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Synthesis and relative binding affinity to human steroid receptors of substituted 3-aryloxycoumarins

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Abstract

The synthesis of a set of substituted 3-aryloxycoumarins was performed. The study of the relations between their structure and their relative binding affinity to human androgen, progesterone, α and β estrogen receptors was achieved.

Keywords: Coumarins; Binding affinity; Steroid receptors

1. Introduction

The selective estrogen receptor modulators (SERMs) represent various compounds exemplified by Tamoxifene and Raloxifene presenting mixed agonist/antagonist activities depending on target tissues [1,2].

Tamoxifene and other triarylethylenes are widely used to treat hormone dependent breast cancer (promoted by estrogen receptors) due to their antiestrogen activity [3]. Furthermore, like estrogens, they present additional health benefits such as the prevention of postmenopausal decrease of bone mineral density leading to osteoporosis, fractures, vertebral crush [4,5] and to coronary heart disease [6]. However, their proliferative properties on the endometrium and the mammary cells [7] limits their long-term clinical use. Therefore, the availability of new SERMs is of great interest.

Most of the benefits of estrogens in the treatment of postmenopausal osteoporosis are associated with their ability to regulate bone turnover and to decrease bone resorption.

Estrogen regulation of bone resorption has been proposed to result from the ability of these hormones to prevent synthesis of, or response to several proinflammatory cytokines such as Il-α, Il-β, Il-6, or inflammatory cytokines (TGF-β) [8].

More recently, estrogens were also shown to regulate the expression or activity of different members of the TNFα superfamily critically involved in the control of osteoclastogenesis [9]. On the other hand, Iproflavone **1** and Genistein **2** [10] are potent bone resorption inhibitors, the former being already used in some countries. Their mechanism of action involves further the indirect increasing of calcitonin secretion by enhancing the effect of estrogen [11].

Previous research dedicated to 3-substituted coumarinic derivatives **3** [12] as well as to Coumestrol **4** [13], showed the presence of an estrogenic activity related to their structural analogy with the natural hormone estradiol **5**. Furthermore, a study of the relationships between the structure of various 3-substituted coumarins and their relative binding affinities (RBAs) to steroid receptors and to their antiproliferative activity

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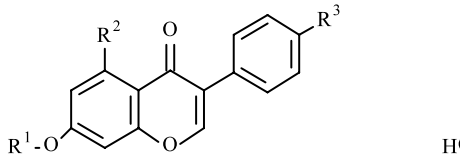
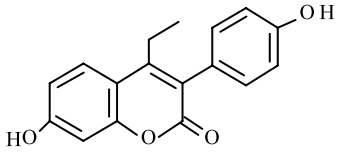
on human breast cancer MCF-7 cells, showed the importance of the substituents at positions 3 and 7 [14].

Taking into consideration these results and the fact that the estrogen receptor (ER) is regulated allosterically by association to a hydrophobic ligand [15], we decided to investigate the influence of a 3-phenoxy substituent in order to complete our knowledge on the relations between the structure of 3-aryloxycoumarins **6a**–**l** and their RBAs to human androgen, progesterone, α and β ERs. Furthermore, these derivatives could help to select compounds presenting favourable RBAs to the ERs leading to the design of new SERMs.



1 : R^1 : $CH(CH_3)_2$; $R^2 = R^3 = H$ = Iproflavone

2 : $R^1 = H$; $R^2 = R^3 = OH$: Genistein



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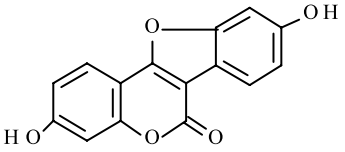

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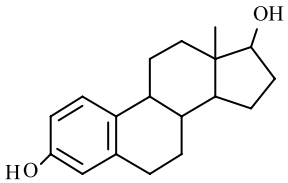

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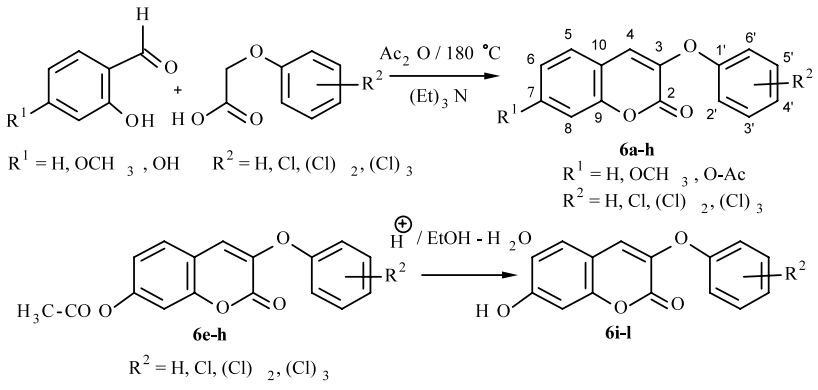
2. Synthesis

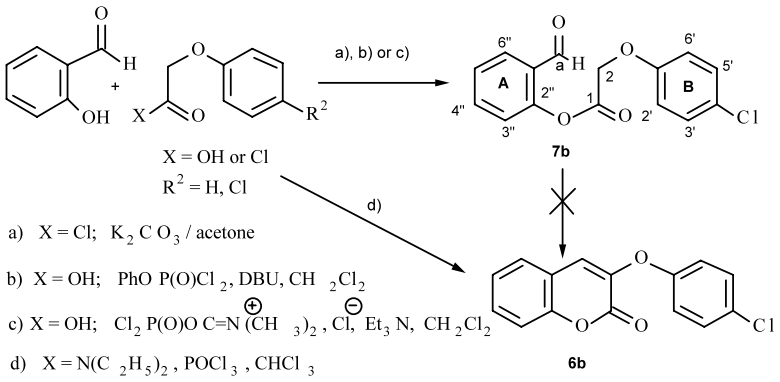
The preparations of the 3-aryloxycoumarins **6a**–**h** with various substitution patterns were performed via the modified Perkin–Oglialoro reaction [16] by condensation of conveniently susbstituted salicylaldehydes with aryloxyacetic acids in presence of triethylamine and acetic anhydride. The reactions using 2,4-dihydroxybenzaldehyde afforded the 7-acetoxycoumarins **6e**–**h** which were hydrolysed further to the corresponding 7-hydroxycoumarins **6i**–**l** (Scheme 1).

The yields of 3-substituted coumarins obtained with triethylamine as base, are generally better than those obtained in the classical Perkin–Ogliarolo reaction using the sodium salts of the corresponding aryloxyacetic acids both as reagent and as base [17]. However, they are not very high, due to the formation of various by-products including cinnamic type derivatives [18].

Several recent procedures allowing to increase the yields of coumarins are described. All these methods are based on the observation that in the Perkin condensation, the formation of cinnamic-type by-products is due to an intermolecular aldol type reaction prior to the cyclisation step. In order to avoid this possibility, these procedures use various conditions of in situ esterification of the 2-hydroxyl group of salicylaldehyde followed



Scheme 1.



Scheme 2.

by an intramolecular aldol condensation. One of them uses acid chloride in acetone/potassium carbonate [19]; the others are performed with various phosphorous reagents: *N,N*-dimethyl(dichloro)phosphoryloxymethylene)-ammonium chloride [20], phenyl dichlorophosphate [21] to condense acids, or phosphorous trioxychloride to condense the corresponding *N,N*-diethylamides [22].

In order to improve the yields of the desired 3-aryloxycoumarins **6**, we applied all these procedures to the synthesis of a model molecule, 3-(4-chlorophenoxy)coumarin (**6b**). Only in one case, we succeeded in its preparation starting from the amide, according to the latter technique [22]. The spectral properties and the melting point obtained for this compound are in agreement with those recorded for the same compound obtained by the Perkin reaction. However, this method turned to be unapplicable to the hydroxylated salicylaldehydes.

On the other hand, despite the literature data, none of the three other methods applied to the (4-chlorophenoxy)acetic acid and the salicylaldehyde as starting products and described on Scheme 2 led to the expected compound (no traces of the expected coumarin were detected by TLC), but an ester-type non-cyclized intermediate **7b** was always obtained as the major product (Scheme 2).

The compound **7b** was identified by spectral methods. Thus, its mass spectrum indicates a weak molecular pic at $m/z = 290$ (6.6%) corresponding to $C_{15}H_{11}O_4Cl$. The ester-aldehyde structure is confirmed by IR spectroscopy (carbonyl bands at 1690 and 1772 cm^{-1}); the aldehyde proton appears as a singlet at 10.0 ppm on ^1H NMR spectrum, both the aldehyde and the ester carbonyl signals are clearly distinguished at 189.3 and 167.1, respectively on ^{13}C NMR spectrum.

The structures of all prepared coumarin derivatives were established by elemental analysis or mass spectra and by IR, ^1H and ^{13}C NMR spectroscopy.

3. Experimental

3.1. Chemistry

The purity of all the compounds was routinely checked on the 'Riedel-de Haën 60 F_{254} special' silica gel plates (0.2 mm) and spots were located by UV lamp and/or by iodine vapors.

Melting points were taken on a Kofler bench and are uncorrected. Analyses (C,H) are within $\pm 0.4\%$ of the theoretical values. The ^1H and ^{13}C NMR spectra were recorded on a Brucker AC 300 in $CDCl_3$ or DMSO-d_6 using tetramethylsilane (TMS) as internal reference. Chemical shifts δ are in ppm and J in Hz. Splitting patterns are described as follows: (s) singlet; (d) doublet; (dd) doublet of doublet; (t) triplet; (q) quadruplet; (m) multiplet. The multiplicity of carbons was determined by J-modulation or DEPT experiences. The mass spectra (MS) were run on a 'Nermag R-1010C' mass spectrometer under electron impact (EI) conditions. Infrared spectra were obtained from potassium bromide pellets containing 0.5% of the product on a 'Perkin–Elmer' spectrophotometer (in cm^{-1}). Analyses (C,H) are within $\pm 0.5\%$ of the theoretical values.

The (3,4-dichlorophenoxy)acetic acid was prepared according to a described procedure [23]. All other aryloxyacetic acids are commercially available.

3.1.1. Preparation of 3-aryloxycoumarins 6: general method

In a round bottom flask, equipped with a magnetic stirring bar and a reflux condenser, aryloxyacetic acid (20 mmol), the substituted salicylic aldehyde (10 mmol), triethylamine (2.11 ml, 4.25 g, 42 mmol) and acetic anhydride (4.7 ml, 5.1 g, 50 mmol) were mixed and refluxed at 180–190 °C for 15–18 h. After cooling at room temperature, ice-water (100 ml) was added and the mixture stirred for 1 h. After extraction with ethyl acetate (3×50 ml), the mixed organic extracts were washed with a saturated $NaHCO_3$ solution, then with

brine, dried on Na_2SO_4, filtered and evaporated under reduced pressure. The resulting solid was washed with a small portion of methanol, filtered and the expected coumarin **6** was recristallized in ethanol.

The corresponding 7-acetoxycoumarins **6e**–**h** (0.5 g), were suspended in a mixture of ethanol (40 ml)–water (15 ml). A 15% sulfuric acid (5 ml) was added and the mixture heated at 40–50 °C (the progress of the reaction was monitored by TLC). At the end of the reaction, the solvent was evaporated under reduced pressure and the product recrystallized in ethanol.

*3.1.1.1. 3-Phenoxycoumarin (**6a**) ($C_{15}H_{10}O_3$).* Prepared from phenoxyacetic acid and salicylaldehyde. Yield: 27%. M.p.: 114–115 °C (ethanol), 115–6 [20], Lit.: 114–5 [21]. MS (EI): *m/z* (%): 238 (M^+; 29), 210 ($M^+ - C{=}O$; 3), 181 (11), 133 (13), 105 (5), 77 (18.5), 51 (100), 39 (30). IR (KBr cm^{-1}): 1730 (C=O); ^1H NMR (DMSO-d_6): δ 7.06–7.14 (m, 3H); 7.31 (m, 1H); 7.33–7.38 (m, 2H); 7.35 (m, 1H); 7.56 (m, 1H); 7.59 (s, 1H); 7.67 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 116.1 (1CH), 116.99 (1C), 118.1 (2CH), 122.5 (1CH), 124.8 (1CH), 128.1 (1CH), 129.2 (1C), 129.7 (2CH), 130.4 (1CH), 140.1 (1C), 151.8 (1C), 154.6 (1C), 156.2 (1C).

*3.1.1.2. 3-(4-Chlorophenoxy)coumarin (**6b**) ($C_{15}H_9O_3Cl$).* This compound was prepared as above from (4-chlorophenoxy)acetic acid and 2-hydroxybenzaldehyde**.** Yield: 35%. M.p.: 177–179 °C (ethanol). Lit.: 178–80 [24]. MS (EI): *m/z* (%): 272 (M^+; 9), 244 ($M^+ - C{=}O$; 0.6), 181 (4), 133 (13), 105 (15), 77 (33), 51 (100), 39 (30). ^1H NMR (DMSO-d_6): δ 7.22 (d, 2H, $^3J = 9.0$ Hz); 7.30 (m, 1H); 7.36 (m, 1H); 7.45 (d, 2H, $^3J = 9.0$ Hz); 7.56 (m, 1H); 7.69 (s, 1H); 7.67 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 116.0 (1CH), 116.99 (1C), 119.8 (2CH), 124.9 (1CH), 127.9 (1CH), 128.1 (1CH), 129.1 (1C), 129.9 (2CH), 130.4 (1CH), 139.9 (1C), 151.7 (1C), 154.7 (1C), 155.9 (1C). IR (KBr, cm^{-1}): 1728 (C=O).

*3.1.1.3. 3-Phenoxy-7-methoxycoumarin (**6c**) ($C_{16}H_{12}O_4$).* Prepared from phenoxyacetic acid and 2-hydroxy-4-methoxybenzaldehyde. Yield: 24%. M.p.: 121–2 °C (ethanol), Lit.: 122 [22]. ^1H NMR (DMSO-d_6):δ 6.97 (dd, 1H, $^3J = 8.8$, 7.07 (d, 1H, $^4J = 2.4$ Hz); 7.05–7.12 (m, 3H); 7.09 (d, 1H, $^4J = 2.4$ Hz); 7.32–7.37 (m, 2H, $^3J = 8.8$ Hz); 7.78 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 100.7 (1CH), 112.3 (1C), 112.8 (1CH), 117.1 (2CH), 122.4 (1CH), 128.2 (1CH), 129.2 (1C), 129.6 (2CH), 137.9 (1C), 154.3 (1C), 155.1 (1C), 161.0 (1C), 161.4 (1C). IR (KBr, cm^{-1}): 1728 (C=O).

*3.1.1.4. 3-(4-Chlorophenoxy)-7-methoxycoumarin (**6d**) ($C_{16}H_{11}O_4Cl$).* Prepared from (4-chlorophenoxy)acetic acid and 2-hydroxy-4-methoxybenzaldehyde Yield: 26%. M.p.: 138 °C (ethanol). ^1H NMR (DMSO-d_6): δ 3.86 (s, 3H, OCH_3); 6.98 (dd, 1H, $^3J = 8.7$ Hz); 7.07 (d, 1H, $^4J = 2.4$ Hz); 7.16 (d, 2H, $^3J = 9.0$ Hz); 7,26 (d, 2H, $^3J = 9.0$ Hz); 7.59 (d, 1H, $^3J = 8.7$ Hz); 7.76 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 100.7 (1CH), 112.2 (1C), 112.8 (1CH), 119.0 (2CH), 127,4 (1CH), 128.3 (1CH), 129.1 (1C), 129.8 (2CH), 137.4 (1C), 152.9[b] (1C), 155.2[b] (1C), 160.9 (1C), 161.5 (1C).

3.1.2. Preparation of 3-aryloxy-7-acetoxycoumarins and 3-aryloxy-7-hydroxycoumarins

These derivatives were prepared from 2,4-dihydroxybenzaldehyde and the corresponding aryloxyacetic acids. The coumarins were obtained as acetates at the 7-position. After separation, they were hydrolyzed to the corresponding 7-hydroxy derivatives.

*3.1.2.1. 3-Phenoxy-7-acetoxycoumarin (**6e**) ($C_{17}H_{12}O_5$).* Prepared from 2,4-dihydroxybenzaldehyde and phenoxyacetic acid. Yield: 28%. M.p.: 153–4 °C (ethanol). ^1H NMR (DMSO-d_6): δ 2.30 (s, 3H, CH_3), 7.13–7.21 (m, 4H); 7.32 (d, 1H, $^4J = 2.2$ Hz); 7.34–7.39 (m, 2H); 7.62 (s, 1H); 7.69 (d, 1H, $^3J = 8.5$ Hz); ^{13}C NMR (DMSO-d_6): δ 20.9 (1C, CH_3), 109.9 (1CH), 117.0 (1C), 117.8 (2CH), 119.0 (1CH), 124.1 (1CH), 125.3 (1CH), 128.7 (1C), 130.2 (2CH), 140.2 (1C), 151.2 (1C), 151.6 (1C), 155.6 (1C), 156.6 (1C), 169.1 (1C, C=O acetate).

*3.1.2.2. 3-(4-Chlorophenoxy)-7-acetoxycoumarin (**6f**) ($C_{17}H_{11}O_5Cl$).* Prepared from (4-chlorophenoxy)acetic acid and 2,4-dihydroxy-benzaldehyde. Yield: 36%. M.p.: 189–90 °C (ethanol). ^1H NMR (DMSO-d_6): δ 2.30 (3H, CH_3), 7.16 (dd, 1H, $^3J = 8.4$, $^4J = 2.2$ Hz); 7.18 (d, 2H, $^3J = 9.0$ Hz); 7.32 (d, 1H, $^4J = 2.2$ Hz); 7.39 (d, 2H, $^3J = 9.0$ Hz); 7.70 (d, 1H, $^3J = 8.4$ Hz); 7.71 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 20.9 (1C, CH_3), 109.9 (1CH), 117.0 (1C, 119.0 (1CH), 119.6 (2CH), 125.9 (1CH), 127.9 (1CH), 128.7 (1C), 129.9 (2CH), 139.9 (1C), 151.4 (1C), 151.7 (1C), 154.1 (1C), 154.7 (1C), 168.9 (1C, C=O acetate). IR (KBr, cm^{-1}): 1764 (C=O, acetate), 1733 (C=O, lactone), 1211 (C=O arom. ether); 3067 (C–H arom.).

*3.1.2.3. 3-(3,4-Dichlorophenoxy)-7-acetoxycoumarin (**6g**) ($C_{17}H_{10}O_5C$).* Prepared from (3,4-dichlorophenoxy)acetic acid and 2,4-dihydroxybenzaldehyde**.** Yield: 32%. M.p.: 180–1 °C (ethanol). ^1H NMR (DMSO-d_6): δ 2.31 (s, 3H, CH_3); 6.66 (dd, 1H, $^3J = 8.4$ Hz), 6.92 (d, 1H, $^4J = 2.2$ Hz); 7.72 (d, 1H, $^3J = 8.4$ Hz); 7.86 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 20.9 ($1CH_3$), 109.9 (1CH), 113.7 (1CH), 116.9 (1C), 118.7 (1CH), 119.1 (1CH), 125.2 (1C), 125.4 (1CH), 128.8 (1C), 131.2 (1CH), 131.8 (1C), 140.2 (1C), 151.3 (1C), 153.3 (1C), 155.5 (1C), 156.5 (1C), 169.2 (1C, C=O acetate). IR (KBr, cm^{-1}): 1754 (C=O, acetate), 1744 (C=O, lactone), 1205 (C–O, ether arom.); 3102 and 3067 (vC–H arom.).

*3.1.2.4. 3-(2,4,5-Trichlorophenoxy)-7-acetoxycoumarin (**6h**) ($C_{17}H_9O_5Cl_3$).* Prepared from 2,4,5-trichlorophenoxyacetic acid and 2,4-dihydroxybenzaldehyde. Yield: 20%. M.p.: 201–3 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 3H, CH_3); 7.19 (dd, 1H, $^3J = 8.4$ Hz); 7.35 (d, 1H, $^4J = 2.2$ Hz); 7.70 (d, 1H, $^3J = 8.4$ Hz); 7.82 (s, 1H), 7.90 (s, 1H); 8.01 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 20,9 (1C, CH_3), 109.9 (1CH), 116.9 (1C), 119.2 (1CH), 119.4 (1C), 119.4 (1CH), 125.5 (1CH), 126.1 (1C), 128.9 (1CH), 130.8 (1C), 131.3 (1CH), 140.1 (1C), 151.4 (1C), 151.6 (1C), 155.4 (1C), 156.4 (1C), 169,0 (1C, C=O acetate). IR (KBr, cm^{-1}): 1780 (C=O), 1732 (C=O), 3076 (C–H), 1187 (C–O).

*3.1.2.5. 3-Phenoxy-7-hydroxycoumarin (**6i**) ($C_{15}H_{10}O_4$).* Prepared by hydrolysis. Yield: 92%. M.p.: 174–5 °C (ethanol). MS (EI): *m/z* (%): 254 (M^+; 65), 226 ($M^+ - C{=}O$; 10), 197 (21), 149 (90), 121 (47), 105 (18), 77 (70), 65 (40), 51 (100), 39 (29). ^{1}H NMR (DMSO-d_6): δ 6.78 (d, 1H, $^4J = 2.2$ Hz); 6.81 (dd, 1H, $^3J = 8.4$, $^4J = 2.2$ Hz); 7.06–7.14 (m, 3H); 7.34–7.39 (m, 2H); 7.48 (d, 1H, $^3J = 8.4$ Hz); 7.67 (s, 1H); 10.6 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 102.1 (1CH), 110.9 (1C), 113.5 (1CH), 116.9 (2CH), 123.3 (1CH), 128.5 (1C), 129.4 (1CH), 129.9 (2CH), 136.7 (1C), 152.9 (1C), 156.1 (1C), 156.4 (1C), 160.1 (1C).

*3.1.2.6. 3-(4-Chlorophenoxy)-7-hydroxycoumarin (**6j**) ($C_{15}H_9O_4Cl$).* Prepared by hydrolysis. Yield: 95%. M.p.: 204 °C (ethanol). MS (EI): *m/z* (%): 288 (M^+; 70), 260 ($M^+ - C{=}O$; 3), 197 (10), 149 (100), 121 (43), 105 (13.5), 75 (89), 65 (91), 43 (74), 39 (44). ^{1}H NMR (DMSO-d_6): δ 7.08 (d, 1H, $^4J = 2.2$ Hz); 7.08 (d, 2H, $^3J = 9.0$ Hz); 7.13 (dd, 1H, $^3J = 8.5$, $^4J = 2.2$ Hz); 7.39 (d,2H, $^3J = 9.0$ Hz); 7.81 (d, 1H, $^3J = 8.5$ Hz); 8.07 (s, 1H), 10.62 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 102.2 (1CH,), 110.9 (1C), 113.7 (1CH), 118.7 (2CH), 127.2 (1CH), 129.4 (2C), 129.8 (2CH), 136.3 (1C), 153.1 (1C), 155.5 (1C), 157.2 (1C), 160.4 (1C). IR (KBr, cm^{-1}): 1688 (C=O).

*3.1.2.7. 3-(3,4-Dichlorophenoxy)-7-hydroxycoumarin (**6k**) ($C_{15}H_8O_4Cl_2$).* Prepared by hydrolysis. Yield: 93%. M.p.: 244–5 °C (ethanol). MS (EI): *m/z* (%): 324 ($M^+ + 2$; 100, 2 ^{37}Cl), 322 (M^+; 100, 2 ^{35}Cl), 149 (89), 121 (35), 105 (13), 65 (24), 51 (67). ^{1}H NMR (DMSO-d_6): δ 6.77 (d, 1H, $^4J = 2.3$ Hz); 6.82 (dd, 1H, $^3J = 8.6$, $^4J = 2.3$ Hz); 7.15 (dd, 1H–, $^3J = 8.6$, $^4J = 2.9$ Hz); 7.49 (d, 1H–, $^4J = 2.9$ Hz); 7.50 (d, 1H, $^3J = 8.6$ Hz); 7.59 (d, 1H, $^3J = 8.6$ Hz); 7.85 (s, 1H), 10.68 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 102.2 (1CH, C-8), 110.9 (1C), 113.6 (2CH), 118.7 (1CH), 125.2 (1C), 129.6 (1CH), 128.6 (1CH), 131.3 (1CH), 131.9 (1C), 135.7 (1C), 153.3 (1C), 156.1 (1C), 156.9 (1C), 160.6 (1C). IR (KBr, cm^{-1}): 1708 (C=O), 3320 (O–H).

*3.1.2.8. 3-(2,4,5-Trichlorophenoxy)-7-hydroxycoumarin (**6l**) ($C_{15}H_7O_4Cl_3$).* Prepared by hydrolysis. Yield: 88%. M.p.: 248–50 °C (ethanol). MS (EI): *m/z* (%): 358 ($M^+ + 2$; 8.6, 3 ^{37}Cl), 356 (M^+; 8.7, 3 ^{35}Cl), 323 (100, 3 ^{37}Cl), 321 (63, 3 ^{35}Cl), 149 (21), 121 (18), 105 (7), 65 (14). ^{1}H NMR (DMSO-d_6): δ 6.83 (dd, 1H, $^3J = 8.5$, $^4J = 2.2$ Hz); 6.78 (d, 1H, $^4J = 2.2$ Hz); 7.49 (d, 1H, $^3J = 8.5$ Hz); 7.68 (s, 1H), 7.86 (s, 1H–); 7.96 (s, 1H); 10.62 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ 102.2 (1CH, C), 110.9 (1C), 113.7 (1CH), 119.4 (1C), 119.4 (1CH), 126.1 (1C), 129.5 (1CH), 129.9 (1CH), 130.9 (1CH), 131.2 (1CH), 135.7 (1C), 151.5 (1C), 153.4 (1C), 156.6 (1C), 160.7 (1C).

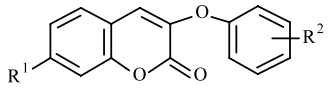
*3.1.2.9. 1-Formyl-2-hydroxybenzene 2-(4′-chlorophenoxy)ethanoate (**7b**) ($C_{15}H_{11}O_4Cl$).* Obtained according to a described procedure [20], (4-chlorophenoxy)acetic acid was condensed with salicylic aldehyde in presence of a readily available phosphorylating agent *N,N*-dimethyl(dichloro)phosphoryloxymethylene)ammonium chloride and triethylamine. The work-up led to a red oil which was treated with a small amount of pentane to afford a solide product identifed as 1-formyl-2-hydroxybenzene 2-(4′-chlorophenoxy)ethanoate (**7b**).

Obtained quantity: 8.25 g, yield: 86%. white needles, M.p.: 80 °C. MS (EI): *m/z* (%): 290 (M^+; 6.6), 135 (100), 111 (30), 92 (6), 65 (32), 39 (31). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.01 (s, 1H); 7.00 (d, 2H, $^3J = 9.0$ Hz), 7.21 (dd, 1H), 7.29 (d, 2H, $^3J = 9.0$ Hz), 7.47 (td, 1H), 7.67 (td, 1H), 7.88 (dd, 1H); 10.0 (s, 1H, OH). ^{13}C NMR (300 MHz, $CDCl_3$): δ 65.5 ($1CH_2$,), 116.1 (2CH), 123.4 (1CH), 126.91 (1CH), 126.93 (1CH), 127.8 (1CH), 129.5 (2CH), 133.4 (1CH), 135.4 (1CH), 149.8 (1C), 156.3 (1C), 167.1 (1C), 189.1 (1C, C=O). IR (KBr pellet, cm^{-1}): 3083, 2943, 2786, 1776 (C=O, ester), 1694 (C=O, arom. aldehyde).

3.2. Biological assay

The studies of the RBAs to the human progesterone, androgen and α and β ERs of the various prepared 3-aryloxycoumarins **6a**–**l** were determined according to previously reported procedures [25–28]. These are based on the competitive displacement of the tritium-labeled hormone from the receptor. The determination of RBAs to the α and β ERs were determined with Cos cells transiently transfected with expression plasmids for human ERs (pSG5ERα, obtained from Professor P. Chambon, IGBMC, Strasbourg, France and pSG5ERβ). RBAs were determined by incubating Cos cell cytosol for 24 h at 0 °C with either [^{3}H]-Estradiol (NEN Life Science products) with or without different concentrations of competitor steroids. Bound and free ligands were separated by the dextran-coated charcol

Table 1
Relative binding affinites to human steroid receptors of the various 3-aryloxycoumarins **6a**–**l**



Compounds	R^1	R^2	Human steroid receptors (24 h at 0 °C)		
			Progester. [a]	Androgen [b]	α and [β] Estrogen [c])
6a	H	H	0	0	0, [0]
6b	H	4-Cl	0	0	0, [0]
6c	OCH_3	H	0	0	0, [0]
6d	OCH_3	4-Cl	0	0	0, [0]
6e	OAc	H	0	0	0, [0]
6f	OAc	4-Cl	0	0	0, [0]
6g	OAc	m,p-(Cl_2)	< 0.1 B/B0 = 70% at 25 μM	0	0, [0]
6h	OAc	o,m,p-(Cl_3)	0	0	< 0.1 B/B0 = 75% at 25 μM, [0]
6i	OH	H	0	0	0, [< 0.1 B/B0 = 63% at 25 μM]
6j	OH	4-Cl	0	0	0, [0]
6k	OH	m,p-(Cl_2)	0	0	0, [0]
6l	OH	o,m,p-(Cl_3)	0	0	< 0.1 B/B0 = 60% at 25 μM, [0]

[a] Progesterone = 100.
[b] Testoterone = 100.
[c] Estradiol = 100 for α and β receptors.

method [26]. The RBAs of progestérone, testostérone and estradiol were taken as reference.

4. Results and discussion

The obtained results of RBAs to human progesterone, androgen and α and β ERs are indicated in Table 1.

The examination of the data presented in Table 1 shows that some of the studied 3-aryloxycoumarins present a weak RBA to human steroid receptors, lacking in selectivity. The derivative **6g**, shows a weak RBA to the progesterone receptor (PG) while the derivatives **6h**, **6i** and **6l** present a weak RBA to the ERs α or β. The unsubstituted 3-phenoxy-7-hydroxycoumarin **6i** presents a weak RBA to the ER β while the coumarins **6h** and **6l**, substituted by a lipophilic 3-(2,4,5-trichlorophenoxy) group, present a RBA to the ER α. Furthermore, the coumarins **6f** substituted by a 3-(4-chlorophenoxy) group and **6k** substituted by a 3-(3,4-dichlorophenoxy) group does not lead to a RBA to any type of receptor.

5. Conclusion

From this study it could be concluded that despite its limited yields, the Perkin type condensation showed to be the best procedure for the preparation of the whole set of the desired 3-aryloxycoumarins **6a**–**l**.

Furthermore, the coumarinic derivatives, substituted by a 3-aryloxy group lead to weak RBAs to human steroid receptors, lacking in selectivity and the presence of a lipophilic substituent on the 3-phenoxycoumarins does not increase the RBA to the ERs.

The synthesis of new derivatives is now in progress in order to improve our knowledge of the relationships between the structure and RBAs of substituted coumarins to ERs.

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