Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 34 (2006) 1–14
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

2-Pyridin-2-yl-1*H*-indole derivatives: Synthesis, estrogen receptor binding affinity, and photophysical properties

Konstantinos M. Kasiotis, Serkos A. Haroutounian *

Chemistry Laboratory, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

Received 20 July 2005
Available online 1 December 2005

Abstract

A series of novel 2-pyridin-2-yl-1*H*-indole derivatives (**4a**–**f**) was prepared by intramolecular cyclodehydration of α-anilinyl (or 3-anisidyl)-2-pyridin-2-yl-ethanones (**2a**–**f**) and their optical spectroscopy and estrogen receptor (ER) binding properties were studied. These compounds showed long wavelength fluorescent emission, which is sensitive to solvent polarity and pH, while indol-6-ols **4b**, **e**, and **f** displayed reasonably good binding affinities to ER.

Keywords: Indoles; Cyclization; Fluorescence; Estrogen receptor

1. 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]. In this regard, particularly interesting is the development of a fluorescence-based assay of the estrogen receptor (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 or fluorescence microscopy, providing useful information for the prediction

* Corresponding author. Fax: +30 210 5294265.
E-mail address: sehar@aua.gr (S.A. Haroutounian).

0045-2068/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2005.10.005

of responsiveness to hormonal therapy [4–6]. 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.

To date three major classes of fluorescent estrogenic probes have been developed: estrogen–fluorophore conjugates, inherently fluorescent estrogens, and photofluorogenic estrogens [7–9]. 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 attractive research goal.

Part of our earlier work in this field concerned the synthesis of novel indole derivatives, since compounds of this structural framework have been found to possess pronounced estrogenic and tumor inhibition properties [10–13]. In this context, we have synthesized various novel 2,3-bis-(4-hydroxyphenyl)-indole derivatives that exhibit good binding affinities and interesting photophysical properties. Among the compound synthesized, 2,3-bis-(4-hydroxyphenyl)-1*H*-indol-6-ol (DPI) was the most efficient since has been found to posses the best photophysical properties and receptor binding affinity [14]. However, the design of such fluorescent probes must include a donor–acceptor system. Since the presence of phenol ring (donor) is a prerequisite for good binding affinity to the ER, our attention was focused on the study of the acceptor moiety. Thus, we have considered substituting one of the phenyl rings with a pyridine ring. The latter is expected to act as a potent acceptor moiety because of the additional involvement of a (n, π, and *) state that significantly enhances its photophysical properties. In this report, we describe the synthesis of derivatives of 2-pyridin-2-yl-1*H*-indoles, their ER binding affinity measurements and photophysical property studies.

2. Materials and methods

2.1. General

Air- and/or moisture-sensitive reactions were carried out under an argon atmosphere 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. 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 ethanolic solution of anisaldehyde. Products were purified by flash chromatography on Merck silica gel 60 (230–400 mesh ASTM). Prior the performance of photophysical experiments and RBA assays, all compounds were purified by semi-preparative HPLC (Column: Kromasil 10-5 C18 (25 cm × 10 mm); mobile phase: CH_3CN; detector: UV λ 300 nm; flow: 1.5 mL/min; load: 3 mg/100 μL solution in mobile phase). Melting points (uncorrected): Büchi melting point apparatus. FT-IR: Nicolet Magna 750, series II. Samples were recorded as KBr pellets, unless otherwise stated. ^{1}H NMR: Bruker DRX-400 (400 MHz) spectrometer, in the indicated solvents. Chemical shifts are referenced to internal TMS. HPLC: Hewlett–Packard 1100 series instrument with a variable wavelength UV detector and coupled to HP Chem. Station utilizing the manufacturer's 5.01 software package.

2.2. 2-Bromo-1-(4-methoxy-phenyl)-2-pyridin-2-yl-ethanone (**1**)

To a stirred solution of 1-(4-methoxy-phenyl)-2-pyridin-2-yl-ethanone (0.3 g, 1.32 mmol) and catalytic amount of anhydrous aluminum trichloride (2 mg), in anhydrous THF (2 mL), was added bromine (0.07 mL, 1.32 mmol). The reaction was run at room temperature for 1 h, then the mixture was partitioned between EtOAc (20 mL) and H_2O (15 mL). The organic layer was separated, washed with brine, and dried over $MgSO_4$. Concentration under reduced pressure produced a yellow solid, which was purified by flash chromatography (hexane/EtOAc 7:3) to afford 0.32 mg of bromide **1** (yield 80%) as a pale yellow solid. Mp 78–79 °C (diethylether/hexane). $R_f = 0.5$ (EtOAc/hexane 2:3). IR: $\tilde{\nu} = 1698\ cm^{-1}$ (C=O). ^1H NMR ($CDCl_3$): $\delta = 3.83$ (s, 3H, –OCH_3), 6.45 (s, 1H, H-2); pyridinyl: 7.21 (m, 2H, H-3, H-5), 7.70 (m, 1H, H-4), 8.51 (d, $J = 4.8$ Hz, 1H, H-6); phenyl: 6.90 (d, $J = 9.1$ Hz, 2H, Ar-H), 8.02 (d, $J = 9.1$ Hz, 2H, Ar-H). $C_{14}H_{12}BrNO_2$ (305.9): calcd: C, 54.92; H, 3.95; N. 4.58. Found: C, 54.76; H, 4.09; N, 4.71.

2.3. General procedure for the synthesis of amino 2-pyridin-2-yl-ethanones (**2a** and **b**)

To a stirred solution of 2-bromo-1-(4-methoxy-phenyl)-2-pyridin-2-yl-ethanone **1** (7.46 mmol) and triethylamine (9 mmol) in anhydrous CH_2Cl_2 (10 mL) was added aniline or *m*-anisidine (9 mmol). The reaction was run at room temperature 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, and dried over $MgSO_4$. Concentration under reduced pressure and flash chromatographic purification furnished the desired products.

2.4. 1-(4-Methoxy-phenyl)-2-phenylamino-2-pyidin-2-yl-ethanone (**2a**)

This compound was obtained as a yellow solid (0.22 g, 53%). Mp 114–115 °C (diethylether/hexane). $R_f = 0.65$ (EtOAc/hexane 2:3). IR: $\tilde{\nu} = 3384\ cm^{-1}$ (N–H), 1673 (C=O). ^1H NMR ($CDCl_3$): $\delta = 3.82$ (s, 3H, –OCH_3), 6.14 (s, 1H,–NH), 6.90 (s, 1H, H-2); pyridinyl: 7.08 (m, 1H, H-5), 7.38 (d, $J = 7.7$ Hz, 1H, H-3), 7.54 (ddd, $J = 1.8$, 7.7, 7.7 Hz, 1H, H-4), 8.56 (d, $J = 4.8$ Hz, 1H, H-6); phenyl: 6.73 (m, 3H, Ar-H), 6.88 (d, $J = 8.8$ Hz, 2H, Ar-H), 7.10 (m, 2H, Ar-H), 8.23 (d, $J = 8.8$ Hz, 2H, Ar-H). $C_{20}H_{18}N_2O_2$ (318.4): calcd: C, 75.45; H, 5. 70; N, 8.80. Found: C, 75.61; H, 5.56; N, 8.67.

2.5. 1-(4-Methoxy-phenyl)-2-(3-methoxy-phenylamino)-2-pyidin-2-yl-ethanone (**2b**)

This compound was obtained as an amorphous yellow solid (0.25 g, 55%). $R_f = 0.54$ (EtOAc/hexane 2:3). IR: $\tilde{\nu} = 3356\ cm^{-1}$ (N–H), 1679 (C=O). ^1H NMR ($CDCl_3$): $\delta = 3.81$ (s, 3H, –OCH_3), 3.83 (s, 3H, –OCH_3), 6.16 (s, 1H, –NH), 6.92 (s, 1H, H-2); pyridixnyl: 7.09 (d, $J = 7.7$ Hz, 1H, H-3), 7.36 (dd, $J = 4.5$, 7.7 Hz, 1H, H-5), 7.86 (ddd, $J = 1.8$, 7.7, 7.7 Hz, 1H, H-4), 8.59 (d, $J = 4.5$ Hz, 1H, H-6); phenyl: 6.25 (d, $J = 8.8$ Hz, 1H, Ar-H), 6.57 (m, 1H, Ar-H), 6.85 (d, $J = 8.8$ Hz, 1H, Ar-H), 6.95 (d, $J = 9.1$ Hz, 2H, Ar-H), 7.11 (m, 1H, Ar-H), 7.78 (d, $J = 9.1$ Hz, 2H, Ar-H). $C_{21}H_{20}N_2O_3$ (348.4): calcd: C, 72.40; H, 5.79; N, 8.04. Found: C, 72.54; H, 5.65; N, 8.17.

2.6. General procedure for the reductive alkylation of amines

To a stirred solution of amine (14.8 mmol) and the 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 diethylether (15 mL) and extracted with a saturated solution of $NaHCO_3$ (15 mL). The organic layer was separated, dried over $MgSO_4$, concentrated in vacuo, and purified by flash chromatography.

2.7. 1-(4-Methoxy-phenyl)-2-(methyl-phenylamino)-2-pyridin-2-yl-ethanone (**2c**)

This compound was obtained as a yellow solid (0.22 g, 50%). Mp 121–122 °C (diethylether/hexane). $R_f = 0.66$ (EtOAc/hexane 1:4). IR: $\tilde{\nu} = 3327\ cm^{-1}$ (N–H), 1668 (C=O). ^1H NMR ($CDCl_3$): $\delta = 2.99$ (s, 3H, –NCH_3) 3.82 (s, 3H, –OCH_3), 6.72 (s, 1H, H-2); pyridinyl: 6.99 (dd, $J = 4.8$, 7.6 Hz, 1H, H-5), 7.19 (d, $J = 7.6$ Hz, 1H, H-3), 7.54 (ddd, $J = 4.8$, 7.6, 7.6 Hz, 1H, H-4), 8.35 (d, $J = 4.8$ Hz, 1H, H-6); phenyl: 6.73 (m, 2H, Ar-H), 6.80 (m, 4H, Ar-H), 7.21 (m, 1H, Ar-H), 7.60 (d, $J = 9$ Hz, 2H, Ar-H). $C_{21}H_{20}N_2O_2$ (332.4): calcd: C, 75.45; H, 5.70; N, 8.80. Found: C, 75.29; H, 5.54; N, 8.97.

2.8. 2-(Ethyl-phenylamino)-1-(4-methoxy-phenyl)-2-pyridin-2-yl-ethanone (**2d**)

This compound was obtained as an orange solid (0.44 g, 40%). Mp 85–86 °C (diethylether/hexane). $R_f = 0.62$ (EtOAc/hexane 1:4). IR (neat): $\tilde{\nu} = 3319\ cm^{-1}$ (N–H), 1667 (C=O). ^1H NMR ($CDCl_3$): $\delta = 0.94$ (t, $J = 7.2$ Hz, 3H, –NCH_2CH_3) 3.25 (qd, $J = 7.2$ Hz, 1H, –NCH_2CH$_3$), 3.37 (qd, $J = 7.2$ Hz,1H, -NCH_2CH$_3$), 3.82 (s, 3H, –OCH_3), 6.71 (s, 1H, H-2); pyridinyl: 6.94 (dd, $J = 4.8$, 7.7 Hz, 1H, H-5), 7.21 (d,$J = 7.7$ Hz, 1H, H-3), 7.52 (ddd, $J = 4.1$, 7.7, 7.7 Hz, 1H, H-4), 8.33 (d, $J = 4.1$ Hz, 1H, H-6); phenyl: 6.76 (m, 2H, Ar-H), 6.80 (m, 4H, Ar-H), 7.19 (m, 1H, Ar-H), 7.55 (d, 2H, $J = 9$ Hz, Ar-H). $C_{22}H_{22}N_2O_2$ (346.4): calcd: C, 76.28; H, 6.40; N, 8.09. Found: C, 76.47; H, 6.22; N, 8.28.

2.9. 1-(4-Methoxy-phenyl)-2-[(3-methoxy-phenyl)-methylamino]-2-pyridin-2-yl-ethanone (**2e**)

This compound was obtained as a yellowish solid (0.16 g, 40%). Mp 120–121 °C (diethylether/hexane). $R_f = 0.38$ (EtOAc/hexane 1:4). IR (neat): $\tilde{\nu} = 3340\ cm^{-1}$ (N–H), 1671 (C=O). ^1H NMR ($CDCl_3$): $\delta = 2.99$ (s, 3H, –NCH_3) 3.81 (s, 3H, –OCH_3), 3.82 (s, 3H, –OCH_3), 6.39 (s, 1H, H-2); pyridinyl: 6.99 (d, $J = 7.7$ Hz, 1H, H-3), 7.10 (m, 1H, H-5), 7.51 (ddd, $J = 4.1$, 7.7, 7.7 Hz, 1H, H-4), 8.35 (d, $J = 4.8$ Hz, 1H, H-6); phenyl: 6.33 (m, 2H, Ar-H), 6.80 (m, 2H, Ar-H), 6.82 (d, $J = 9.1$ Hz, 2H, Ar-H), 7.60 (d, $J = 9.1$ Hz, 2H, Ar-H). $C_{22}H_{22}N_2O_3$ (362.4): calcd: C, 72.91; H, 6.12; N, 7.73. Found: C, 72.84; H, 6.22; N, 7.59.

2.10. 2-[Ethyl-(3-methoxy-phenyl)-amino]-1-(4-methoxy-phenyl)-2-pyridin-2-yl-ethanone (**2f**)

This compound was obtained as a yellow amorphous solid (0.17 g, 40%). $R_f = 0.43$ (EtOAc/hexane 1:4). IR (neat): $\tilde{\nu} = 3340\ cm^{-1}$ (N–H), 1671 (C=O). ^1H NMR ($CDCl_3$): $\delta = 0.94$ (t, $J = 7.2$ Hz, 3H, –NCH_2CH_3), 3.25 (qd, $J = 7.2$ Hz, 1H, –NCH_2CH$_3$), 3.37

(qd, $J = 7.2$ Hz, 1H, –NCH_2CH$_3$), 3.77 (s, 3H, –OCH_3), 3.79 (s, 3H, –OCH_3), 6.55 (s, 1H, H-2); pyridinyl: 6.92 (d, $J = 8.2$ Hz, 1H, H-3), 6.99 (dd, $J = 5.1$, 8.2 Hz, 1H, H-5), 7.52 (m, 1H, H-4), 8.33 (d, $J = 5.1$ Hz, 1H, H-6); phenyl: 6.31 (m, 2H, Ar-H), 6.43 (dd, $J = 8.5$, 2.1 Hz, 1H, Ar-H), 6.79 (d, $J = 8.9$ Hz, 2H, Ar-H), 7.13 (t, $J = 8.8$ Hz, 1H, Ar-H), 7.57 (d, $J = 8.9$ Hz, 2H, Ar-H). $C_{23}H_{24}N_2O_3$ (376.4): calcd: C, 73.38; H, 6.43; N, 7.44. Found: C, 73.58; H, 6.57; N, 7.58.

2.11. General procedure for the cyclization of ketones

A mixture of α-anilinyl (or 3-anisidyl)-desoxyanisoin **2a**–**f** (0.5 g) and PPA (10 g) was mechanically stirred at 70 °C for 5–10 h (monitored by TLC). 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 over $MgSO_4$, concentrated in vacuo, and purified by flash chromatography.

2.12. 3-(4-Methoxy-phenyl)-2-pyridin-2-yl-1H-indole (*3a*)

This compound was obtained as pale white needles (0.3 g, 63%). Mp 101–102 °C (diethylether/hexane). $R_f = 0.56$ (EtOAc/hexane 1:4). ^{1}H NMR ($CDCl_3$): $\delta = 3.79$ (s, 3H, –OCH_3), 7.07 (d, $J = 8.7$ Hz, 2H, Ar-H), 7.12 (m, 2H, H-6, H-4), 7.46 (d, $J = 8.7$ Hz, 2H, Ar-H), 7.48 (m, 1H, H-5), 7.52 (d, $J = 7.7$ Hz, 1H, H-4), 9.89 (s, 1H, NH); pyridinyl: 7.27 (ddd, $J = 4.1$, 7.9, 7.9 Hz, 1H, H-4), 7.42 (d, $J = 7.9$ Hz, 1H, H-3), 7.48 (dd, $J = 5.1$, 7.9 Hz, 1H, H-5), 8.62 (d, $J = 5.1$ Hz, 1H, H-6). $C_{22}H_{16}N_2O$ (300.3): calcd: C, 79.98; H, 5.37; N, 9.33. Found: C, 79.81; H, 5.19; N, 9.47.

2.13. 3-(4-Methoxy-phenyl)-1-methyl-2-pyridin-2-yl-1H-indole (*3c*)

This compound was obtained as an amorphous solid (0.26 g, 55%). $R_f = 0.6$ (EtOAc/hexane 1:4). ^{1}H NMR ($CDCl_3$): $\delta = 3.80$ (s, 3H, –NCH_3), 3.86 (s, 3H, –OCH_3), 6.85 (d, $J = 8.2$ Hz, 2H, ArH), 7.19 (m, 3H, H-5, H-6, H-7), 7.41 (d, $J = 8.2$ Hz, 2H, ArH), 7.69 (d, $J = 7.7$ Hz, 1H, H-4); pyridinyl: 7.21 (d, $J = 7.1$ Hz, 1H, H-3), 7.30 (dd, $J = 4.1$, 7.1 Hz, 1H, H-5), 7.51 (ddd, $J = 4.1$, 7.1, 7.1 Hz, 1H, H-4), 8.75 (d, $J = 4.1$ Hz, 1H, H-6). $C_{21}H_{18}N_2O$ (314.4): calcd: C, 65.02; H, 6.21; N, 2.61. Found: C, 65.15; H, 6.32; N, 2.45.

2.14. 1-Ethyl-3-(4-methoxy-phenyl)-2-pyridin-2-yl-1H-indole (*3d*)

This compound was obtained as a white solid (0.23 g, 62%). Mp 98–99 °C (diethylether/hexane). $R_f = 0.47$ (EtOAc/hexane 1:4). ^{1}H NMR ($CDCl_3$) $\delta = 1.25$ (t, $J = 7.1$ Hz, 3H, –NCH$_2$CH_3), 3.79 (s, 3H, –OCH_3), 4.42 (q, $J = 7.1$ Hz, 2H, –NCH_2CH$_3$), 6.85 (d, $J = 8.8$ Hz, 2H, ArH), 7.12 (m, 1H, H-5), 7.21 (d, $J = 8.8$ Hz, 2H, ArH), 7.29 (t, $J = 7.4$ Hz, 1H, H-6), 7.44 (d, $J = 8.1$ Hz, 1H, H-7), 7.69 (d, $J = 8.1$ Hz, 1H, H-4); pyridinyl: 7.14 (m, 2H, H-3, H-5), 7.51 (ddd, $J = 4.2$, 7.7, 7.7 Hz, 1H, H-4), 8.74 (d, $J = 4.2$ Hz, 1H, H-6). $C_{22}H_{20}N_2O$ (328.4): Calcd. C, 80.46; H, 6.14; N, 8.53. Found: C, 80.71; H, 6.29; N, 8.39.

2.15. 6-Methoxy-3-(4-methoxy-phenyl)-1-methyl-2-pyridin-2-yl-1H-indole (*3e*)

This compound was obtained as a yellowish solid (0.28 g, 60%). Mp 74–76 °C (diethylether/hexane). $R_f = 0.21$ (EtOAc/hexane 1:4). ^{1}H NMR ($CDCl_3$): $\delta = 3.79$ (s,

3H, –OCH_3) 3.81 (s, 3H, –OCH_3), 3.92 (s, 3H, –NCH_3), 6.79 (d, $J = 2.1$ Hz, 1H, H-7), 6.82 (m, 3H, Ar-H, H-4), 7.22 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.48 (dd, $J = 7.7$, 2.1 Hz, 1H, H-5); pyridinyl: 7.17 (m, 2H, H-3, H-5), 7.54 (m, 1H, H-4), 8.75 (d, $J = 4.1$ Hz, 1H, H-6). $C_{22}H_{20}N_2O_2$ (344.4): calcd: C, 65.02; H, 6.21; N, 2.61. Found: C, 64.87; H, 6.35; N, 2.47.

2.16. 1-Ethyl-6-methoxy-3-(4-methoxy-phenyl)-2-pyridin-2-yl-1H-indole (**3f**)

This compound was obtained as a yellow solid (65 mg, 68%). Mp 130–131 °C (diethylether/hexane). $R_f = 0.51$ (EtOAc/hexane 1:4). ^{1}H NMR ($CDCl_3$): $\delta = 1.23$ (t, $J = 7.1$ Hz, 3H, –NCH$_2$CH_3), 3.79 (s, 3H, –OCH_3), 3.91 (s, 3H, –OCH_3), 4.36 (q, $J = 7.1$ Hz, 2H, –NCH_2CH$_3$), 6.84 (d, $J = 8.7$ Hz, 2H, Ar-H), 6.88 (d, $J = 2.1$ Hz, 1H, H-7), 7.01 (dd, $J = 2.1$, 8.3 Hz, 1H, H-5), 7.19 (d, $J = 8.7$ Hz, 2H, Ar-H), 7.31 (d, $J = 8.3$ Hz, 1H, H-4); pyridinyl: 7.11 (d, $J = 7.9$ Hz, 1H, H-3), 7.16 (m, 1H, H-5), 7.49 (ddd, $J = 4.1$, 7.9, 7.9 Hz, 1H, H-4), 8.73 (d, $J = 4.1$ Hz, 1H, H-6). $C_{23}H_{22}N_2O_2$ (358.4): calcd: C, 77.07; H, 6.19; N, 7.82. Found: C, 76.88; H, 6.32; N, 7.69.

2.17. General procedure for the deprotection of phenols

To a stirred solution of indole **3a**–**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 temperature and stirred for 16–24 h. The reaction was quenched 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 over $MgSO_4$, concentrated under reduced pressure, and purified by flash chromatography.

2.18. 4-(2-Pyridin-2-yl-1H-indol-3-yl)-phenol (**4a**)

This compound was obtained as yellow needles (44 mg, 81%). Mp 126–128 °C (diethylether). $R_f = 0.49$ (EtOAc/hexane 2:3). IR (neat): $\tilde{\nu} = 3329$ cm^{-1} (OH). ^{1}H NMR (acetone-d_6): $\delta = 3.80$ (s, 3H, –NCH_3), 6.89 (d, $J = 8.6$ Hz, 2H, ArH), 7.23 (d, $J = 8.3$ Hz, 2H, ArH), 7.34 (t, $J = 8.6$ Hz, 2H, ArH), 7.49 (d, $J = 8.1$ Hz, 2H, ArH), 9.41 (br s, 1H, –N*H*); pyridinyl: 7.01 (d, $J = 7.3$ Hz, 1H, H-3), 7.22 (m, 2H, H-4, H-5), 8.44 (d, $J = 4.8$ Hz, 1H, H-6). $C_{19}H_{14}N_2O$ (286.3): calcd: C, 79.98; H, 5.43; N, 4.44. Found: C, 80.18; H, 5.31; N, 4.63.

2.19. 3-(4-Hydroxyphenyl)-2-pyridin-2-yl-1H-indol-6-ol (**4b**)

This compound was obtained as an orange solid (18 mg, 80%). Mp 107–108 °C (diethylether). $R_f = 0.40$ (EtOAc/hexane 1:1). IR (neat): $\tilde{\nu} = 3341$ cm^{-1} (OH). ^{1}H NMR (acetone-d_6): $\delta = 6.63$ (dd, $J = 2.1$, 8.8 Hz, 1H, H-5), 6.96 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.01 (d, $J = 2.1$ Hz, 1H, H-7), 7.27 (d, $J = 8.5$ Hz, 2H, Ar-H), 7.32 (d, $J = 8.8$ Hz, 1H, H-4), 8.11 (s, 1 H, OH), 8.45 (s, 1H, OH), 10.57 (br s, 1H, NH); pyridinyl: 7.09 (m, 1H, H-5), 7.18 (d, $J = 7.9$ Hz, 1H, H-3), 7.49 (ddd, $J = 4.9$, 7.9, 7.9 Hz, 1H, H-4), 8.53 (d, $J = 4.9$ Hz, 1H, H-6). $C_{19}H_{14}N_2O_2$ (302.3): calcd: C, 75.48; H, 9.67; N, 9.27. Found: C, 75.66; H, 9.92; N, 9.37.

2.20. 4-(1-Methyl-2-pyridin-2-yl-1H-indol-3-yl)-phenol (**4c**)

This compound was obtained as pale white crystals (70 mg, 75%). Mp 150–152 °C (diethylether). $R_f = 0.43$ (EtOAc/hexane 2:3). IR (neat): $\tilde{\nu} = 3343\ cm^{-1}$ (OH). ^1H NMR (acetone-d_6): $\delta = 6.98$ (d, $J = 8.6$ Hz, 2H, Ar-H), 7.26 (d, $J = 8.3$ Hz, 2H, Ar-H), 7.38 (d, $J = 8.6$ Hz, 2H, Ar-H), 7.57 (d, $J = 7.9$ Hz, 2H, Ar-H), 9.52 (s, 1H, –N*H*); pyridinyl: 7.02 (d, $J = 7.3$ Hz, 1H, H-3), 7.19 (m, 2H, H-4, H-5), 8.59 (d, $J = 4.8$ Hz, 1H, H-6). $C_{20}H_{16}N_2O$ (300.3): calcd: C, 79.98; H, 5.37; N, 9.33. Found: C, 80.13; H, 5.22; N, 9.73.

2.21. 4-(1-Ethyl-2-pyridin-2-yl-1H-indol-3-yl)-phenol (**4d**)

This compound was obtained as an amorphous yellow solid (110 mg, 80%). $R_f = 0.57$ (EtOAc/hexane 3:2). IR: $\tilde{\nu} = 3338\ cm^{-1}$ (OH). ^1H NMR (acetone-d_6): $\delta = 1.25$ (t, $J = 7.1$ Hz, 3H, –NCH$_2$C*H*$_3$), 3.47 (q, $J = 7.1$ Hz, 1H, –NC*H*$_2$CH$_3$), 4.4 (q, $J = 7.1$ Hz, 1H, –NC*H*$_2$CH$_3$), 6.95 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.13 (m, 1H, Ar-H), 7.30 (d, $J = 8.8$ Hz, 2H, Ar-H), 7.36 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.43 (m, 1H, ArH), 9.65 (br s, 1H, OH); pyridinyl: 6.76 (d, $J = 8.3$ Hz, 1H, H-3), 7.10 (m, 1H, H-5), 7.14 (m, 1H, H-4), 8.57 (d, $J = 4.8$ Hz, 1H, H-6). $C_{21}H_{18}N_2O$ (314.4): calcd: C, 80.23; H, 5.77; N, 8.91. Found: C, 80.36; H, 5.62; N, 8.77.

2.22. 3-(4-Hydroxyphenyl)-1-methyl-2-pyridin-2-yl-1H-indol-6-ol (**4e**)

This compound was obtained as an orange solid (36 mg, 80%). Mp 107–108 °C (diethylether). $R_f = 0.40$ (EtOAc/hexane 1:1). IR (neat): $\tilde{\nu} = 3341\ cm^{-1}$ (OH). ^1H NMR (acetone-d_6): $\delta = 3.69$ (s, 3H, –NC*H*$_3$), 6.58 (dd, $J = 2.1$, 8.8 Hz, 1H, H-5), 6.89 (d, $J = 8.8$ Hz, 2H, Ar-H), 6.96 (d, $J = 2.1$ Hz, 1H, H-7), 7.23 (d, $J = 8.8$ Hz, 2H, Ar-H), 7.32 (d, $J = 8.1$ Hz, 1H, H-4), 8.09 (s, 1H, OH), 8.46 (s, 1H, OH); pyridinyl: 7.03 (m, 1H, H-5), 7.12 (d, $J = 8.3$ Hz, 1H, H-6), 7.44 (ddd, $J = 4.9$, 8.3, 8.3 Hz, 1H, H-4), 8.53 (d, $J = 4.9$ Hz, 1H, H-6). $C_{20}H_{16}N_2O_2$ (316.3): calcd: C, 75.93; H, 5.10; N, 8.86. Found: C, 75.83; H, 5.02; N, 8.97.

2.23. 1-Ethyl-3-(4-hydroxyphenyl)-2-pyridin-2-yl-1H-indol-6-ol (**4f**)

This compound was obtained as pale orange crystals (20 mg, 39%). Mp 201–203 °C (diethylether). $R_f = 0.45$ (EtOAc/hexane 1:1). IR (neat): $\tilde{\nu} = 3347\ cm^{-1}$ (OH); ^1H NMR (acetone-d_6): $\delta = 0.94$ (t, $J = 7.1$ Hz, 3H, –NCH$_2$C*H*$_3$), 4.36 (q, $J = 7.1$ Hz, 2H, –NC*H*$_2$CH$_3$), 6.67 (dd, $J = 2.1$, 8.8 Hz, 1H, H-5), 7.01 (d, $J = 8.3$ Hz, 2H, ArH), 7.05 (d, $J = 2.1$ Hz, 1H, H-7), 7.21 (d, $J = 8.8$ Hz, 1H, H-4), 7.31 (d, $J = 8.3$ Hz, 2H, ArH), 8.12 (s, 1H, OH), 8.51 (s, 1H, OH); pyridinyl: 7.12 (m, 1H, H-5), 7.34 (d, $J = 7.9$ Hz, 1H, H-3), 7.49 (ddd, $J = 4.1$, 7.9, 7.9 Hz, 1H, H-4), 8.55 (d, $J = 4.1$ Hz, 1H, H-6). $C_{21}H_{18}N_2O_2$ (330.4): calcd: C, 76.34; H, 5.49; N, 8.48. Found: C, 76.36; H, 5.62; N, 8.39.

2.24. Determination of the estrogen receptor binding affinity

Relative binding measurements were performed as previously reported, using lamb uterine cytosol, diluted to ~1.5 nM receptor [21]. 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 1:1 (v/v) dimethylformamide/TEA buffer (10 mM Tris, 1.5 mM EDTA, and 3 mM sodium azide, pH 7.4, at 25 °C) to ensure solubility. All data are reported relative to estradiol = 100%.

2.25. UV–Vis and fluorescence spectra

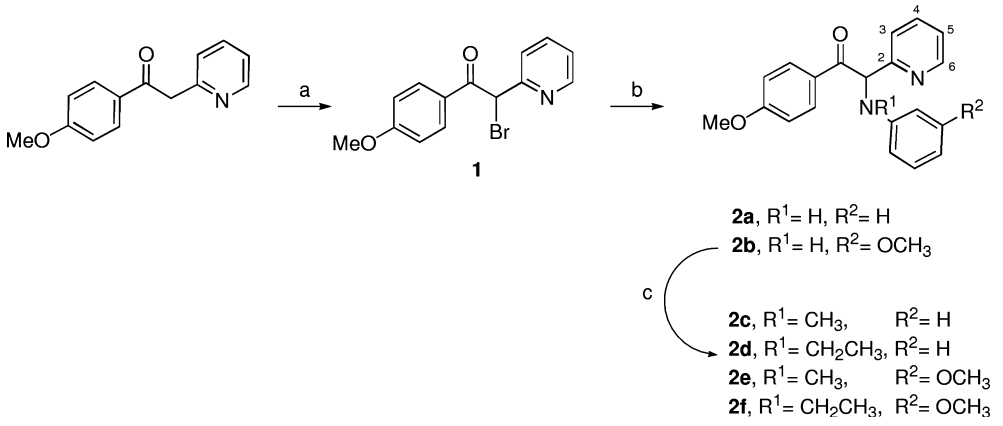
Ultraviolet–visible (UV–Vis) spectra were recorded on a Jasco V-550 spectrophotometer. Fluorescence spectra were acquired by photon counting on a Jobin-Yvon Fluorolog-3 spectrophotometer. All spectra were recorded at room temperature and are corrected for phototube sensitivity and by subtraction of the 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 5×10^{-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.

3. Results and discussion

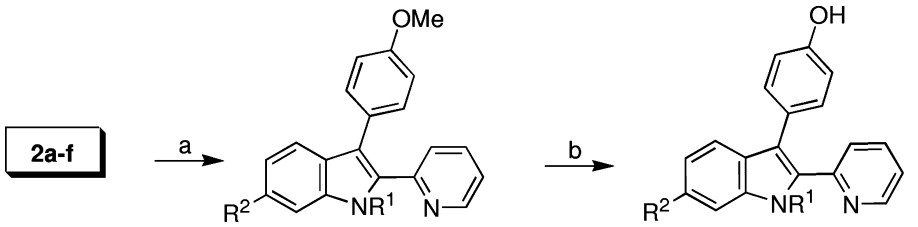
3.1. Chemistry

The synthesis of the amine substrates (**2a**–**f**) is illustrated in Scheme 1. More specifically treatment of 1-(4-methoxy-phenyl)-2-pyridin-2-yl-ethanone [15] with bromine in the presence of $AlCl_3$ furnished the monobromide **1** [16], which was subsequently reacted with aniline (or 3-anisidine) in the presence of triethylamine, to produce the desired α-anilinyl-(or 3-anisydyl)-2-pyridin-2-yl-ethanones **2a** and **b**. The latter, by reductive alkylation, provided efficiently the substrates **2c**–**f**.

The synthesis of indoles was accomplished by an acid catalyzed intramolecular cyclodehydration of the corresponding α-anilinyl (or 3-anisidyl)-2-pyridin-2-yl-ethanone derivatives (**2a**–**f**), a modified version of the classical Bischler method [17].



Scheme 1. Reagents and conditions: (a) Br_2, $AlCl_3$, THF; (b) $RC_6H_4NH_2$, Et_3N, CH_2Cl_2; (c) HCHO or CH_3CHO, $NaBH_3CN$, AcOH, CH_3CN.



3a, R^2= H, R^1= H
3c, R^2= H, R^1= Me
3d, R^2= H, R^1= Et
3e, R^2= OMe, R^1= Me
3f, R^2= OMe, R^1= Et

4a, R^2= H, R^1= H
4b, R^2= OH, R^1= H
4c, R^2= H, R^1= Me
4d, R^2= H, R^1= Et
4e, R^2= OH, R^1= Me
4f, R^2= OH, R^1= Et

Scheme 2. Reagents and conditions: (a) PPA, 70 °C; (b) BBr_3, CH_2Cl_2, −78 °C.

Best yields were obtained when the cyclodehydration reaction was performed using a polyphosphoric acid (PPA) solution at 70 °C, for 5–10 h. Under these experimental conditions the desired indole derivatives (**3a** and **3c**–**f**) were obtained (Scheme 2) in satisfactory yields, while there is no indication for the formation of any resinous side product. However, the molecule of anisidyl derivative **2b** under these (or more intense) experimental conditions failed to undergo the cyclodehydration reaction, furnishing a complex mixture of products.

Finally, the desmethylation of the indole derivatives was accomplished in excellent yields using a slight excess of BBr_3 in methylene chloride. On the other hand, the use of larger excess of BBr_3 resulted in the simultaneous cleavage of the *N*-alkyl bond to produce the 3-(4-hydroxyphenyl)-2-pyridin-2-yl-1*H*-indol-6-ol (**4b**).

3.2. Absorption and fluorescence properties

The ultraviolet/visible absorbance spectra of the synthesized indoles (**4a**–**f**) were measured in tetrahydrofuran, acetonitrile, ethanol, and water, under neutral, acidic (0.1 N HCl), and basic (0.1 N KOH) conditions. Absorbance measurements of indole **4a** in base, as well as all emission measurements in water were excluded from studies due to solubility problems. The results of the measurements are summarized in Table 1 and the spectra of compound **4b** in CH_3CN under neutral, acidic, and basic conditions are shown in Fig. 1. Comparison of the absorption bands of indoles in different solvents indicates that their absorbance spectra showed only limited solvatochromicity under neutral and acidic conditions. However, these compounds show environmental sensitivity, since their absorption intensities and wavelength were dependent on the pH of the solution.

The suitability of fluorescent probes for biological systems depends strongly on the extent to which their fluorescent properties are sensitive to their environment (solvent and pH) [18]. Thus, we have measured the fluorescence emission of all indoles (**4a**–**f**) in each of the aforementioned solvents and pH conditions using the major-longwavelength absorbance maximum as the excitation wavelength; in each case, wavelengths of maximum emission were measured. As expected, the fluorescence emission is relatively

Table 1
Long wavelength absorbance maxima for 2-pyridin-2-yl-1*H*-indol-6-ols (**4a**–**f**)

Compound	Conditions[a]	λ_{abs}^{max} (ε)			
		THF	CH_3CN	EtOH	H_2O
4a	Neutral	266 (4700) 353 (5900)	244 (4800) 280 (4500)	239 (4800) 349 (3700)	243 (5500) 324 (3400)
	Acid	223 (9500) 268 (4100)	259 (5200)	260 (5000)	253 (5400)
	Basic	[b]	[b]	[b]	[b]
4b	Neutral	326 (1600)	247 (6100) 320 (4500)	243 (10,100) 319 (4500)	244 (5100) 318 (3000)
	Acid	326 (6600) 367 (6200)	240 (19,400) 312 (9000)	362 (5600) 242 (10,100)	243 (5400) 320 (2200)
	Basic	275 (12,900) 333 (6800)	361 (11,400) 244 (9800) 313 (5300)	[b]	[b]
4c	Neutral	323 (2100)	247 (6100) 320 (4500)	238 (10,230) 317(4600)	244 (5100) 318 (3000)
	Acid	326 (6720) 367 (6200)	312 (9310) 358 (11,400)	362 (5600) 242 (10,100)	242 (5400) 322 (3110)
	Basic	[b]	243 (10,100) 309 (6100)	[b]	[b]
4d	Neutral	228 (10,700) 323 (5200)	244 (7600) 323 (5500)	229 (31,300) 278 (7600)	243 (7400) 313 (4300)
	Acid	233 (37,300)	247 (7400) 365 (4200)	322 (6000) 226 (51,000) 278 (7600)	249 (4600)
	Basic	[b]	244 (9500) 319 (5900)	[b]	245 (8500) 313 (4700)
4e	Neutral	329 (2430)	249 (6720) 322 (5000)	245 (11,100) 322 (5110)	246 (5870) 321 (3300)
	Acid	330 (7600) 372 (6900)	242 (22,500) 313 (9900)	366 (6100) 245 (11,500)	245 (5400) 321 (2450)
	Basic	277 (13200) 333 (7100)	362 (12,900) 246 (10,100) 315 (6110)	[b]	[b]
4f	Neutral	331 (2490)	250 (6900) 324 (6000)	247 (11,400) 323 (5180)	245 (6000) 322 (3400)
	Acid	331 (8200) 374 (7300)	243 (22,500) 314 (9900) 364 (13,600)	368 (7000) 247 (12,500)	247 (6400) 322 (2652)
	Basic	280 (13,330) 335 (8000)	248 (10,300) 318 (6230)	[b]	[b]
DPI[14]	Neutral	242 (10,100) 325 (14,700)	242 (11,600) 315 (5300)	258 (33,300) 296 (20,500) 330 (9600)	244 (25,400) 311 (11,200)
	Acid	239 (8900) 327 (10,500)	240 (15,200) 315 (6200)	251 (35,000) 297 (21,400) 334 (9500)	245 (23,800) 314 (9700)
	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.

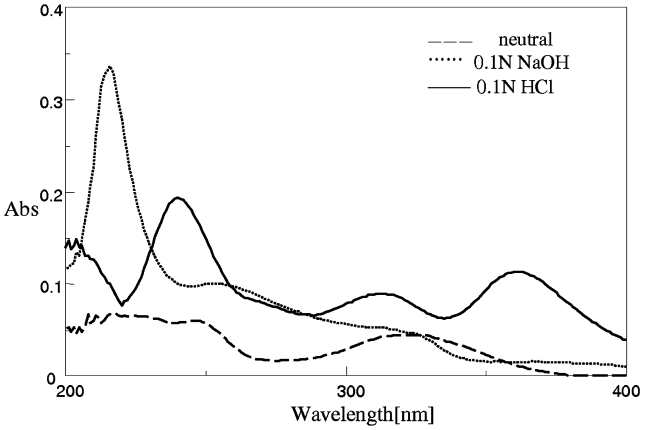


Fig. 1. UV absorbance spectra of 3-(4-hydroxy-phenyl)-2-pyridin-2-yl-1*H*-indol-6-ol (**4b**) in acetonitrile (5×10^{-6} M) under neutral, acidic (0.1 N HCl) and basic (0.1 N NaOH) conditions.

Table 2
Long wavelength emission maxima for indoles (**4a**–**f**)

Compound	Condition[a]	λ_{em}^{max}(relative intensity)[b]		
		THF	CH_3CN	EtOH
4a	Neutral	433 (73)	432 (47)	436 (1.4)
	Acid	[c]	[c]	[c]
	Basic	435 (24)	[d]	[d]
4b	Neutral	437 (84)	434 (55)	445 (2.6)
	Acid	[c]	[c]	[c]
	Basic	441 (26)	437 (23)	[d]
4c	Neutral	436 (78)	434 (49)	438 (2.1)
	Acid	[c]	[c]	[c]
	Basic	437 (27)	[d]	[d]
4d	Neutral	439 (115)	437 (61)	445 (36)
	Acid	[c]	[c]	[c]
	Basic	441 (74)	[d]	[d]
4e	Neutral	437 (79)	431 (48)	440 (2.4)
	Acid	[c]	[c]	[c]
	basic	435 (27)	[d]	[d]
4f	Neutral	439 (99)	438 (67)	445 (3.6)
	Acid	[c]	[c]	[c]
	Basic	442 (76)	[d]	[d]
DPI[14]	Neutral	435 (29)	407[e] (22)	427 (37)
		460[e] (22)	445 (31)	
	Acid	410 (31)	408[e] (23)	438 (46)
		430 (35)	440 (24)	464[e] (40)
		460 (24)		
	Basic	[d]	[d]	460 (39)

[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 ($\times 10^4$ cps) at λ_{em}^{max}.
[c] Quenched.
[d] Not soluble.
[e] Shoulder.

weak, because the aryl and pyridine ring are twisted out of planarity from one another. For comparison reasons, we have included in Tables 1 and 2 the corresponding data of **DPI** (2,3-bis-(4-hydroxyphenyl)-1*H*-indol-6-ol), the most efficient 4-hydroxyphenyl-indole [14].

Fluorescence emission spectra of compound **4b** in THF are shown in Fig. 2 and complete results on fluorescence measurements are given in Table 2 showing that in all solvents their emission maxima are slightly over 430 nm. The fluorescence emission of these compounds however, is environmentally (solvent and pH) dependent indicating that there is a large dipole moment in the excited state [19]. More specifically, the emission intensity was decreased sharply when the polarity of the solvent was increased. On the

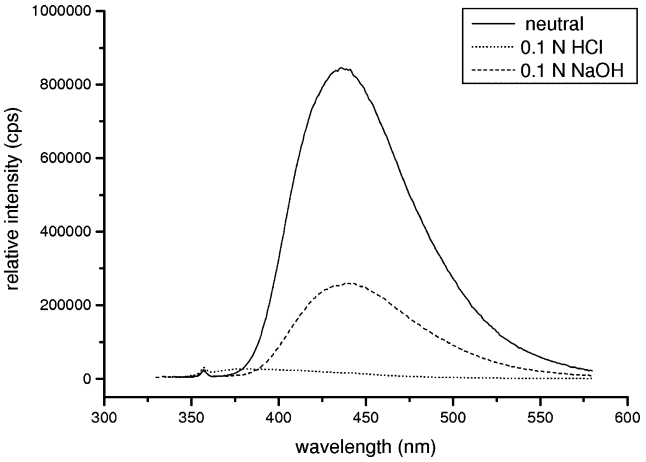
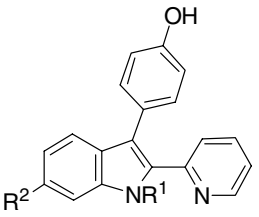


Fig. 2. Fluorescence emission spectra of 3-(4-hydroxy-phenyl)-2-pyridin-2-yl-1*H*-indol-6-ol (**4b**) in THF (5×10^{-6} M) under neutral, acidic (0.1 N HCl) and basic (0.1 N NaOH) conditions.

Table 3
Estrogen receptor binding affinity of indole derivatives (**4a**–**f**)



Compound	RBA	R^1	R^2
4a	0.82	H	H
4b	4.26	H	OH
4c	0.95	Me	H
4d	1.73	Et	H
4e	9.51	Me	OH
4f	14.30	Et	OH
DPI[14]	10.37		

other hand, although in the excited state the formation of the ionic form of the molecules is facilitated, no additional red shift was observed upon going from neutral to acidic and basic conditions [20]. In all cases when indoles were diluted in an acidic solution, the emission was quenched, while in basic pH the emission intensity was significantly decreased.

3.3. Estrogen receptor binding affinity

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

In every case, an increase in RBA is observed as the alkyl steric bulk and the lipophilicity in the center of the molecule are increased. As expected, the absence of a hydroxyl group from the position 6 of the indole ring led to compounds with low binding affinities for the estrogen receptor (compounds **4a**, **c**, and **d**). Thus, the three indol-6-ols (compounds **4b**, **e**, and **f**) displayed much better affinities to the ER than the other indoles. Their RBA values are comparable or better to those of the corresponding 2,3-bis-(4-hydroxyphenyl)-1*H*-indoles (e.g., **DPI**) and may be considered suitable for use in biological assays.

In conclusion, we have used a convenient and simple synthetic pathway to prepare a variety of novel 2-pyridin-2-yl-1*H*-indolol derivatives. The target compounds have been found to possess moderate to good affinities to the estrogen receptor, while their absorbance and fluorescence spectra display strong dependence on solvent polarity and pH. Since their synthesis does not require complicated stereospecific methods or isomer separations, these compounds constitute attractive candidates for further development in order to be used as fluorescent probes. Our current studies are directed towards the extension of these studies by covering additional derivatives and relevant heterocyclic systems.

References

[1] (a) A.S. Waggoner, in: P.L. Taylor, A.S. Waggoner, R.F. Murphy, F. Lanni, R.R. Birge (Eds.), Applications of Fluorescence in the Biomedical Sciences, Alan Liss, New York, 1986;
(b) D.L. Rousseau, Optical Techniques in Biological Research, Academic Press, New York, 1984, chapter 4.
[2] M. Adamazyk, R.E. Reddy, Z. Yu, Bioorg. Med. Chem. Lett. 12 (2002) 1283.
[3] C. Mazur Bowen, J.A. Katzenellenbogen, J. Org. Chem. 62 (1997) 7650, and references within.
[4] N.T. Van, M. Raber, G.H. Barrows, B. Barlogie, Science 224 (1984) 876.
[5] L.P. Pertschuk, K.B. Eisenberg, A.C. Carter, J.G. Feldman, Breast Cancer Res. Treat. 5 (1985) 137.
[6] C.G. Castles, S.A.W. Fuqua, in: J.R. Pasqualini, B.S. Katzenellenbogen (Eds.), Hormone Dependent Breast Cancer, Marcell Dekker, New York, 1996, pp. 81–105, Chapter 3, and references cited therein.
[7] (a) For examples of estrogen–fluorophore conjugates see: T.L. Fevig, J.E. Lloyd, J.A. Zablocki, J.A. Katzenellenbogen, J. Med. Chem. 30 (1987) 156;
(b) A.N. French, S.R. Wilson, M.J. Welch, J.A. Katzenellenbogen, Steroids 58 (1993) 157.
[8] (a) For examples of inherently fluorescent estrogens see: G.M. Anstead, J.L. Ensing, C.S. Peterson, J.A. Katzenellenbogen, J. Org. Chem. 54 (1989) 1485;
(b) G.M. Anstead, S.R. Wilson, J.A. Katzenellenbogen, J. Med. Chem. 32 (1989) 2163;
(c) G.M. Anstead, C.S. Peterson, J.A. Katzenellenbogen, J. Steroid Biochem. 33 (1989) 877;
(d) K.-J. Hwang, K.E. Carlson, G.M. Anstead, J.A. Katzenellenbogen, Biochemistry 31 (1992) 11536;
(e) R.S. Miksicek, K.E. Carlson, K.-J. Hwang, J.A. Katzenellenbogen, Mol. Endocrinol. 9 (1995) 592;
(f) M.J. Meyers, K.E. Carlson, J.A. Katzenellenbogen, Bioorg. Med. Chem. Lett. 8 (1998) 3589.

[9] (a) For examples of photofluorogenic estrogens see: R.D. Bindal, J.A. Katzenellenbogen, J. Steroid Biochem. 23 (1985) 929;
(b) R.D. Bindal, J.A. Katzenellenbogen, Photochem. Photobiol. 9 (1995) 592;
(c) A.W. Scribner, S.A. Haroutounian, K.E. Carlson, J.A. Katzenellenbogen, J. Org. Chem. 62 (1997) 1043.
[10] G.W. Gribble, in: M. Sainsbury (Ed.), Second Supplements to the Second Edition of Rodd's Chemistry of Carbon Compounds—Vol. IV—Heterocyclic Compounds, Pt. B, Elsevier, 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. 27 (1984) 1439.
[13] (a) For examples of novel indole derivatives see: T. Golob, R. Liebl, E. Von Angerer, Bioorg. Med. Chem. 10 (2002) 3941;
(b) G. Walter, R. Liebl, E. Von Angerer, J. Steroid Biochem. Mol. Biol. 88 (2004) 409.
[14] S.D. Koulocheri, S.A. Haroutounian, Eur. J. Org. Chem. (2001) 1723.
[15] S.A. Haroutounian, J.A. Katzenellenbogen, Photochem. Photobiol. 4 (1988) 503.
[16] B.E. Fink, D.S. Mortensen, S.R. Stauffer, Z.D. Aron, J.A. Katzenellenbogen, Chem. Biol. 6 (1999) 205.
[17] M.T. Baumgartner, M.A. Nazareno, M.C. Murguia, A.B. Pierini, R.A. Rossi, Synthesis (1999) 2053, and References therein.
[18] A.C. Stevens, R.P. Frutos, D.F. Harvey, A.A. Brian, Bioconj. Chem. 4 (1993) 19.
[19] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum Press, New York, 1983.
[20] J.F. Ireland, A.H. Wyatt, Adv. Phys. Org. Chem. 12 (1976) 131.
[21] J.A. Katzenellenbogen, H.J. Johnson, H.N. Meyers, Biochemistry 12 (1973) 4085.