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Short communication

Synthesis and binding affinity to human α and β estrogen receptors of various 7-hydroxycoumarins substituted at 4- and 3,4- positions

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Abstract

The study of the relative binding affinity (RBA) to the human α and β estrogen receptors (ERs) of various 7-hydroxycoumarins substituted at 4- and 3,4- positions is weak and lacks in selectivity for both ERα and ERβ. The 4-(4-hydroxyphenyl)-7-hydroxycoumarin shows a weak RBA to ERβ and 3,4-diphenyl-7-hydroxycoumarin presents a stronger RBA to ERα than ERβ.

Keywords: Substituted-7-hydroxycoumarins; Binding affinity; α and β estrogen receptors

1. Introduction

The selective estrogen receptor modulators (SERMs) represent a wide class of compounds ranging from the mixed agonist/antagonist derivatives exemplified by Tamoxifen **1** and Raloxifene **2**, till the pure antagonists represented by ICI 182,780 (Fulvestrant) **3** and RU 58,668 **4** [1] (Fig. 1).

Tamoxifen **1**, is the first SERM belonging to the triarylethylene group, that has been widely used for the adjuvant treatment of hormone-dependant breast cancer, due to its antiestrogen action on mammary cells [2]. Furthermore, like estradiol, Tamoxifen presents additional health benefits such as the prevention of postmenopausal decrease of bone mineral density leading to osteoporosis, fractures, vertebral crush [3] and to coronary heart disease [4]. However, its proliferative properties on the endometrium [5] and the increase in the incidence of endometrial carcinoma [6] limits its long-term clinical use. Raloxifene **2** is another SERM presenting mixed agonist/antagonist activities. It is now currently recommended for hormone replacement therapy to increase bone mineral density and its serum cholesterol lowering properties would be interesting for the prevention of postmenopausal risk factors [7].

Among the pure antiestrogens, only Fulvestrant **3** is now used in the treatment of advanced breast cancer [8,9] although the pure antiestrogen RU 58,668 **4** might present the same therapeutic potential [10].

The bone formation and resorption is influenced by cytokines, growth factors and hormones. Most of the health benefits of estrogens in the treatment of postmenopausal osteoporosis are associated with their ability to regulate bone turnover and to decrease bone resorption. Estrogens regulate bone remodeling by modulating the synthesis of cytokines and growth factors, decreasing interleukine 6 (Il-6) mRNA expression [11] and inhibiting Il-6 induction by tumor necrosis factor [12].

Since many years, important research studies are devoted to natural phyto-estrogens and to their synthetic analogues. These derivatives can bind to the ERs and lead to agonist or antagonist activities through the activation or inactivation of certain genes [13–15]. In the case of Ipriflavone **6a** and Genisteine **6b** (Fig. 1) [16], their bone resorption inhibitory potency is already used in some countries and involves the indirect increasing of calcitonin secretion by enhancing the effect of estrogen [17].

The availability of new SERMs presenting agonist and/or antagonist activities would thus be of great therapeutic interest. Furthermore, the identification and cloning of the se-

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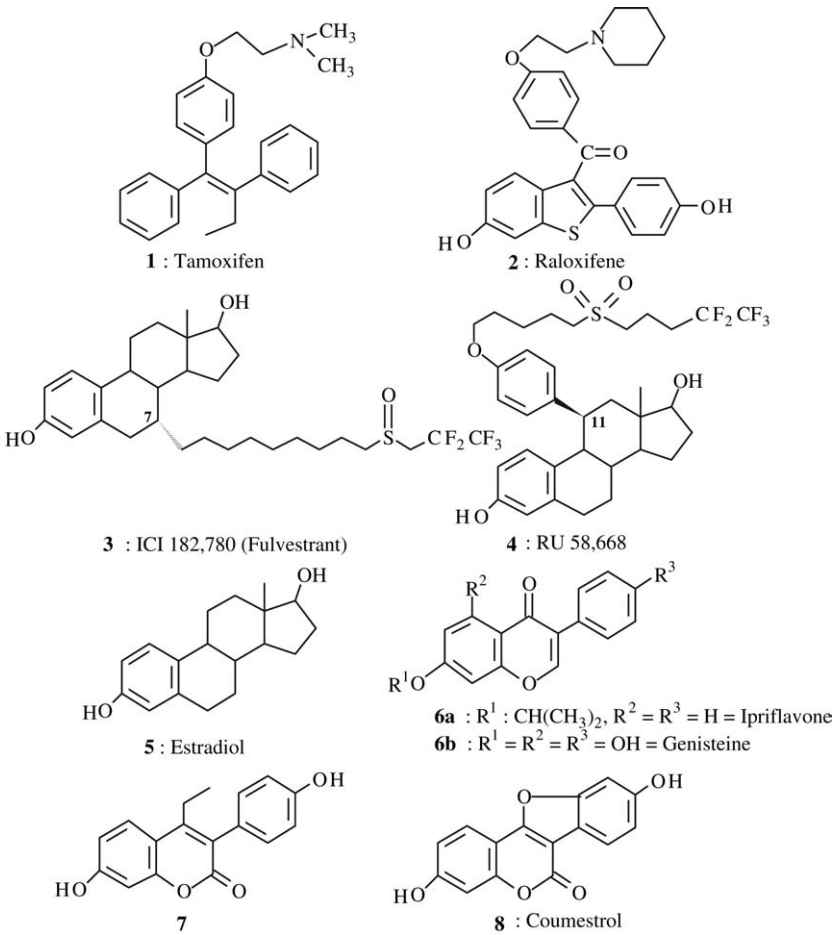


Fig. 1. Reference structures.

cond estrogen receptor (ERβ) in addition to the known (ERα) [18] opens up the perspective to prepare new selective ligands to either ERα and/or ERβ, with new potential biologic and pharmacologic applications, in relationship with the targeted tissue [19]. The research in this area has already led to ligands exhibiting a greater selectivity to ERα [20,21] and/or to ERβ [22] as well as for synthetic derivatives than for natural phytoestrogens [23].

Previous research dedicated to the 3-substituted coumarin **7** [24] and to coumestrol **8** [25] (Fig. 1) showed 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 substituted coumarins and their RBAs to the ERs, showed the importance of the substituents at positions 3 and 7 [26,27] and in the case of 3-(4-hydroxyphenyl)-7-hydroxycoumarin, the presence of a five times more potent RBA for the ERβ than for the ERα [28].

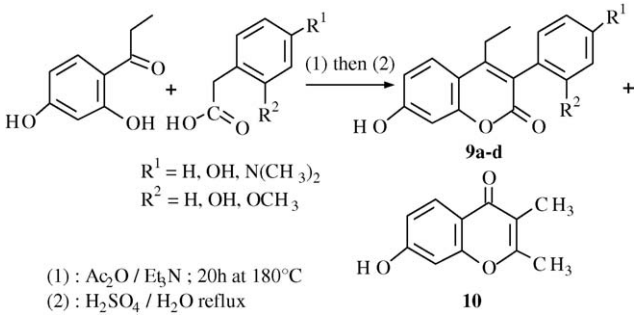
Taking into consideration these results, we decided to investigate the relationships between the structure and the RBAs to the human ERα and ERβ of some 4- and 3,4-disubstituted coumarins. Our objective was to look out for compounds capable to present selective RBAs to either ERα or Erβ which could lead to the design of potential tissue-selective SERMs. Therefore, the synthesis and the study of the RBAs to the ERs of various substituted coumarins was decided. Compounds **9a–e** were selected in order to study the influence of a 4-ethyl or a 4-hydroxy group and derivatives **11a–c** were chosen to study the effect of the presence of a 4-aryl and that of a 3,4-diphenyl group substituted on 7-hydroxycoumarin.

2. Synthesis

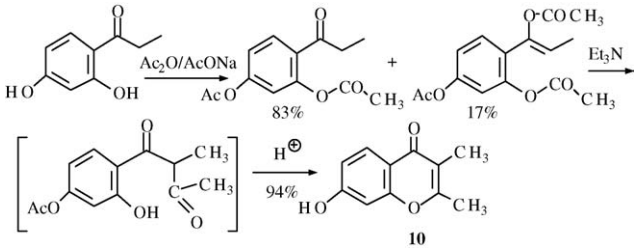
The preparation of the 3-aryl-4-ethyl-7-hydroxycoumarins **9a–d** with various substitution patterns was performed using the modified Perkin–Oglialoro reaction [29] by condensation of 2,4-dihydroxypropiophenone with conveniently substituted phenylacetic acids in presence of triethylamine and acetic anhydride. The reaction leads to a mixture of 7-acetoxy derivatives. After acid hydrolysis, the corresponding 7-hydroxycoumarins **9a–d** were obtained accompanied always by 2,3-dimethylchromone **10** (Scheme 1).

These results can be explained by the participation of two different reactions:

- the first one, is the Perkin–Oglialoro reaction leading to the formation of the expected coumarins **9a–d** [30,31],
- the second one, is the result of the acetylation of 2,4-dihydroxypropiophenone and that of its enol group lea-



Scheme 1.



Scheme 2.

ding to a mixture of 1-(2,4-diacetoxyphenyl)propane-1-one and 2-(α-acetoxy-propenyl)benzene-1,3-diol diacetate. These latter form by a Baker–Venkataraman rearrangement the intermediate 1,3-diketone which gives by cyclization the 2,3-dimethylchromone **10** [32,33] (Scheme 2). The mechanism of this reaction was confirmed by the acetylation of 2,4-dihydroxypropiophenone without the presence of the arylacetic acid. Only the mixture of 1-(2,4-diacetoxyphenyl)propane-1-one (83%) and 2-(α-acetoxy-propenyl)benzene-1,3-diol diacetate (17%) was obtained. On treatment of this mixture of acetates with triethylamine followed by acidification led to the expected pure 2,3-dimethylchromone **10** in 94% yield (Scheme 2).

The structure of the prepared coumarins **9a–d** and that of the chromone **10** was established by elemental analysis, IR, ^{1}H-NMR 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 not corrected. Analyses (C, H) are within ±0.4% of the theoretical values. The ^{1}H-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 proton at position 6 gives a doublet of doublet due to the ortho and meta couplings, respectively, with protons 5 and 8.

The infrared spectra (IR) were recorded on a Bruker "Vector 22" (ν in cm^{-1}). They present characteristic bands of the hydroxyl and the conjugated carbonyl groups.

The 3-(4-hydroxyphenyl)-4,7-dihydroxycoumarin **9e** [34], 4-aryl-7-hydroxycoumarins **11a–b** [35,36] and 3,4-diphenyl-7-hydroxycoumarin **11c** [37,38] are obtained according to the previously described procedures.

*3.1.1. Preparation of 3-aryl-4-ethyl-7-hydroxycoumarins **9a–d** and 2,3-dimethyl-chromone **10***

*3.1.1.1. 3-(2-Hydroxyphenyl)-4-ethyl-7-hydroxycoumarin **9d** ($C_{17}H_{14}O_4$).* Typical procedure

In a round bottom dry flask, equipped with a magnetic stirring bar and a reflux condenser topped by a calcium chloride drying tube, a solution of 2,4-dihydroxypropiophenone (1.66 g, 10 mmol), 2-hydroxyphenylacetic acid (3.04 g, 20 mmol), triethylamine (5.62 ml, 40 mmol) and acetic anhydride (4.71 ml, 50 mmol) are refluxed at 160 °C during 23 h. After cooling, the reaction mixture is poured in cold water (100 ml) and the product recovered by filtration, washed with water and dried. The hydrolysis is performed during 5 h (monitored by TLC) with a mixture of 10% sulfuric acid (20 ml) in a solution of ethanol (80 ml) and water (100 ml). After cooling, the mixture is poured in cold water (100 ml) and the solid is filtered off, washed till neutrality with water and dried. Recrystallization in an ether–chloroform mixture (7/3) gives 2,3-dimethylchromone **10**.

Yield: 114 mg (6.0%). mp 270 °C, Litt. 270 °C [39]; 271–272 °C [33].

IR: 3185, 1631, 1568, 1405, 1245, 859, 690.

^{1}H-NMR (DMSO-d_6): 1.9 (s, 3H, CH_3); 2.35 (s, 3H, CH_3); 6.75 (d, 1H, J = 2.2 Hz, arom.); 6.85 (d, 1H, J = 8.8 Hz, arom.); 7.85 (d, 1H, J = 8.8 Hz, arom.); 10.6 (s, 1H, OH).

^{13}C-NMR (DMSO-d_6): 176.17, 162.41, 161.61, 157.39, 127.08, 115.35, 114.84, 102.05, 18.44, 9.99.

The recovered solution upon concentration gives a crystalline solid of the expected coumarin **9d**. Yield 535 mg (19%) mp 262–264 °C (ethanol–water: 8/2).

IR: 3307, 1674, 1614, 1559, 1141, 1000, 757.

^{1}H-NMR (DMSO-d_6): 1.0 (t, 3H, CH_3), 2.5 (q, 2H, CH_2), 6.7–6.9 (m, 4H, arom.), 7.0 (d, 1H, arom.), 7.1 (t, 1H, arom.), 7.7 (d, 1H, arom.).

^{13}C-NMR (DMSO-d_6): 160.45, 155.45, 154.94, 154.42, 131.44, 131.12, 129.43, 127.04, 122.33, 119.67, 119.16, 115.99, 113.41, 111.25, 102.59, 22.86, 13.80.

*3.1.1.2. 3-(2-Methoxyphenyl)-4-ethyl-7-hydroxycoumarin **9b** ($C_{18}H_{16}O_4$).* Performed according to the typical procedure.

Yields: 123 mg (6.5%) of 2,3-dimethylchromone **10** and 590 mg (20%) of coumarin **9b** mp: 199 °C (ethanol–water: 8/2).

IR: 3412, 1683, 1566, 1470, 1245, 855, 755.

^{1}H-NMR (DMSO-d_6): 1.2 (t, J = 7.4 Hz, 3H, CH_3), 2.6 (q, 2H, CH_2), 3.7 (s, 3H, OCH_3), 6.74 (d, J = 2.2 Hz, 1H arom.), 6.83 (dd, J = 8.8 Hz and 2.4 Hz, 1H arom.), 6.97–7.19 (m, 3H arom.), 7.39 (td, J = 8.8 Hz and 1.8 Hz, 1H arom.), 7.65 (d, J = 8.8 Hz, 1H arom.)-7.6 (m, 7H, arom.), 10.47 (s, 1H, OH).

^{13}C-NMR (DMSO-d6): 161.02, 160.23, 157.33, 154.82, 154.32, 131.30, 129.86, 127.15, 123.98, 120.66, 119.46, 113.42, 111.64, 111.19, 102.61, 55.69, 22.84, 13.74.

3.1.1.3. 3-(4-Dimethylaminophenyl)-4-ethyl-7-hydroxycoumarin ***9a*** *(C_{19} H_{19} N O_3).* A mixture of 2,4-dihydroxypropiophenone (1.66 g, 10 mmol), 4-dimethylamino-phenylacetic acid (3.58 g, 20 mmol), acetic anhydride (4.2 ml, 50 mmol) and triethylamine (4.72 ml, 40 mmol) is refluxed during 23 h under anhydrous conditions according to the typical procedure. After cooling, the mixture is poured on cold water (100 ml) and the precipitate recovered by filtration. The black residue is hydrolysed overnight with a mixture of 10% sulfuric acid (50 ml) in a solution of ethanol (140 ml) and water (80 ml). The reaction mixture is poured in cold water (100 ml) and stirred during 4 h. The precipitate of 2,3-dimethylchromone 10 is collected and washed with water (yield 1.04 g, 54%). The solution after neutralization with sodium bicarbonate (4% solution) gives a brown precipitate of 3-(4-dimethylaminophenyl)-4-ethyl-7-hydroxycoumarin **9a**, which is recovered by filtration and washed with water.

Yield 1.40 g (46%); mp: 233 °C (ethanol–water: 8/2).

IR: 3367, 1674, 1611, 1559, 1518, 1347, 1136, 1097, 819, 803, 788.

^{1}H-NMR ($CDCl_3$): 1.1 (t, 3H, CH_3); 2.8 (q, 2H, CH_2); 3.0 (s, 6H, $N(CH_3)_2$; 6.7 -7.8 (m, 7H, arom.)

^{13}C-NMR (DMSO-d_6): 161.21, 160.77, 154.88, 153.34, 150.01, 130.72 (2C), 127.18, 122.65, 122.52, 113.35, 112.17 (2C), 111.43, 106.56, 102.51, 39.89, 22.62, 14.51.

3.1.1.4. 3-(4-Hydroxyphenyl)-4-ethyl-7-hydroxycoumarin 9c (C_{17} H_{14} O_4). Performed according to the typical procedure.

Yields: 2,3-dimethylchromone **10**: 110 mg (5.8%), and coumarin **9c** 507 mg (18%); mp: 315–317 °C (ethanol–water 8/2); 316–320°C [40,41].

IR: 3356, 1687, 1609, 1554, 1511, 1220, 1137, 829, 789.

^{1}H-NMR (DMSO-d_6): 1.2 (t, 3H, CH_3), 2.7 (q, 2H, CH_2), 6.8–7.1 (m, 4H, arom.), 7.2 (d, 2H, arom.), 7.8 (d, 1H, arom.), 9.7 (s br, 1H, OH), 10.7 (s br, 1H, OH).

3.1.2. Preparation of 2,3-dimethyl-7-hydroxychromone ***10*** *(C_{11} H_{10} O_3)*

A mixture of 2,4-dihydroxypropiophenone (1.66 g, 10 mmol) acetic anhydride (38 ml, 400 mmol) and sodium acetate (4.92 g, 60 mmol) is refluxed during 3 h. After cooling, the solution is filtered and the solvent evaporated. A visquous oil is obtained (1.10 g) constituted by a mixture of two acetates: 1-(2,4-diacetoxyphenyl)propane-1-one (83%) and 2-(α-acetoxy-propenyl)benzene-1,3-diol diacetate (17%).

^{1}H-NMR ($CDCl_3$) of 1-(2,4-diacetoxyphenyl)propane-1-one: 1.10 (t, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.9 (q, 2H, CH_2), 6.9 (d, 1H, arom.), 7.10 (dd, 1H, arom.), 7.8 (d, 1H, arom.).

^{1}H-NMR ($CDCl_3$) of 2-(α-acetoxy-propenyl)benzene-1,3-diol diacetate: 1.7 (d, 3H, CH_3), 2.0 (s, 3H, CH_3), 2.10 (s, 3H, CH_3),), 2.20 (s, 3H, CH_3), 5.70 (m, 1H, ethylenic), 6.90 (d, 1H, arom.), 7.00 (dd, 1H, arom.), 7.4 (d, 1H arom.).

To the previously obtained mixture of acetates (910 mg), triethylamine (1.01 ml, 7.2 mmol) is added and the mixture refluxed overnight under anhydrous conditions. After cooling, the reaction mixture is poured on a cold N hydrochloric acid solution (200 ml) and the mixture stirred during 4 h. The formed precipitate is recovered by filtration and washed with an ethanol–water mixture (9–1) giving 640 mg (94%) of pure 7-hydroxy-2,3-dimethylchromone **10**, (physico-chemical data reported previously).

3.2. Biological assay

The determination of the RBAs to the human ERα and ERβ was performed according to previously reported procedures [15,42,43] using Cos cells transiently transfected with expression plasmids for human estrogen receptors (pSG5ERα and pSG5ERβ, obtained from Prof. P. Chambon, IGBMC, Strasbourg, France). 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 charcoal method [44]. The estradiol was taken as reference compound with RBAs of 100% for both ERα and ERβ.

4. Results and discussion

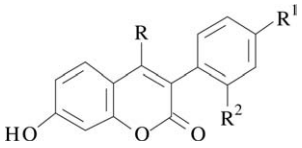
The obtained results are reported in Table 1 for the 3-phenyl-4-ethyl-7-hydroxycoumarins **9a–d** and for 3-(4-hydroxyphenyl)-4,7-dihydroxycoumarin **9e** and Table 2 for the derivatives **11a–c**.

The examination of the data reported in Table 1 indicates that the RBAs of these derivatives to both ERα and ERβ are weak and lacking in selectivity. However, the 3-(4-hydroxyphenyl)-4-ethyl-7-hydroxycoumarin **9c** shows stronger RBAs to both ERα and ERβ than the coumarins **9b, 9d** and **9a** substituted, respectively, by 3-(2-methoxyyphenyl), 3-(2-hydroxyphenyl) and by 3-(4-dimethylaminophenyl) groups. The same result is also observed with the coumarin **9e** presenting a 4-hydroxy substituent instead of the 4-ethyl group.

The RBAs obtained with the coumarins **11a–c** presenting a 4-phenyl or 3,4-diphenyl group are reported in Table 2.

The data show that 4-phenyl-7-hydroxycoumarin **11a** is devoid of RBA to any ER. However, the substitution of the 4-phenyl ring by a 4-hydroxy group as in the case of 4-(4-hydroxyphenyl)-7-hydroxycoumarin **11b** leads to the appearance of a weak RBA to the ERβ. This result is interesting as

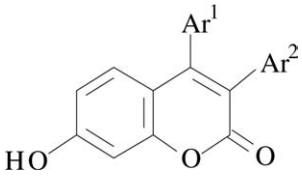
Table 1
RBAs to human α and β estrogen receptors of 3-aryl-4-hydroxy- and 3-aryl-4-ethyl-7-hydroxycoumarins



Compounds	R	R^1	R^2	Human estrogen receptors RBAs 24 h at 0 °C [a]	
				α receptors	β receptors
9a	C_2H_5	$N(CH_3)_2$	H	0.03	<0.02
9b	C_2H_5	H	OCH_3	0.2	0.08
9c	C_2H_5	OH	H	0.7	0.65
9d	C_2H_5	H	OH	0.02	0.04
9e	OH	OH	H	0.02	0.1

[a] Estradiol, 100 for both α and β estrogen receptors.

Table 2
RBAs to human α and β estrogen receptors of various 4-phenyl- and 3,4-diphenyl-7-hydroxycoumarinsv **11a–c**



Compound	Ar^1	Ar^2	Human estrogen receptors RBA 24 h at 0 °C [a]	
			α receptors	β receptors
11a	C_6H_5	H	00	00
11b	(p)HO-C_6H_4	H	00	0.2
11c	C_6H_5	C_6H_5	56.0	5.0

[a] Estradiol, 100 for both α and β estrogen receptors.

ERβ signalling might induce opposite effects to those of ERα [45] and play significant roles in the central nervous, cardiovascular and immune systems as well as on urogenital tract, bone, kidney and lung [46]. The substitution by a second phenyl group at position 4 as in the case of 3,4-diphenyl-7-hydroxycoumarin **11c** leads to a significant improvement of the RBAs to both ERs but with 10 times more selectivity to ERα than ERβ.

5. Conclusion

This study indicates that in comparison to estradiol, the 3-phenyl-4-ethyl-7-hydroxycoumarins and 3-(4-hydroxyphenyl)-4,7-dihydroxycoumarin present weak RBAs to both α and β ERs lacking in selectivity. The substitution by a second phenyl group at position 4 as for 3,4-diphenyl-7-hydroxycoumarin increases the RBAs to both estrogen receptors but with more selectivity to ERα than ERβ. More work is now in progress in the study of the RBAs of coumarins presenting various substitution patterns.

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