 mg, 0.15 mmol) in 2 ml benzene. The reaction was brought to reflux for 2 h under dry nitrogen. The solvent was removed under reduced pressure, and the residual mass was dissolved in 5% triethylamine in benzene (2 ml) and percolated through 2 g of silica gel to furnish compound **8** (40 mg, 94%), m.p. 214–216°C (as hydrochloride). [^{1}H]-NMR ($CDCl_3$), δ: 1.83 (m, 4, —CH_2—CH_2—), 2.67



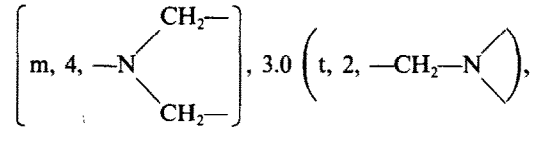
3.95 (s, 3, Ar—O—CH_3), 4.10 (t, 2, —O—CH_2—), 6.7–7.9 (m, 14, Ar—H). Mass spectrum (10 eV) *m/e* (rel. intensity): 423 (25.5, M^+), 98 (3.7%), 84 (100%). Analysis:

$C_{29}H_{29}NO_2 . HCl$

Calculated: C 75.74, H 6.53, N 3.05
Found: C 75.46, H 6.51, N 3.15.

1-[4-(2-N-pyrrolidinoethoxy)phenyl]-2-phenyl-6-hydroxynapthalene (**9**)

A stirred solution of diethylene glycol (1 ml) and solid potassium hydroxide (300 mg) was heated to 230°C under dry nitrogen. To the hot solution was added compound **8** (46 mg, 0.1 mmol). The reaction mixture was stirred at this temperature for 10 min, cooled and then mixed with 10 ml water. After the reaction mixture was neutralized with acetic acid, the aqueous phase was extracted with ethyl acetate (3 × 15 ml). The combined organic phase was washed with water, and dried over anhydrous sodium sulfate. After the solvent was removed by distillation under reduced pressure, the residual oil was chromatographed over 2 g of silica (5% triethylamine/benzene) to afford compound **9** (20 mg, 50%), m.p. 174–176°C (benzene). [^{1}H]-NMR ($CDCl_3$), δ: 1.86 (m, 4, —CH_2—CH_2—), 2.68



4.35 (t, 2-Ar-OCH_2—), 7.05–8.85 (m, 14, Ar—H). Mass spectrum (10 eV) *m/e* (rel. intensity): 409 (5.5%, M^+), 405 (9.0), 312 (3.4%), 98 (8.6%), 84 (100%). Analysis:

$C_{28}H_{27}NO_2$

Calculated: mol. wt = 409.2041
Found: mol. wt = 409.2042.

RESULTS

Preparation and photocyclization–oxidation of photofluorogenic estrogens

The photofluorogenic estrogens we have studied (Fig. 2) are related to the Upjohn antiestrogen,

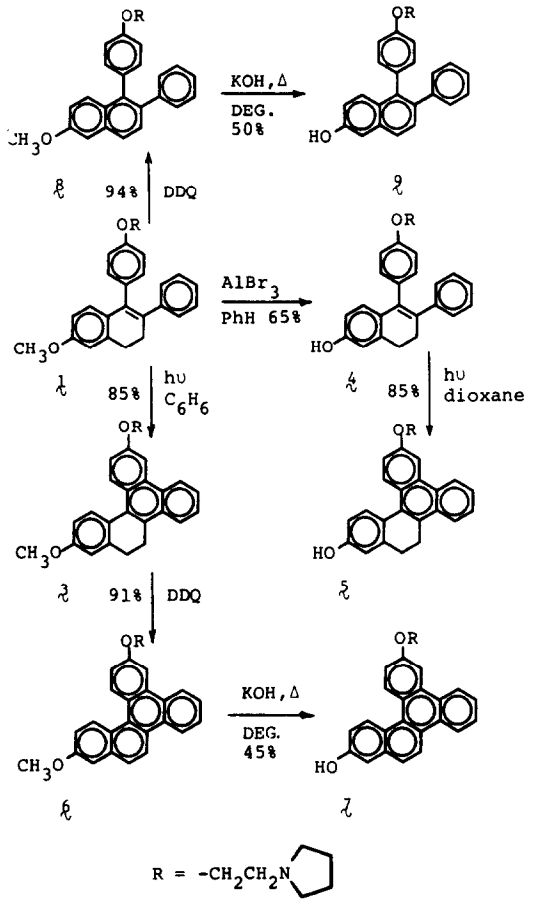


Fig. 2. Chemical and photochemical conversions of photofluorogenic estrogens.

nafoxidine (U11, 100A) (**1**) [20]. When an ethanol solution of nafoxidine (**1**) is irradiated at 254 nm, it is converted rapidly and efficiently (85%) into the phenanthrene system (**3**) (Figs 1 and 2). This conversion is presumed to involve first a photochemical cyclization to a dihydrophenanthrene system (**2**), followed by a spontaneous oxidation to the aromatic system (cf. Fig. 1) (**3**). While other workers have reported that this photocyclization–oxidation is assisted by the presence of iodine or cupric salts [21], we find that the reaction proceeds well simply when it is conducted in air. Since free phenols bind to the estrogen receptor with higher affinity than aryl methyl ethers, we prepared the free phenol analog of nafoxidine [desmethylnafoxidine (**4**)], by exposure of compound **1** to aluminum bromide (Fig. 2). Irradiation of compound **4** also results in an efficient (85%) photocyclization–oxidation (Fig. 3), giving the phenanthrene system **5** (Fig. 2).

The photocyclized–oxidized nafoxidine analog **3** is a dihydrobenzochrysene system, and brief exposure to dichlorodicyanoquinone produces the fully aromatic benzochrysene **6** (Fig. 2). The corresponding phenol **7** can also be prepared by demethylation of compound **6** with potassium hydroxide in diethylene glycol (Fig. 2).

Nafoxidine can also be aromatized to the 1,2-diarylnaphthalene system **8**, from which the free phenol **9** can also be produced (Fig. 2). These analogs, however, do not undergo photocyclization–oxidation to the benzochrysenes, **6** and **7**, presumably because the double bond in the *cis*-stilbene structure is now part of the more extensively delocalized naphthalene system [22, 23], or possibly because the reverse reaction, also photochemical, proceeds more rapidly than the oxidation [16].

Absorption and fluorescence properties of the photofluorogenic estrogens

The absorption and fluorescence properties of the compounds described above are summarized in Tables 1–3; selected spectra are presented in Figs 3–6. Photocyclization of nafoxidine (**1**) results in a u.v. shift from adsorption maxima at 304 nm to a more complex spectrum having strong bands at 269, 327 and 339 nm, with weaker bands extending to nearly 380 nm (Fig. 3); desmethylnafoxidine (**4**) also undergoes a similar change in the u.v. spectrum (Fig. 4). The clear isosbestic points in the photoconversion of compounds **1** to **3** (Fig. 3) and **4** to **5** (Fig. 4) are consistent with the chemical efficiency of these reactions. The naphthalene systems **8** and **9** show u.v.

Table 1. Absorbance properties of photofluorogenic estrogens

Methyl ethers[a]			*Phenols*[a]				
	Ethanol			Ethanol		EtOH + 0.1 N KOH	
Compound no.	λ_{max} (nm)	ε	Compound no.	λ_{max} (nm)	ε	λ_{max} (nm)	ε
1	258	1.1×10^4	**4**	258	1.3×10^4	270	1.3×10^4
	304	1.3×10^4		309	1.8×10^4	340	2.0×10^4
3	269	2.6×10^4	**5**	270	7.2×10^4	258	7.6×10^4
	327	1.0×10^4		327	1.4×10^4		
	339	1.0×10^4		340	1.3×10^4	352	1.7×10^4
	376	1.5×10^3		376	4.0×10^3		
6	290	2.8×10^3	**7**	270	3.4×10^3	285	6.9×10^3
	336	2.0×10^3		328	2.1×10^3		
	342	1.6×10^3		340	2.4×10^3	360	2.4×10^3
	378	3.2×10^2		376	6.2×10^2		
8	288	8.8×10^3	**9**	288	9.0×10^3	312	7.0×10^3
	322	2.6×10^3		328	2.8×10^3		
	336	2.3×10^3		340	2.7×10^3	364	2.7×10^3

[a]Spectra of methyl ethers are pH independent and were measured in ethanol only.

Table 2. Fluorescence properties of photofluorogenic estrogens

Methyl ethers[a]				*Phenols*[a]					
Compound no.	Solvent	λ^{excit}_{max} (nm)	$\lambda^{emiss.}_{max}$ (nm)	Compound no.	Solvent	$\lambda^{excit.}_{max}$ (nm)	$\lambda^{emiss.}_{max}$ (nm)	λ^{excit}_{max} (nm) (+0.1 N KOH)	$\lambda^{emiss.}_{max}$ (nm)
3	Heptane	336, 375	388, 407, 430 (s)	5	Heptane[b]	340, 376	387, 407	—	—
	EtOH	336, 375	388, 406		EtOH	340, 376	394, 410	352	510
	H_2O	335, 375	415		H_2O	340, 376	416	352	493
6	Heptane	340, 378	391, 409, 430 (s)	7	Heptane[b]	340, 376	391, 412, 430 (s)	—	—
	EtOH	340, 378	390, 409, 430 (s)		EtOH	340, 376	398, 418	364	522
	H_2O	340, 378	394, 409		H_2O	340, 376	420	358	523
8	Heptane	288, 336	388, 408	9	Heptane[b]	330	375	—	—
	EtOH	294, 336	374		EtOH	340	394, 412	360	450
	H_2O	290, 334	387		H_2O	343	405, 412	358	443

[a]As the emission of the methyl ethers is not pH dependent, their fluorescence spectra were not determined under basic conditons.
[b]Because of insolubility, spectra under basic conditions were not determined in heptane.

spectra remarkably similar to those of the dihydronaphthalenes **1** and **4**, and the spectra of the benzochrysenes **6** and **7** also are very similar to the dihydrobenzochrysenes **3** and **5**. All the phenolic systems (**4**, **5**, **7** and **9**) display different spectra at alkaline pH (cf. Table 1).

Table 3. Fluorescence quantum-yields of compound **5** in various solvents

Solvent	Quantum-yields[a,b]
Ethanol	0.43
Dichloromethane	0.31
Water	0.19
Heptane	0.14
Aqueous-alkali	0.002
Ethanolic-alkali	0.12

[a]Excitation wavelength for all determinations is 340 nm. Quantum-yield determinations are considered accurate within 10%.
[b]Reference is Quinine sulfate (0.1 N H_2SO_4); quantum-yield = 0.55.

The maxima for fluorescence excitation and emission for these compounds in three solvents are listed in Table 2. The dihydrobenzochrysenes **3** and **5** are highly fluorescent, showing excitation maxima at 263 nm and emission maxima at 407 nm (Table 2, Fig. 5 for compound **5**). Changing solvents has a moderate effect on the emission spectra (Fig. 6): In heptane, compound **5** shows three emission bands, but in solvents of increasing polarity, the spectrum shifts towards the red. This shift is the result of both a movement of the bands as well as an increase in intensity of the long-wavelength bands relative to the shorter-wavelength ones, with the result that the emission band in water is almost structureless. In base, compound **5** has its excitation and emission maxima shifted to 352 and 493 nm, respectively.

The quantum yields of these fluorescent compounds are moderately high and are somewhat solvent dependent (cf. Table 3). It is difficult to deter-

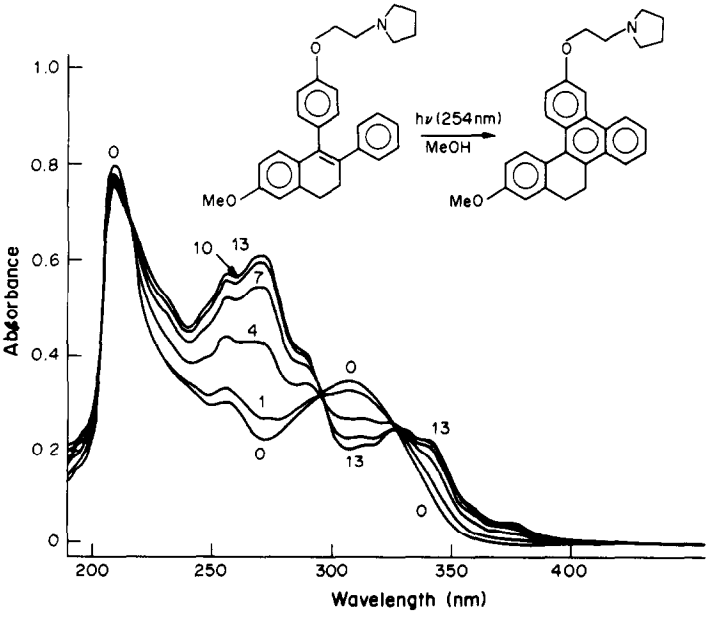


Fig. 3. u.v. Spectrum showing the time-course of nafoxidine (**1**) photocyclization to compound **3** in ethanol. Concentration of compound **1** was 2.7×10^{-5} M. Irradiation was conducted in a quartz cuvette suspended in a Rayonet photochemical reactor equipped with four 8 W bulbs emitting at 254 nm.

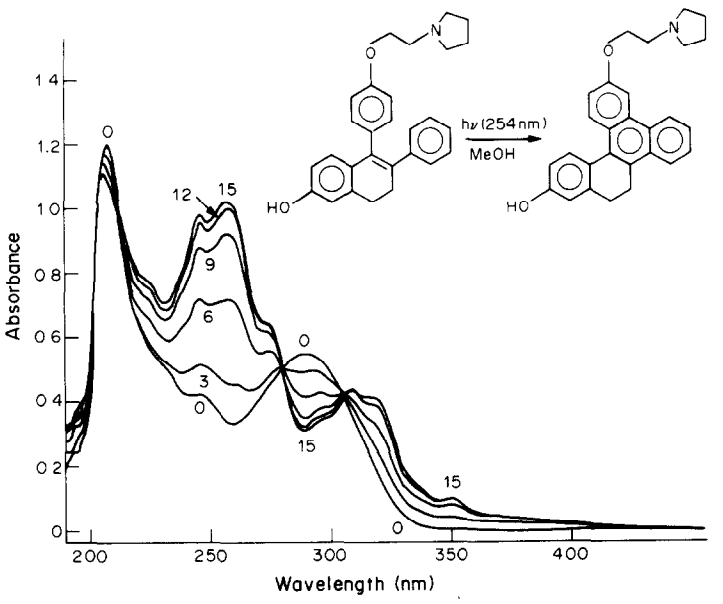


Fig. 4. u.v. Spectrum showing the time-course of desmethylnafoxidine (**4**) photolysis to compound **5** in methanol. Concentration of compound **4** was 3.1×10^{-5} M. Irradiation was conducted in a quartz cuvette suspended in a Rayonet photochemical reactor equipped with four 8 W bulbs emitting at 254 nm.

mine whether the dihydronaphthalenes **1** and **4** are fluorescent, since they undergo photocyclization–oxidation to the highly fluorescent dihydrobenzochrysenes in the fluorometer, during the measurement of their fluorescence spectra.

Binding affinity for the estrogen receptor

The binding affinity of these compounds for the uterine estrogen receptor was determined in a competitive binding assay, using lamb uterine cytosol as a source of receptor and charcoal–dextran as an adsorbant of free ligand [18]. The binding affinities expressed as RBA (i.e. relative binding affinity) values are summarized in Table 4. As expected, the binding affinities of the free phenols are much higher than those of the methyl ethers. The free phenol analog of nafoxidine **4** has the highest affinity, 110% that of estradiol.

In both the free phenol and methyl ether series there is a very pronounced decrease in receptor binding affinity that results from the introduction of a double bond into the dihydronaphthalene system and from cyclization of the stilbene system. From many studies of structure–binding affinity or structure–bioactivity relationships, it is known that the receptor has an affinity for compounds that are

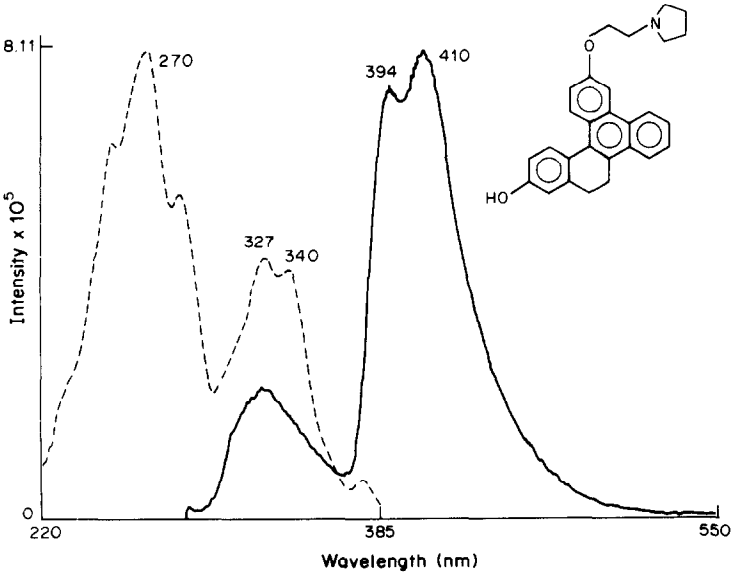


Fig. 5. Fluorescence excitation and emission spectrum of compound **5** in ethanol. Concentration of compound **5** was 8.1×10^{-8} M. The ordinate scale is photon counts/s.

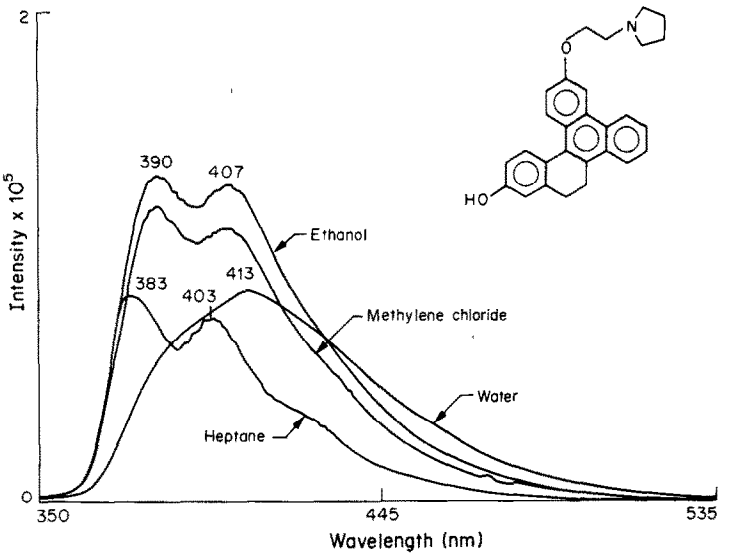


Fig. 6. Fluorescence emission spectra of 8.1×10^{-8} M of compound **5** in various solvents. Excitation was at 340 nm.

Table 4. RBA and surface area of the compounds

Methyl ethers			*Phenols*		
Compound no.	Surface area (cm^2/mol $\times 10^9$)[a]	RBA (%)[b]	Compound no.	Surface area (cm^2/mol $\times 10^9$)	RBA (%)
1	28.64	2.65	**4**	27.44	108.5
3	26.87	0.02	**5**	27.67	1.33
6	25.81	0.01	**7**	24.61	0.60
8	27.75	0.27	**9**	26.55	10.60

[a]Calculated by the method of Bondi [25].
[b]Assayed by a radiometric competitive binding assay, using [^{3}H]estradiol as tracer [cf. 18].

generally non-planar. Thus, it is not surprising that double bond introduction and cyclization, both of which would increase planarity, would reduce binding affinity.

In order to explore this point in more detail, we conducted a regression analysis relating the receptor binding affinity of the two series of four compounds with their molecular areas, as calculated from the parameters of Bondi[24]. As can be seen in Fig. 7, these area–binding affinity correlations are good, with both series of compounds showing similar sensitivities to changes in this structural parameter (i.e. nearly equal slopes).

DISCUSSION

Fluorescent estrogens that have been described up to now have been of three types: estrogen–fluorophore conjugates [3, 6], macromolecule-linked estrogen–fluorophore conjugates [25, 27] and inherently-fluorescent ligands [1, 14, 15, 28]. Fluorescent estrogens of the first two types have been bulky molecules with low affinity for the estrogen receptor. Reports of high-affinity conjugates may be suspect, since free estrogenic ligand may contaminate the preparation [12, 13] or may be formed during the binding assay due to hydrolytic lability of the linking function. The design of the third type, the inherently-fluorescent ligands, presents the challenge of achieving within the same molecule the properties of high

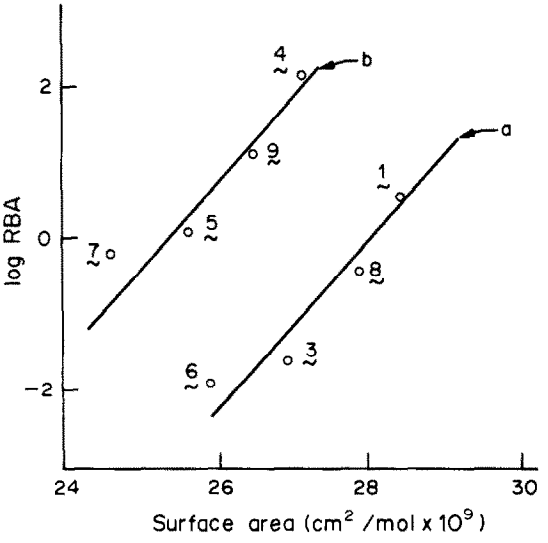


Fig. 7. Plot of log RBA vs surface area of the methyl ether (a) or free phenol (b) compounds.

receptor affinity and high fluorescence. In this paper, we have described the preparation of a fourth type of ligand for the estrogen receptor, one that can be converted from a high-affinity, non-fluorescent form to a highly fluorescent form by a photocyclization–oxidation process, hence the term photofluorogenic ligand.

The photochemical cyclization–oxidation of *cis*-stilbenes to phenanthrenes is a reaction that has been known since 1950 [29], and many examples of this conversion are known [16]. In fact, the photolytic conversion of tamoxifen to a fluorescent phenylphenanthrene system forms the basis of clinical assays of plasma levels of tamoxifen and some of its metabolites [30, 31], and a related reaction is used to assay for diethylstilbestrol [32]. In the specific application we are making of this reaction, we are converting one structure (the precursor), optimized in terms of its binding affinity for receptor, into a photocyclized–oxidized product, that has desirable fluorescence properties.

We have selected the free phenol analog of the antiestrogen nafoxidine as the starting point for investigating photofluorogenic ligands for the estrogen receptor. Compared to hydroxytamoxifen, the additional ring in desmethylnafoxidine avoids complications associated with *cis–trans* isomerism in the tamoxifens. Furthermore, the photoproduct **5** appears to have fluorescent properties that are superior to those of the corresponding phenanthrene systems derived from tamoxifen or hydroxytamoxifen ($\lambda_{max}^{emiss.} = 310$ nm; $\lambda_{max}^{emiss.} = 368$–$387$ nm) [30].

The photocyclization–oxidation sequence is exemplified in our series by the conversion of compound **4** to **5**; this results in the generation of a product with desirable fluorescence properties, but also results in a 100-fold decrease in binding affinity of the product for the estrogen receptor. Thus, the use of photofluorogenic estrogens of this type for the measurement of estrogen receptor will need to be coupled with an appropriately designed assay protocol, perhaps one in which relocation of the ligand after photocyclization is retarded by working in a viscous medium or at low temperatures.

The availability of photofluorogenic estrogens of this type offers a new approach to the development of fluorescent estrogens that may prove useful in assaying estrogen receptors. We are actively engaged in such studies.

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