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Synthesis, pharmacological evaluation, and structure–activity relationships of benzopyran derivatives with potent SERM activity

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Abstract—The synthesis, binding affinity for estrogen receptor subtypes (ERα and ERβ) and pharmacological activity on rat uterus of a new class of potent ligands, characterized by a 3-phenylbenzopyran scaffold with a basic side chain in position 4, are reported. Some of these compounds, endowed with very high receptor affinity, showed potent inhibition of agonist-stimulated uterine growth, with no or limited proliferative effect. Binding affinity mostly depended on the nature and position of substituents at the 3-phenyl ring, while the uterine activity seems to be affected by basic chain length. Compound **9c** (CHF4227) showed excellent binding affinity and antagonist activity on the uterus. The docking of benzopyran derivatives explained the structure–affinity relationships observed for 3-phenyl substitution: a small, hydrophobic 4′-substituent could interact with a small accessory binding cavity, while di-substitution at 4′ and 3′ led to some ERα selectivity. This selectivity can be ascribed to differences in amino acid composition and side chain conformation in the region accommodating the 3-phenyl ring at human ERα and ERβ ligand-binding domain.
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1. Introduction

Estrogens are known to play an important role in the regulation of the development and maintenance of the female reproductive system, in particular of the uterus, ovaries and breast, through interaction with two different receptor subtypes, named ERα and ERβ,[1] which are members of the nuclear receptor superfamily of ligand-inducible transcription factors. Moreover, estrogens are involved in the growth and/or function of several other tissues such as the bone, liver, brain, and the cardiovascular system.[2] In fact, the decreased production of ovarian steroids, occurring after the climacteric, has been linked to typical menopausal disturbances, such as osteoporosis and coronary artery diseases.[3,4] Estrogen replacement therapy (ERT) and hormone replacement therapy (HRT), used for the prevention and treatment of these pathologies, are characterized by important side effects, greatly reducing the compliance of such treatments.[5–7] This has led to an increased interest in the search for new pharmacological approaches, such as the tissue-selective modulation of estrogen receptors.

In this light, at first tamoxifen,[8] and later a second generation of compounds with an agonist/antagonist behavior[9] were developed: raloxifene[10] (Fig. 1) was the first approved selective estrogen receptor modulator (SERM), marketed for the treatment and prevention of osteoporosis. The efforts to obtain compounds with a better activity profile led to a third generation of SERMs characterized by improved potency and absence of adverse effects, such as those on breast and the cardiovascular system.[11] These new compounds, briefly summarized in Figure 1, belong to different chemical classes, such as benzothiophenes (arzoxifene), indoles (pipindoxifene, bazedoxifene), tetrahydronaphthalenes (lasofoxifene), and benzopyrans (EM-800), albeit sharing with raloxifene the known pharmacophoric elements for SERM activity. These are a core scaffold, mimicking the

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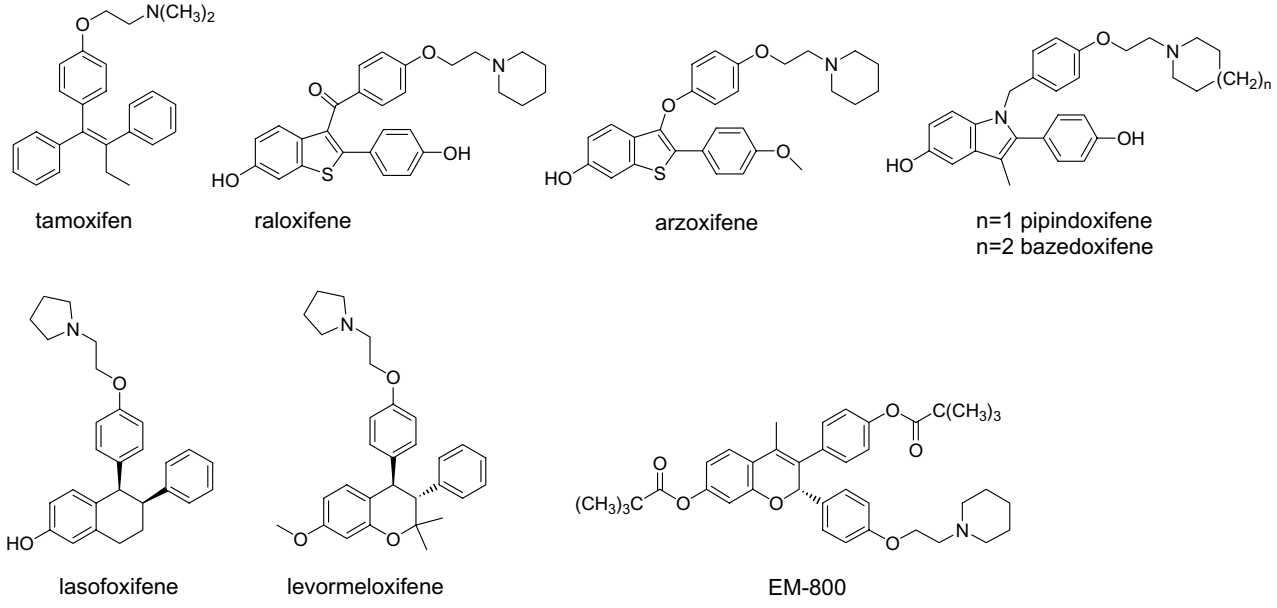


Figure 1. Structure of SERMs.

17β-estradiol structure (often in a stilbene-like arrangement)[9] with at least one hydroxyl group, known to confer high binding affinity for estrogen receptors (ERs).[12,13] Additionally, the feature most characteristic of many SERMs is a basic chain, necessary to achieve an antagonist action in some tissues, projecting from the middle of the core, generally through a phenolic spacer. In fact, SERMs appear to achieve their antiestrogen effect by inducing a conformational change on helix-12 of both the estrogen receptor subtypes, α and β, because of the necessity to accommodate the basic chain in the binding site.[14,15] Chemical modulations at the polar side chain of SERMs widely affect their activity, as has been shown by SAR exploration.[16] In particular, a coplanar arrangement between the basic side chain and the stilbene moiety, as in tamoxifen, produces uterine proliferating effects, while an orthogonal orientation between the two substructures, like that conferred by the carbonyl hinge of raloxifene, leads to an antagonist effect on the uterus.

In this light, we undertook a project aimed at the discovery of a new class of third generation SERMs, endowed with good binding affinity for the α and β estrogen receptors and with an improved pharmacological profile compared to that of raloxifene, for the treatment of postmenopausal pathologies such as osteoporosis, and of estrogen-dependent cancer. Our approach started from the hypothesis that 7-hydroxyisoflavone (7-hydroxy-3-phenyl-4*H*-1-benzopyran-4-one, **A** in Fig. 2), a metabolite of ipriflavone (Osteofix™, **B** in Fig. 2),[17] marketed for the treatment of osteoporosis, could be a good starting point for the preparation of compounds able to bind to estrogen receptors, even though the precise mechanism of ipriflavone action was not clear.[18,19] This hypothesis was supported by the presence of a stilbene-like substructure, typical of classical ER ligands, within the isoflavone structure, while the hydroxyl group at position 7 may mimic the phenolic hydroxyl of 17β-estradiol and raloxifene, which is known to be strongly involved in electrostatic interactions with a carboxyl group at both the receptor subtypes. Moreover, the carbonyl group at position 4 of the benzopyranone nucleus could be easily modified through a C–C forming reaction, and this gave us the possibility to attach a piperidinyl-alkoxy-phenyl side chain at that position. We decided to make the connection through a methylene hinge, thus allowing for an orthogonal disposition of the side chain with respect to the plane of the stilbene scaffold, in analogy with the carbonyl group of raloxifene. As stated above, it had been speculated that this specific arrangement could lead to a reduction of the uterotrophic effect.[16] As expected, the resulting compound (CHF4056, **9g** in Table 1) behaved like a new nonsteroidal estrogen agonist/antagonist, binding with high affinity to human ERα and ERβ, and displaying a very interesting pharmacological profile, because of its ability to prevent bone loss and its positive action on cholesterol levels, while maintaining antagonist effects on the uterus.[20] Moreover, **9g** and its derivatives presented the additional advantage over other benzopyran derivatives such as EM800 (Fig. 1) of being devoid of chiral centers, thus avoiding the problems related to racemization and optical purity.

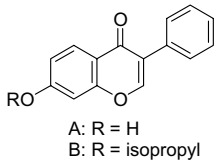


Figure 2. Isoflavone derivatives.

The present work reports the synthesis, pharmacological characterization, and structure–activity relationship (SAR) studies of a series of compounds belonging to the

Table 1. Binding affinity (pK_i) for ERα and ERβ, uterotrophic (% increase in uterine weight, IUW), and antiuterotrophic (% inhibition of EE2-induced uterine weight increase) activity of the tested compounds

					$pK_i\alpha$[a]	$pK_i\beta$[a]	$K_i\beta/K_i\alpha$	IUW[b,c]	Inhib.[b,d]
	raloxifene				10.15 ± 0.05	8.79 ± 0.09	22.9	23 ± 1	80 ± 4[e]
	revormeloxifene				9.57 ± 0.04	8.74 ± 0.12	6.8	112 ± 9	41 ± 5[f]
	4-hydroxytamoxifen				10.70 ± 0.02	9.97 ± 0.19	5.4	n.a.[g]	n.a.[g]
	tamoxifen				9.29 ± 0.04	9.20 ± 0.03	1.2	153[h]	50[h]
	R_1	R_2	*n*	*m*					
9g	H	OH	2	1	10.39 ± 0.12	9.80 ± 0.08	3.9	—[i]	11 ± 6
10a	H	OCH_3	2	1	9.59 ± 0.09	8.17 ± 0.09	26.0	—[i]	35 ± 4
9b	4-OH	OH	2	1	9.68 ± 0.03	9.28 ± 0.07	2.5	11 ± 1	25 ± 1
9c	4-OCH_3	OH	2	1	10.77 ± 0.05	10.00 ± 0.02	5.9	5 ± 1	86 ± 1
9d	4-CH_3	OH	2	1	10.49 ± 0.03	10.15 ± 0.03	2.2	20 ± 1	71 ± 4
9e	4-O-*n*-C_4H_9	OH	2	1	9.95 ± 0.03	8.96 ± 0.04	9.8	8 ± 1	59 ± 3
9f	3-OCH_3, 4-OCH_3	OH	2	1	10.15 ± 0.03	8.80 ± 0.03	22.4	—[j]	21 ± 1
9a	2-OCH_3	OH	2	1	10.35 ± 0.03	10.08 ± 0.03	1.9	—[j]	62 ± 3
9h	3-OCH_3	OH	2	1	9.87 ± 0.03	9.52 ± 0.03	2.2	2 ± 1[f]	10 ± 1
9i	4-OCH_3	OH	2	0	10.48 ± 0.03	10.04 ± 0.03	2.8	26 ± 1	54 ± 2
9l	4-OCH_3	OH	2	2	10.40 ± 0.03	9.82 ± 0.03	3.8	—[j]	42 ± 2
9j	H	OH	3	1	10.12 ± 0.03	9.74 ± 0.03	2.4	24 ± 2	20 ± 1
9k	4-OCH_3	OH	3	1	10.34 ± 0.03	9.53 ± 0.03	6.5	85 ± 3	30 ± 1

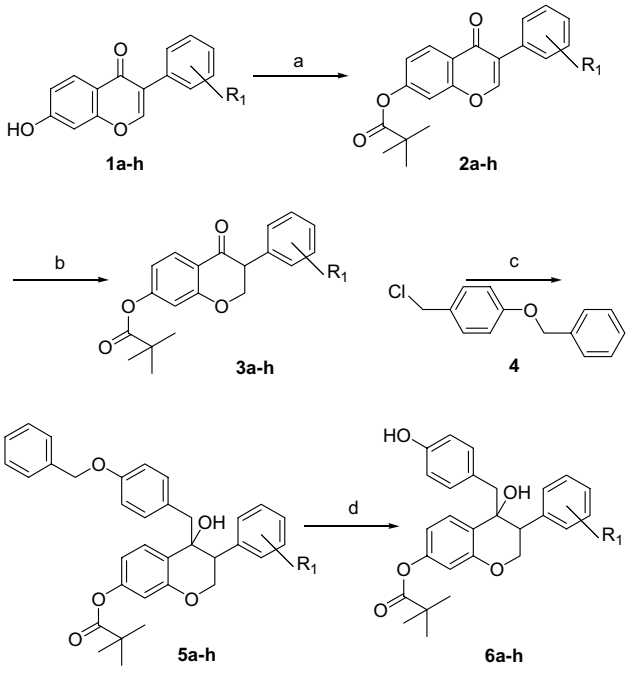
[a] Average of three determinations made in duplicate.
[b] $n = 8$.
[c] % Increase in uterine weight at 1 mg/kg, unless differently indicated.
[d] % Inhibition of 0.05 mg/kg day EE2-induced uterine weight increase at 0.1 mg/kg, unless differently indicated.
[e] At 1 mg/kg.
[f] At 10 mg/kg.
[g] Data not available.
[h] Ref. 8. $ED_{50} = 0.1$ mg/kg.
[i] Inactive at 1 mg/kg.
[j] Inactive at 10 mg/kg.

benzopyran class, developed to explore the possibility to obtain new derivatives with an improved SERM profile, lacking stimulating effects on the reproductive system. Starting from the reference compound **9g**, we investigated the importance of some chemical modulations on the binding affinity at the two estrogen receptor subtypes, and on in vivo uterotrophic and antiuterotrophic activity. The hydroxyl group at position 7 on the benzopyran nucleus was masked by an ether to evaluate its importance for receptor binding, and some substituents were added to the phenyl ring at position 3 (also called the 'distal ring'), to look for additional interactions at the binding site, in analogy with what had been observed for the 4′-OH group of raloxifene. Minor modifications were applied to the basic side chain, preparing longer derivatives and modulating the basic ring hindrance, in order to test the influence on binding affinity and pharmacological profile. Moreover, in order to rationalize the binding affinity data obtained, we built 3D-models of the ligand-binding domains of human ERα and ERβ, employing the crystallographic coordinates present in the Protein Data Bank (PDB)[21] and we performed docking studies with our compounds.

2. Chemistry

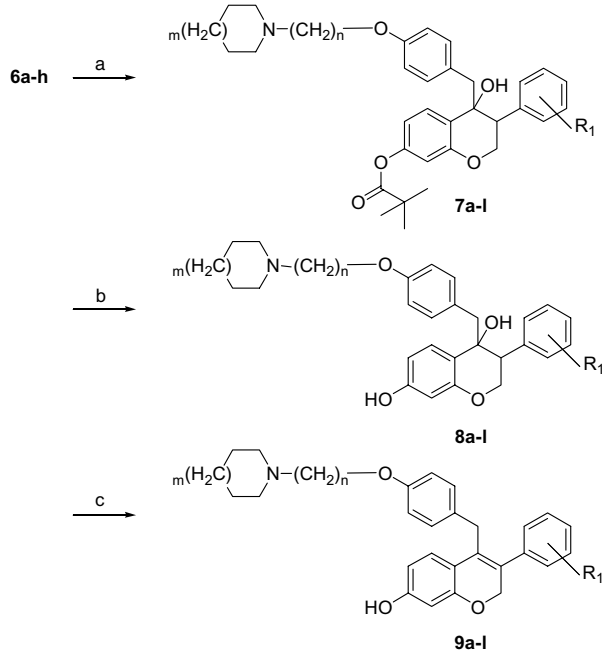
The benzopyran derivatives **9a**–**l** were obtained following a common synthetic route, which involves seven steps (Schemes 1 and 2), starting from the commercially available 7-hydroxy-isoflavone derivatives (compounds **1a**–**h**). Of all the possible protecting groups of the phenol hydroxyl in position 7, we chose the pivaloyl group since it was simple to introduce and remove, and it was not reactive in the Grignard addition.[22]

In detail, protection of the phenolic group of 7-hydroxy-isoflavones (**1a**–**h**) as a pivaloyl ester (step a in Scheme 1) gave compounds **2a**–**h**; the subsequent catalytic selective reduction of the carbon–carbon double bond gave compounds **3a**–**h**, which yielded compounds **5a**–**h** by reaction with the Grignard reagent obtained from compound **4**. After catalytic deprotection of the benzyl group, the free phenol hydroxyl of compounds **6a**–**h** was alkylated by reaction with the desired tertiary chloroalkyl amine in the presence of a base, to obtain compounds **7a**–**l**. For the synthesis of the benzopyran products **9a**–**l**, compounds **7a**–**l** were first deprotected from the pivaloyl group, furnishing compounds **8a**–**l**,



Compounds	R_1
1a, 2a, 3a, 5a, 6a	R_1= 2-OCH_3
1b	R_1= 4-OH
2b, 3b, 5b, 6b	R_1= 4-$OCOC(CH_3)_3$
1c, 2c, 3c, 5c, 6c	R_1= 4-OCH_3
1d, 2d, 3d, 5d, 6d	R_1= 4-CH_3
1e, 2e, 3e, 5e, 6e	R_1= 4-O-nC_4H_9
1f, 2f, 3f, 5f, 6f	R_1= 3-OCH_3, 4-OCH_3
1g, 2g, 3g, 5g, 6g	R_1= H
1h, 2h, 3h, 5h, 6h	R_1= 3-OCH_3

Scheme 1. Reagents and conditions: (a) K_2CO_3, pivaloyl chloride, acetonitrile, room temperature; (b) H_2, Pd/C, dioxane, room temperature; (c) Mg, THF dry, 70 °C then −20 °C to room temperature; (d) H_2, Pd/C, ethyl acetate, room temperature.



Compounds	R_1	n	m
7a, 8a, 9a	R_1= 2-OCH_3	2	1
7b	R_1= 4-$OCOC(CH_3)_3$	2	1
8b, 9b	R_1= 4-OH	2	1
7c, 8c, 9c	R_1= 4-OCH_3	2	1
7d, 8d, 9d	R_1= 4-CH_3	2	1
7e, 8e, 9e	R_1= 4-O-nC_4H_9	2	1
7f, 8f, 9f	R_1= 3-OCH_3, 4-OCH_3	2	1
7g, 8g, 9g	R_1= H	2	1
7h, 8h, 9h	R_1= 3-OCH_3	2	1
7i, 8i, 9i	R_1= 4-OCH_3	2	0
7j, 8j, 9j	R_1= H	3	1
7k, 8k, 9k	R_1= 4-OCH_3	3	1
7l, 8l, 9l	R_1= 4-OCH_3	2	2

Scheme 2. Reagents and conditions: (a) *N*-chloroalkyl-cycloalkylamine, K_2CO_3, acetone, reflux; (b) CH_3OH/H_2O, K_2CO_3 room temperature; (c) acetonitrile or ethanol, HCl, reflux.

which were dehydrated to give the desired products **9a**–**l** (Scheme 2). Compound **9b** was obtained by protecting (step a of Scheme 1) and deprotecting (step b of Scheme 2) both the phenolic hydroxyls as pivaloyl esters. Compound **10a** was obtained by methylation of compound **9g** with methyl iodide.

3. Pharmacology

Binding affinity to human ERα and ERβ was determined by the inhibition of [^{3}H]-17β-estradiol binding, in accordance with the reported procedure for estrogen receptors.[23] pK_i values were calculated from concentration response curves of test compounds.

Estrogen agonist and antagonist activity was tested in vivo using an immature female rat model[24] since in these animals, in the absence of endogenous estrogen, the uterus is fully responsive to exogenous estrogen and allows for the ready measurement of an agonist and antagonist action. We used the increase in uterine wet weight as a measure of estrogenic activity, while antiestrogenic activity was assessed by evaluating the ability of a compound to block estrogen-stimulated uterine weight gain.

4. Results and discussion

Table 1 reports the pK_i values for the benzopyran derivatives at ERα and ERβ and their in vivo effects. The uterine pharmacological profile was investigated because the uterotrophic effects represent one of the most important drawbacks for SERMs. Agonist activity is expressed as percent of uterine weight gain at 1 mg/kg of tested compounds and the antagonist effect as percent inhibition of 17α-ethynylestradiol (EE2) induced weight gain on immature female rat, at 0.1 mg/kg. For comparison, pK_i values and uterine profile activity were also evaluated for two reference SERMs: the marketed raloxifene, and levormeloxifene. In addition, we measured the binding affinity of tamoxifen and of its metabolite 4-hydroxytamoxifen (OHT). All the benzopyran derivatives **9a**–**l** and **10a** showed good binding affinity for both receptor subtypes and inhibited estrogen-stimulated uterus growth at doses lower than 1 mg/kg (po). Interestingly, some derivatives (e.g., **9c** and **9a**) resulted as being significantly more potent in vivo than the unsubstituted compound **9g**, despite the small differences in their pK_i values; this could be due to differing metabolic stability or access to the target. Most of the tested com-

pounds showed uterotrophicity similar to raloxifene, giving no or limited weight increase; the only exception was compound **9k**, characterized by a longer side chain.

The role of the basic side chain in the modulation of receptor intrinsic activity has been widely recognized. As a result of the side chain modulation, we found a good agreement with the SARs previously reported for raloxifene derivatives. In fact, an optimal pharmacological profile was achieved when the chain attached to the methylene hinge was a 4-(piperidinoethoxy)phenyl one.[16] Compounds **9j** and **9k**, having a propoxy chain, showed a limited decrease in binding affinity and in vivo antiuterotrophic activity, although, as stated before, compound **9k** led to an increase in uterus weight. At the same time we observed that, although cyclic amine modifications are tolerated by the receptor binding site, ring contraction or enlargement, as in compounds **9i** and **9l**, led to a decrease in antiuterotrophic activity compared to compound **9c**.

The 7-OH group, mimicking the 3-OH one of the A-ring of estradiol, was silenced by a methyl ether in order to evaluate its importance on binding affinity. In analogy with SAR studies on estradiol and 2-arylbenzothiophene[12] derivatives, a loss in binding affinity at ERα and ERβ was observed, with a 10-fold reduction of potency between **10a** and **9g**.

Observing the crystal structures of the complexes ERs-raloxifene, it is possible to note that the 2-phenyl ring is accommodated in a hydrophobic region, and that the 4′-OH group undertakes a hydrogen bond interaction with a flexible histidine. Grese et al.[12] have underlined the importance of this interaction, in particular observing that the replacement of the raloxifene 4′-hydroxyl group with a hydrogen atom caused a 5-fold affinity decrease versus ERα. In contrast, compound **9b** in our benzopyran series, having a 4′-hydroxyl group, exhibited lower affinity, on both ERα and ERβ, than the unsubstituted compound **9g**. We thought that SAR differences between 2-arylbenzothiophenes and 3-arylbenzopyrans could arise from different binding modes. This was in accordance with the observations of Egner et al.[25] arising from the superposition of the ligand-binding domain of all known human ERα structures, co-crystallized with different ligands. In fact, this superposition has evidenced that the steroidal A-ring was tightly fixed, in contrast to the D-ring region, where substituents of differing size and shape could be accommodated, suggesting greater flexibility than in other regions of the ligand-binding site. In fact, comparing the crystal structures of the complexes ERα-OHT and ERα-raloxifene, it can be observed that His524, the last amino acids of helix 11 and a β-turn, linking helices 6 and 7, show different conformations. In particular, the absence of the hydroxyl group in the 'estradiol D-ring region' induces His524 to move backward, allowing for a deeper penetration of the OHT phenyl ring into the hydrophobic pocket at the ligand-binding domain site.

In this context, we supposed that our benzopyran class may arrange the distal phenyl ring in the ligand-binding domain like OHT rather than like raloxifene, as the stilbene substructure in the 3-phenylbenzopyran scaffold can be superimposed to the unconstrained stilbene fragment of OHT with a better fitting than to that of the 2-phenylbenzothiophene in raloxifene. This binding mode, which is illustrated in Figure 3, may explain the affinity decrease observed for **9b**, having a 4′-OH group, compared to the parent compound **9g**, owing to the placement of a polar group in a hydrophobic environment. We thus explored the possibility of increasing affinity and in vivo activity by introducing a small hydrophobic group at the 4′-position of the distal ring. Compounds **9c** and **9d**, having a methoxy and a methyl group, respectively, gained a significant increase of affinity compared to **9b**, resulting as being more potent than the parent **9g**. A butoxy group was introduced at the 4′-position to test the steric tolerance of the binding pocket; the significant decrease in affinity of compound **9e** indicated that there is not enough room to accommodate bulky groups. The methoxy group was also moved to position 2′ (**9a**) or 3′ (**9h**), and a 3′,4′-disubstituted derivative (**9f**) was prepared. As a result of this limited exploration, we observed that while the substitution in 2′ was tolerated by both receptors, the methoxy group in 3′ caused a 3-fold reduction in binding affinity on ERα and a 2-fold one on ERβ, compared to compound **9g**. Although compound **9f** had an intermediate affinity between compounds **9c** and **9h** on ERα, it showed an unexpected drop of affinity on ERβ. The pharmacological data in Table 1 show that, by introducing a small lipophilic group in 2′ and 4′, the antagonist effect on uterine growth is improved: in fact, **9a** and **9c** gave higher inhibition of EE2-induced uterine weight increase. Moreover, these compounds showed low (**9c**) or no (**9a**) uterotrophic activity at the highest doses tested.

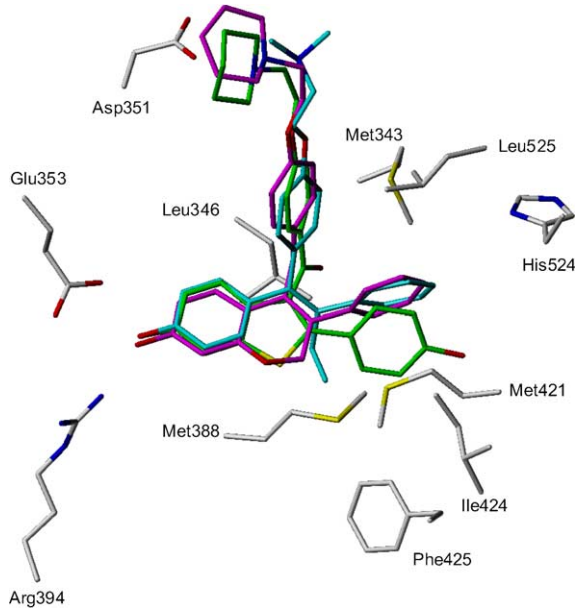


Figure 3. Superposition of 4-hydroxytamoxifen (cyan carbons), raloxifene (green carbons), and **9g** (magenta carbons) into ERα ligand-binding site deriving from the crystal structure 3ERT, displaying the side chains of the principal amino acids involved in ligand recognition.

In order to rationalize the SAR data obtained for the benzopyran compounds, the models of the ligand-binding

domains of human ERα and ERβ were built, starting from the crystallographic coordinates of ER-ligand complexes, and used for docking studies. The observation that the introduction of a 4′-OH decreases the binding affinity, while, at same position, small lipophilic groups are generally tolerated, suggested that the binding of benzopyran derivatives does not involve His524 as a H-bond acceptor, similarly to what was observed for the ERα-OHT complex. Based on this hypothesis, we used the high resolution crystal structure of the complex ERα-OHT (PDB code 3ERT) to build a refined model of human ERα, while the building of the human ERβ model started from the coordinates of the rat ERβ-raloxifene complex (PDB code 1QKN), the crystallographic coordinates of the human ERβ in complex with OHT being unavailable.

Compound **9g** and its derivatives were docked into the ER binding sites superimposing their benzopyran scaffold to the styrene fragment of OHT. While this scaffold was rather rigid, the basic side chain was much more flexible; the conformation that provided the best fit into the binding channel, allowing for interaction of the basic nitrogen with Asp351 (in ERα, corresponding to Asp303 in ERβ), was chosen. In particular, the methylene hinge allowed for an orthogonal arrangement of the side chain with respect to the benzopyran scaffold, and the ethyloxy chain adopted a *gauche* conformation. After ligand orientation, a minimization of the ligand-receptor interaction energy was performed. The result obtained for **9g** docked into ERα is illustrated in Figure 3, where the minimized structures of OHT and raloxifene are also represented for a comparison. Although the side chain of **9g** better fitted that of raloxifene, the distal phenyl ring of **9g** resulted as being strictly superimposed to that of OHT. The distal ring was surrounded by several hydrophobic amino acids, in particular Met343 and Leu346, lining the so-called beta face[14] of the ligand-binding domain pocket, Leu384, Met388, and Ile424, delimiting the alpha one; at the same time, Met421 defined the bottom wall, while His524 and Leu525 formed the side border. The docking of **9g** into the ERβ binding site evidenced an analog network of interactions, where most amino acids were of the same type, with the exception of Met336 in helix-5 of ERβ, corresponding to Leu384 in ERα, and Ile373 in helix-7 of ERβ, corresponding to Met421 in ERα. In the ERβ ligand-binding domain, Phe377 and Ile376 adopted conformations different from those assumed in ERα by the corresponding Phe425 and Ile424; their spatial disposition is that observed in the crystal structures of the beta ligand-binding domain in complex with raloxifene, genisteine, and the pure antagonist ICI164,384;[15,26] This could be due to the different steric properties of Met421 (in ERα) and Ile373 (in ERβ), and probably reflects a conformational equilibrium, which can be affected by ligand binding. This difference resulted in a projection of the phenyl ring of Phe377 into the hydrophobic binding pocket of ERβ, whose volume is further reduced by the side chain conformation of Ile376.

Docking simulations for compounds having a substituent in position 4′ showed that it can be accommodated in an accessory binding pocket created by the backward shift of His524/475 not involved in H-bonding with the ligand, opposite to what was observed in the complexes with raloxifene. This supposed binding mode, resembling that of OHT, may explain the small increase in affinity observed for the methoxy and methyl derivatives, **9c** and **9d**, and the significant decrease in affinity of the hydroxy derivative **9b**. Moreover, the conformation of the distal phenyl ring was a little different for **9c** (green structure in Fig. 4) in the two binding sites, owing to the differing shapes of the hydrophobic pockets. In both cases, however, the limited size of the accessory binding pocket led to bumping interactions when the 4′-butoxy derivative **9e** was docked into the ligand-binding domain. The docking of compound **9h** (orange structure in Fig. 4) showed that the 3′-methoxy group can be accommodated at the ERα binding site close to Met343, and steric clashes could account for the slight decrease in affinity; this also happened at the ERβ binding site, the 3′-methoxy group being positioned close to Met295, owing to a slight rotation of the distal phenyl ring of **9h**. On ERα, the 3′,4′-dimethoxy derivative **9f** had an intermediate affinity, between those of compounds **9c** and **9h**, and it assumed the same binding orientation as the other derivatives represented in Figure 4A; in contrast, on ERβ the two methoxy groups could not be accommodated in the same way, mainly because of the different orientation of Phe377, and they were forced into a crowded region of the hydrophobic binding pocket (yellow structure in Fig. 4), leading to a decrease in affinity. The differing interactions led to a 22-fold selectivity of **9f** for ERα, and this result suggests that additional steric hindrance in 3′ and 4′ regions may be useful to further increase subtype selectivity, as also suggested by the small selectivity observed for the 4′-butoxy derivative **9e**. The differences in steric tolerance at ERα and ERβ may also explain the different selectivity observed for some recently reported compounds of the tetrahydroisoquinoline series:[27] in fact, those derivatives having a bulky substituent at the 2-phenyl ring, such as a *p*-*i*Pr or a *m*-NMe_2, are characterized by the greatest α selectivity, mainly due to a drop in ERβ affinity. The similarity between the binding modes of this class and ours is evidenced by the comparison of the crystal structure of 2-phenyl-1-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-1,2,3,4-tetrahydroisoquinolin-6-ol in complex with ERα ligand-binding domain (PDB code 1UOM) with that of OHT and raloxifene (PDB codes 3ERT and 1ERR,[14] respectively). In fact, the 2-phenyl ring of the tetrahydroisoquinoline derivative occupies the same region of space as that of OHT, slightly apart from the 2-phenyl ring of raloxifene, as proposed for the benzopyran derivatives by our docking models.

The *ortho*-substituted derivative **9a** (not shown) placed the methoxy group in a small cavity, corresponding to that occupied by the ethyl tail emerging from the stilbene scaffold of OHT.

5. Conclusions

3-Phenyl-benzopyran derivatives having a basic side chain, connected to position 4 through a methylene

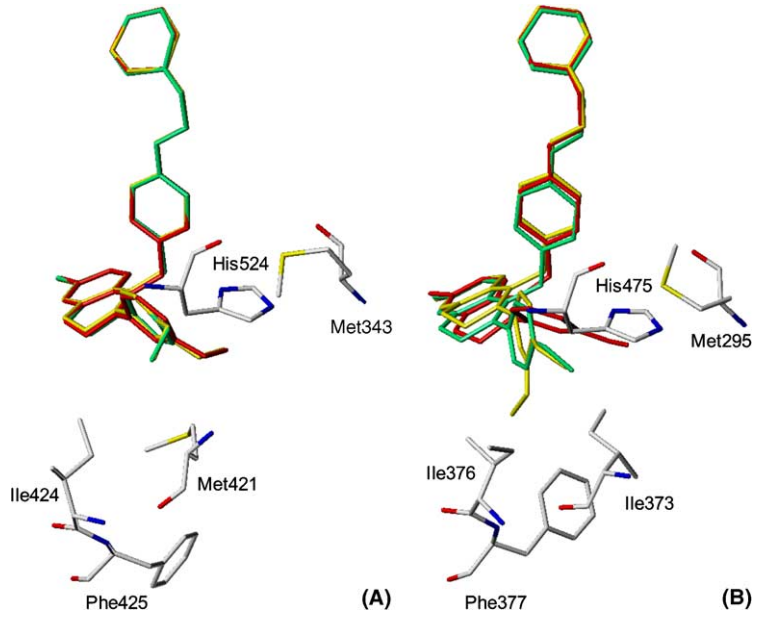


Figure 4. Compounds **9c** (green), **9f** (yellow), and **9h** (orange) docked into the binding site of ERα (A) and ERβ (B). Residues interacting with the distal ring of the ligands are shown. A small lipophilic cavity between His524/475 and Ile424/376 can accommodate a substituent of limited size.

hinge, represent a new class of potent ERα and β ligands, with high antiuterotrophic activity and no or reduced stimulation of uterine proliferation. The 4′-methoxy derivative **9c** is endowed with a receptor affinity higher than that of known SERMs, and therefore warrants further investigation for its therapeutic potential.[28,29] This series of compounds probably binds to ERs in a manner similar to the traditional classes, like those of OHT and raloxifene. On the other hand, some differences were observed in their SAR profile: a 4′-hydroxy group seems not to interact with His524/475, which interacts with the phenol hydroxyl of raloxifene, and a 4′-methoxy or methyl group is optimal for receptor binding. Moreover, the 3′,4′-dimethoxy derivative evidenced some selectivity for the ERα subtype, and the docking into receptor models suggested a possible way to design selective compounds. In fact, it was possible to observe a reduced steric tolerance of the so-called D-ring region at the ERβ ligand-binding domain, compared to the ERα one, which may be ascribed to different amino acids and side chain conformations. Therefore, steric modulation of fragments occupying this region can be critical for subtype selectivity, as observed in other series of ER ligands.

6. Experimental

6.1. Chemistry

All solvents and reagents were used as obtained from commercial sources, unless otherwise indicated. Dry tetrahydrofuran was purchased from Fluka and used without further distillations. Flash chromatography was carried out on Isolute columns SI (silica cartridges). TLC analyses were performed on Merck Silica gel 60 F_{254} UV sensitive plates. Melting points were measured on a Büchi 535 instrument. Purity of compounds **9a**–**l**, and **10a** were analyzed by two different HPLC methods. Method 1: on a Rainin-Dinamax HPLC instrument; Column: Lichrochart C18 RP Select B (4.6×250 mm; 5 μm); isocratic elution A/B = 40/60 (where A was a pH = 3 phosphate buffer and B was a mixture CH_3CN/CH_3OH = 8/2); flow 1 mL/min; λ = 230 nm. Method 2: on a Waters 2695 HPLC system equipped with a DAD detector and a ZQ 2000 single quadrupole MS spectrometer (used in ES+mode); column: Waters C18 XTerra MS (4.6×50 mm; 2.5 μm); gradient elution: at 0 min 100% A, at 5 min 100% B, at 6 min 100% A, at 10 min 100% A (where A = water/CH_3CN/formic acid = 95/5/0.05 and B = CH_3CN/water/formic acid = 95/5/0.05); flow = 0.5 mL/min. All samples were >98% pure with both methods. The ES^+/MS spectra were obtained on a QUATTRO LC Micromass and on a ZQ Waters instrument; the high resolution mass spectra (HRMS) were obtained on a Q-TOF micro Micromass instrument (ES^+ mode). NMR spectra were recorded on a Bruker AC200 spectrometer. The abbreviations THF and DMSO refer to tetrahydrofuran and dimethyl sulfoxide.

6.2. 2,2-Dimethyl-propanoic acid 3-(2-methoxyphenyl)-4-oxo-4*H*-1-benzopyran-7-yl ester (2a)

K_2CO_3 (7.8 g; 56.4 mmol) was added to a suspension of compound **1a** (10.2 g; 38 mmol) in acetonitrile (170 mL). After some min at rt, when basic pH was achieved, pivaloyl chloride (6.8 g, 56.4 mmol) was added dropwise. After 1 h at rt, water was added (350 mL) to obtain a precipitate, which was filtered. The solid was washed with water, then dissolved in methylene chloride (100 mL); residual water was separated and the organic fraction was dried on Na_2SO_4 and evaporated under vacuum, obtaining a yellow solid (12.44 g; yield = 93%).

The product was used for the subsequent steps without any purification.

^{1}H NMR (200 MHz, DMSO-d_6) 1.34 (s, 9H, 3CH_3), 3.72 (s, 3H, Ar–O–CH_3), 6.93–7.15 (m, 2H, Ar-*H*), 7.20–7.32 (m, 2H, Ar-*H*), 7.33–7.45 (m, 1H, Ar-*H*), 7.52–7.56 (d, $J = 2.26$ Hz, 1H, Ar-*H*), 8.10–8.18 (d, $J = 8.76$ Hz, 1H, Ar-*H*), 8.34 (s, 1H, Ar–O–C*H*=C); MS (ESI) m/e 352 (M^+).

6.3. 2,2-Dimethyl-propanoic acid 4-[7-(2,2-dimethyl-1-oxopropoxy)-4-oxo-4*H*-1-benzopyran-3-yl]-phenyl ester (2b)

The title compound was prepared in quantitative yield from **1b** by a method similar to that described for **2a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.32–1.34 (2s, 18H, 6CH_3), 7.13–7.22 (d, $J = 2.07$ Hz, 2H, Ar-*H*), 7.26–7.34 (dd, $J = 2.07$ Hz, $J = 8.62$ Hz, 1H, Ar-*H*), 7.56–7.59 (d, $J = 2.07$ Hz, 1H, Ar-*H*), 7.61–7.69 (d, $J = 8.96$ Hz, 2H, Ar-*H*), 8.15–8.23 (d, $J = 8.62$ Hz, 1H, Ar-*H*), 8.59 (s, 1H, Ar-*H*); MS (ESI) m/e 422 (M^+).

6.4. 2,2-Dimethyl-propanoic acid 3-(4-methoxyphenyl)-4-oxo-4*H*-1-benzopyran-7-yl ester (2c)

The title compound was prepared in quantitative yield from **1c** by a method similar to that described for **2a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.34 (s, 9H, 3CH_3), 3.79 (s, 3H, Ar–O–CH_3), 6.95–7.05 (d, $J = 8.97$ Hz, 2H, Ar-*H*), 7.23–7.31 (dd, $J = 2.07$ Hz, $J = 8.96$ Hz, 1H, Ar-*H*), 7.48–7.58 (m, 3H, Ar-*H*), 8.13–8.21 (d, $J = 8.96$ Hz, 1H, Ar-*H*), 8.49 (s, 1H, Ar–C=C*H*); MS (ESI) m/e 352 (M^+).

6.5. 2,2-Dimethyl-propanoic acid 3-(4-methylphenyl)-4-oxo-4*H*-1-benzopyran-7-yl ester (2d)

The title compound was prepared in quantitative yield from **1d** by a method similar to that described for **2a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.35 (s, 9H, 3CH_3), 2.35 (s, 3H, Ar–CH_3), 7.20–7.31 (m, 3H, Ar-*H*), 7.44–7.55 (m, 3H, Ar-*H*), 8.14–8.21 (d, $J = 8.86$ Hz, 1H, Ar-*H*), 8.48 (s, 1H, Ar–O–C*H*=C); MS (ESI) m/e 336 (M^+).

6.6. 2,2-Dimethyl-propanoic acid 3-(4-butoxyphenyl)-4-oxo-4*H*-1-benzopyran-7-yl ester (2e)

The title compound was prepared in quantitative yield from **1e** by a method similar to that described for **2a**.

^{1}H NMR (200 MHz, DMSO-d_6) 0.88–1.00 (t, $J = 7.19$ Hz, 3H, CH_3), 1.34 (s, 9H, C–[CH_3]$_3$), 1.38–1.56 (m, 2H, CH$_3$–CH_2–CH$_2$), 1.62–1.80 (m, 2H, CH$_3$–CH$_2$–CH_2), 3.95–4.05 (t, $J = 6.46$ Hz, Ar–O–CH_2), 6.95–7.03 (d, $J = 8.85$ Hz, 2H, Ar-*H*), 7.23–7.31 (d, d, $J = 2.15$ Hz, $J = 8.61$ Hz, 1H, Ar-*H*), 7.48–7.56 (m, 3H, Ar-*H*), 8.13–8.21 (d, $J = 8.61$ Hz, 1H, Ar-*H*), 8.48 (s, 1H, Ar–O–C*H*=C); MS (ESI) m/e 394 (M^+).

6.7. 2,2-Dimethyl-propanoic acid 3-(3,4-dimethoxyphenyl)-4-oxo-4*H*-1-benzopyran-7-yl ester (2f)

The title compound was prepared in quantitative yield from **1f** by a method similar to that described for **2a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.34 (s, 9H, 3CH_3), 3.79 (s, 6H, Ar–[O–CH_3]$_2$), 6.98–7.05 (d, $J = 8.38$ Hz, 1H, Ar-*H*), 7.13–7.23 (m, 2H, Ar-*H*), 7.24–7.32 (dd, $J = 2.15$ Hz, $J = 8.61$ Hz, 1H, Ar-*H*), 7.53–7.56 (d, $J = 1.92$ Hz, 1H, Ar-*H*), 8.15–8.22 (d, $J = 8.86$ Hz, 1H, Ar-*H*), 8.53 (s, 1H, Ar–O–C*H*–C=C); MS (ESI) m/e 382 (M^+).

6.8. 2,2-Dimethyl-propanoic acid 3-phenyl-4-oxo-4*H*-1-benzopyran-7-yl ester (2g)

The title compound was prepared in quantitative yield from **1g** by a method similar to that described for **2a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.34 (s, 9H, 3CH_3), 7.25–7.33 (dd, $J = 2.09$ Hz, $J = 8.64$ Hz, 1H, Ar-*H*), 7.37–7.50 (m, 3H, Ar-*H*), 7.52–7.64 (m, 3H, Ar-*H*), 8.13–8.24 (d, $J = 8.64$ Hz, 1H, Ar-*H*), 8.54 (s, 1H, Ar–O–C*H*–C=C); MS (ESI) m/e 322 (M^+).

6.9. 2,2-Dimethyl-propanoic acid 3-(3-methoxyphenyl)-4-oxo-4*H*-1-benzopyran-7-yl ester (2h)

The title compound was prepared in quantitative yield from **1h** by a method similar to that described for **2a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.34 (s, 9H, 3CH_3), 3.80 (s, 3H, Ar–O–CH_3), 6.90–7.00 (m, 1H Ar-*H*), 7.12–7.20 (m, 2H, Ar-*H*), 7.25–7.40 (m, 2H, Ar-*H*), 7.55–7.60 (d, $J = 2.00$ Hz, 1H, Ar-*H*), 8.15–8.22 (d, $J = 8.75$ Hz, 1H, Ar-*H*), 8.55 (s, 1H, Ar–O–C*H*=C); MS (ESI) m/e 352 (M^+).

6.10. 2,2-Dimethyl-propanoic acid 3,4-dihydro-3-(2-methoxyphenyl)-4-oxo-2*H*-1-benzopyran-7-yl ester (3a)

Compound **2a** (4.2 g; 11.9 mmol) was dissolved in acetone (120 mL), Pd 5%/C (50% water) (3 g) was added and the mixture was hydrogenated (30 psi, rt) for 9 h. After filtering, the solution was evaporated under vacuum and purified by flash chromatography (CH_2Cl_2/petroleum ether = from 90/10 to 70/30). After evaporation of the solvent, ~2.5 g of **3a** were obtained (yield = 60%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.25–1.90 (2m, 6H, 3CH_2), 2.80–3.10 (m, 2H, N$^+$–CH_2), 3.32–3.55 (m, 4H, N$^+$–[CH_2]$_2$), 3.72 (s, 2H, Ar–CH_2–C=C), 4.24–4.42 (t, $J = 5.18$ Hz, 2H, Ar–O–CH_2–CH$_2$–N$^+$), 4.91 (s, 2H,

Ar–O–CH_2–C≡C), 6.17–6.31 (m, 2H, Ar-*H*), 6.81–6.96 (m, 3H, Ar-*H*), 7.04–7.17 (d, $J = 8.63$ Hz, 2H, Ar-*H*), 7.23–7.47 (m, 5H, Ar-*H*), 9.58 (s, 1H, Ar–O*H*), 10.18–10.48 (br, 1H, N^+–*H*); MS (ESI) *m/e* 354 (M^+).

6.11. 2,2-Dimethyl-propanoic acid 4-[7-(2,2-dimethyl-1-oxopropoxy)-3,4-dihydro-4-oxo-2*H*-1-benzopyran-3-yl]-phenyl ester (3b)

The title compound was prepared in 70% yield from **2b** by a method similar to that described for **3a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.29–1.31 (2s, 18H, 6CH_3), 4.21–4.36 (dd, $J = 5.21$ Hz, $J = 9.46$ Hz, 1H, CO–C*H*–CH_2), 4.62–4.88 (m, 2H, Ar–O–CH_2–CH), 6.82–6.92 (d+m, $J = 2.13$ Hz, 2H, Ar-*H*), 7.04–7.11 (d, $J = 9.31$ Hz, 2H, Ar-*H*), 7.29–7.36 (d, $J = 9.31$ Hz, 2H, Ar-*H*), 7.83–7.90 (dd, $J = 8.51$ Hz, $J = 0.47$ Hz, 1H, Ar-*H*); MS (ESI) *m/e* 424 (M^+).

6.12. 2,2-Dimethyl-propanoic acid 3,4-dihydro-3-(4-methoxyphenyl)-4-oxo-2*H*-1-benzopyran-7-yl ester (3c)

The title compound was prepared in 85% yield from **2c** by a method similar to that described for **3a**.

^{1}H NMR (200 MHz, DMSO-d_6) 3.73 (s, 3H, Ar–O–CH_3), 4.07–4.20 (dd, $J = 5.02$ Hz, $J = 9.29$ Hz, 1H, Ar–C*H*–CO), 4.60–4.82 (m, 2H, Ar–O–CH_2–CH–CO), 6.80–6.96 (d+m, $J = 2.01$ Hz, 4H, Ar-*H*), 7.15–7.25 (d, $J = 8.79$ Hz, 2H, Ar-*H*), 7.83–7.89 (dd, $J = 8.29$ Hz, $J = 0.50$ Hz, 1H, Ar-*H*); MS (ESI) *m/e* 354 (M^+).

6.13. 2,2-Dimethyl-propanoic acid 3,4-dihydro-3-(4-methylphenyl)-4-oxo-2*H*-1-benzopyran-7-yl ester (3d)

The title compound was prepared from **2d** by a method similar to that described for **3a** (yield = 75%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.03 (s, 9H, 3CH_3), 2.28 (s, 3H, Ar–CH_3), 4.10–4.20 (dd, $J = 5.11$ Hz, $J = 9.02$ Hz, Ar–C*H*–CO), 4.56–4.88 (m, 2H, Ar–CH_2–CH–CO), 6.81–6.90 (d+m, $J = 2.10$ Hz, 2H, Ar-*H*), 7.15 (s, 4H, Ar-*H*), 7.81–7.88 (dd, $J = 0.60$ Hz, $J = 8.11$ Hz, 1H, Ar-*H*); MS (ESI) *m/e* 338 (M^+).

6.14. 2,2-Dimethyl-propanoic acid 3-(4-butoxyphenyl)-3,4-dihydro-4-oxo-2*H*-1-benzopyran-7-yl ester (3e)

The title compound was prepared from **2e** by a method similar to that described for **3a** (yield = 60%).

^{1}H NMR (200 MHz, DMSO-d_6) 0.88–0.99 (t, $J = 7.23$ Hz, 3H, CH_3), 1.30 (s, 9H, C–[CH_3]), 1.33–1.53 (m, 2H, CH_3–CH_2–CH_2), 1.58–1.78 (m, 2H, CH_3–CH_2CH_2), 3.88–3.99 (t, $J = 6.65$ Hz, 2H, Ar–O–CH_2), 4.08–4.18 (dd, $J = 5.21$ Hz, $J = 9.26$ Hz, 1H, Ar–C*H*–CO), 4.59–4.82 (m, 2H, Ar–O–CH_2–CH), 6.80–6.94 (m, 4H, Ar-*H*), 7.15–7.22 (d, $J = 8.67$ Hz, 2H, Ar-*H*), 7.81–7.88 (d, $J = 8.57$ Hz, 1H, Ar-*H*); MS (ESI) *m/e* 396 (M^+).

6.15. 2,2-Dimethyl-propanoic acid 3-(3,4-dimethoxyphenyl)-3,4-dihydro-4-oxo-2*H*-1-benzopyran-7-yl ester (3f)

The title compound was prepared from **2f** by a method similar to that described for **3a** (yield = 65%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 3.71 and 3.73 (2s, 6H, Ar–[O–CH_3]$_2$), 4.08–4.20 (dd, $J = 4.94$ Hz, $J = 9.88$ Hz, 1H, Ar–O–CH_2–C*H*–CO), 4.58–4.88 (m, 2H, Ar–O–CH_2–CH–CO), 6.74–6.81 (dd, $J = 1.98$ Hz, $J = 8.40$ Hz, 1H, Ar-*H*), 6.82–6.94 (m, 4H, Ar-*H*), 7.82–7.89 (dd, $J = 0.50$ Hz, $J = 8.40$ Hz, 1H, Ar-*H*); MS (ESI) *m/e* 384 (M^+).

6.16. 2,2-Dimethyl-propanoic acid 3,4-dihydro-3-phenyl-4-oxo-2*H*-1-benzopyran-7-yl ester (3g)

The title compound was prepared in 60% yield from **2g** by a method similar to that described for **3a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.31 (s, 9H, 3CH_3), 4.15–4.30 (dd, $J = 5.20$ Hz, $J = 9.61$ Hz, 1H, Ar–C*H*–C=O), 4.62–4.88 (m, 2H, Ar–O–CH_2–CH), 6.81–6.93 (m, 2H, Ar-*H*), 7.21–7.42 (m, 5H, Ar-*H*), 7.80–7.92 (dd, $J = 0.52$ Hz, $J = 8.31$ Hz, 1H, Ar-*H*); MS (ESI) *m/e* 324 (M^+).

6.17. 2,2-Dimethyl-propanoic acid 3,4-dihydro-3-(3-methoxyphenyl)-4-oxo-2*H*-1-benzopyran-7-yl ester (3h)

The title compound was prepared from **2h** by a method similar to that described for **3a** (yield = 55%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 3.73 (s, 3H, Ar–O–CH_3), 4.12–4.25 (m, Ar–C*H*–CO), 4.60–4.88 (m, 2H, Ar–O–CH_2–CH), 6.76–6.94 (m, 5H, Ar-*H*), 7.18–7.34 (m, 1H, Ar-*H*), 7.80–7.90 (dd, $J = 0.56$ Hz, $J = 8.40$ Hz, 1H, Ar-*H*); MS (ESI) *m/e* 354 (M^+).

6.18. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(2-methoxyphenyl)-4-[[4-(phenylmethoxy)phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (5a)

A solution of **4** (6 g; 25.8 mmol) in dry THF (37 mL) was added dropwise to Mg turnings (1.8 g; 75 mmol) heating to reflux. The reaction was carried out under nitrogen flux. On completion of the addition, the mixture was left to reach 30 °C, and then further cooled to −20 °C. A solution of compound **3a** (3.5 g; 10 mmol) in dry THF (20 mL) was added dropwise in 20 min keeping $t = -20$ °C. On completion of the addition, the mixture was kept stirred until rt was reached, and it was then quenched with water (5 mL) and filtered.

The solution was evaporated to dryness under vacuum, and ethyl acetate (20 mL) was added to the residue; the

Wurtz coupling by-product of the Grignard reaction ([[4-[2-[4-(phenylmethoxy)phenyl]ethyl]phenoxy]methyl]benzene) crystallized and was filtered off. The solution was evaporated to dryness and purified by flash chromatography (petroleum ether/ethyl acetate = from 90/10 to 60/40). Compound **5a** (1.85 g, 3.35 mmol) were obtained as a viscous oil (yield = 34%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.85–3.00 (d, $J = 14.03$ Hz, 1H, Ar–CH–C–OH), 3.25–3.40 (d, $J = 14.03$ Hz, 1H, Ar–CH–C–OH), 3.58–3.70 (s+m, 4H, Ar–CH–CH_2, Ar–O–CH_3), 3.98–4.34 (m, 2H, Ar–O–CH_2–CH), 5.02 (s, 2H, Ar–O–CH_2–Ar), 5.40 (s, 1H, C–OH), 6.47–6.51 (d, $J = 2.47$ Hz, 1H, Ar-H), 6.62–6.71 (dd, $J = 2.47$ Hz, $J = 8.52$ Hz, 1H, Ar-H), 6.73–6.77 (s, 4H, Ar-H), 6.80–7.00 (m, 2H, Ar-H), 7.15–7.47 (m, 6H, Ar-H), 7.53–7.62 (d, $J = 8.52$ Hz, 1H, Ar-H), 7.66–7.74 (dd, $J = 1.65$ Hz, $J = 7.70$ Hz, 1H, Ar-H); MS (ESI) m/e 552 (M^+).

6.19. 2,2-Dimethyl-propanoic acid 4-[7-(2,2-dimethyl-1-oxopropoxy)-3,4-dihydro-4-hydroxy-4-[[4-(phenylmethoxy)phenyl]methyl]-2*H*-1-benzopyran-3-yl]phenyl ester (5b)

The title compound was prepared in 30% yield from **3b** by a method similar to that described for **5a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30–1.31 (2s, 18H, 6CH_3), 2.75–2.88 (d, $J = 14.52$ Hz, 1H, Ar–CH–C–OH), 3.08–3.20 (dd, $J = 3.41$ Hz, $J = 11.12$ Hz, 1H, OH–C–CHAr), 3.26–3.39 (d, $J = 14.52$ Hz, 1H, Ar–CH–C–OH), 4.08–4.44 (dd+t, $J = 3.41$ Hz, $J = 11.12$ Hz, 1H, Ar–O–CH_2–CH), 5.02 (s, 2H, Ar–O–CH_2–Ar), 5.48 (s, 1H, Ar–CH_2–COH), 6.53–6.57 (d, $J = 2.49$ Hz, 1H, Ar-H), 6.65–6.74 (dd, $J = 2.49$ Hz, $J = 8.40$ Hz, 1H, Ar-H), 6.78–6.85 (br, 3H, Ar-H), 6.96–7.05 (d, $J = 8.62$ Hz, 2H, Ar-H), 7.24–7.47 (m, 8H, Ar-H), 7.51–7.61 (d, $J = 8.62$ Hz, 1H, Ar-H); MS (ESI) m/e 622 (M^+).

6.20. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(4-methoxyphenyl)-4-[[4-(phenylmethoxy)phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (5c)

The title compound was prepared in 50% yield from **3c** by a method similar to that described for **5a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.74–2.88 (d, $J = 14.19$ Hz, 1H, Ar–CH–C–OH), 2.98–3.12 (dd, $J = 3.60$ Hz, $J = 9.91$ Hz, 1H, O–CH_2–CH–Ar), 3.22–3.37 (d, $J = 14.19$ Hz, 1H, Ar–CH–C–OH), 3.73 (s, 3H, Ar–O–CH_3), 4.00–4.13 (dd, $J = 3.60$ Hz, $J = 9.91$ Hz, 1H, O–CH–CH–Ar), 4.25–4.43 (t, $J = 10.14$ Hz, 1H, O–CH–CH–Ar), 5.02 (s, 2H, Ar–O–CH_2–Ar), 5.35 (s, 1H, Ar–CH_2–C–OH), 6.51–6.55 (d, $J = 2.26$ Hz, 1H, Ar-H), 6.65–6.73 (dd, $J = 2.26$ Hz, $J = 8.56$ Hz, 1H, Ar-H), 6.78–6.90 (s+d, $J = 8.78$ Hz, 6H, Ar-H), 7.16–7.25 (d, $J = 8.78$ Hz, 2H, Ar-H), 7.29–7.44 (m, 5H, Ar-H), 7.53–7.61 (d, $J = 8.56$ Hz, Ar-H); MS (ESI) m/e 552 (M^+).

6.21. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(4-methylphenyl)-4-[[4-(phenylmethoxy)phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (5d)

The title compound was prepared in 30% yield from **3d** by a method similar to that described for **5a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_2), 2.27 (s, 3H, Ar–CH_3), 2.66–2.82 (d, $J = 14.46$ Hz, 1H, Ar–CH–C–OH), 3.00–3.14 (dd, $J = 3.43$ Hz, $J = 9.56$ Hz, 1H, Ar–CH–C–OH), 3.16–3.30 (d, $J = 14.46$ Hz, 1H, Ar–CH–C–OH), 3.99–4.12 (dd, $J = 3.43$ Hz, $J = 9.56$ Hz, 1H, Ar–O–CH–CH–C–OH), 4.25–4.42 (t, $J = 10.05$ Hz, 1H, Ar–O–CH–CH–C–OH), 5.02 (s, 2H, Ar–O–CH_2–Ar), 5.29 (s, 1H, C–OH), 6.50–6.73 (2m, 6H, Ar-H), 7.03–7.40 (m, 9H, Ar-H), 7.51–7.60 (d, $J = 8.58$ Hz, 1H, Ar-H), 9.12 (s, 1H, Ar–OH); MS (ESI) m/e 536 (M^+).

6.22. 2,2-Dimethyl-propanoic acid 3-(4-butoxyphenyl)-3,4-dihydro-4-hydroxy-4-[[4-(phenylmethoxy)phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (5e)

The title compound was prepared in 35% yield from **3e** by a method similar to that described for **5a**.

^{1}H NMR (200 MHz, DMSO-d_6) 0.85–1.02 (t, $J = 7.25$ Hz, 3H, CH_3), 1.30 (s, 9H, 3CH_3), 1.34–1.54 (m, 2H, CH_3–CH_2–CH_2), 1.59–1.78 (m, 2H, CH_3–CH_2–CH_2), 2.72–2.90 (d, $J = 14.50$ Hz, 1H, Ar–CH–C–OH), 2.99–3.12 (dd, $J = 3.34$ Hz, $J = 9.47$ Hz, 1H, Ar–CH–CH_2–O), 3.22–3.39 (d, $J = 14.50$ Hz, 1H, Ar–CH–C–OH), 3.86–4.15 (t+dd, $J = 6.41$ Hz, $J = 3.34$ Hz, $J = 10.59$ Hz, 3H, Ar–O–CH_2–CH_2, Ar–O–CH–CH–Ar), 4.24–4.43 (t, $J = 10.03$ Hz, 1H, Ar–O–CH–CH–Ar), 4.97–5.05 (s, 2H, Ar–O–CH_2–Ar), 5.34 (s, 1H, C–OH), 6.50–6.57 (d, $J = 2.23$ Hz, 1H, Ar-H), 6.65–6.74 (dd, $J = 2.23$ Hz, $J = 8.36$ Hz, 1H, Ar-H), 6.77–6.89 (br, 6H, Ar-H), 7.14–7.24 (d, $J = 8.92$ Hz, 2H, Ar-H), 7.30–7.47 (m, 5H, Ar-H), 7.52–7.63 (d, $J = 8.36$ Hz, 1H, Ar-H); MS (ESI) m/e 594 (M^+).

6.23. 2,2-Dimethyl-propanoic acid 3-(3,4-dimethoxyphenyl)-3,4-dihydro-4-hydroxy-4-[[4-(phenylmethoxy)phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (5f)

The title compound was prepared in 35% yield from **3f** by a method similar to that described for **5a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.77–2.90 (d, $J = 14.10$ Hz, 1H, Ar–CH–C–OH), 2.98–3.10 (dd, $J = 3.52$ Hz, $J = 10.14$ Hz, 1H, Ar–O–CH_2–CH–C–OH), 3.27–3.40 (d, $J = 14.10$ Hz, 1H, Ar–CH–C–OH), 3.66 (s, 3H, Ar–O–CH_3), 3.73 (s, 3H, Ar–O–CH_3), 4.02–4.15 (m, 1H, Ar–OCH–CH–C–OH), 4.29–4.42 (t, $J = 10.36$ Hz, 1H, Ar–O–CH–CH–C–OH), 5.02 (s, 2H, Ar–O–CH_2–Ar), 5.34 (s, 1H, C–OH), 6.52–6.57 (d, $J = 2.21$ Hz, 1H, Ar-H), 6.65–6.73 (dd, $J = 2.21$ Hz, $J = 8.37$ Hz, 1H, Ar-H), 6.77–6.91 (m, 8H, Ar-H), 7.31–7.43 (m, 4H, Ar-H), 7.53–7.61 (d, $J = 8.37$ Hz, 1H, Ar-H); MS (ESI) m/e 582 (M^+).

6.24. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-phenyl-4-[[4-(phenylmethoxy)phenyl]methyl]-2*H*-1-benzopyran-7-yl ester (5g)

The title compound was prepared in 30% yield from **3g** by a method similar to that described for **5a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.75–2.90 (d, $J = 14.38$ Hz, 1H, Ar–CH–C–OH), 3.06–3.20 (dd, $J = 3.53$ Hz, $J = 9.22$ Hz, 1H, Ar–CH–CH_2–O), 3.24–3.40 (d, $J = 14.38$ Hz, 1H, Ar–CH–C–OH), 4.06–4.21 (dd, $J = 3.52$ Hz, $J = 10.85$ Hz, 1H, Ar–OCH–CH–Ar), 4.30–4.47 (t, $J = 10.04$ Hz, 1H, Ar–O–CH–CH–Ar), 5.02 (s, 2H, Ar–O–CH_2–Ar), 5.41 (s, 1H, C–OH), 6.51–6.59 (d, $J = 2.17$ Hz, 1H, Ar-H), 6.65–6.75 (dd, $J = 2.17$ Hz, $J = 8.68$ Hz, 1H, Ar-H), 6.82 (s, 4H, Ar-H), 7.20–7.48 (m, 10H, Ar-H), 7.52–7.63 (d, $J = 8.68$ Hz, 1H, Ar-H); MS (ESI) m/e 522 (M^+).

6.25. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(3-methoxyphenyl)-4-[[4-(phenylmethoxy)phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (5h)

The title compound was prepared in 32% yield from **3h** by a method similar to that described for **5a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.78–2.93 (d, $J = 14.36$ Hz, 1H, Ar–CH–C–OH), 3.00–3.15 (dd, $J = 3.33$ Hz, $J = 8.97$ Hz, 1H, Ar–CH–CH_2–O), 3.26–3.40 (d, $J = 14.36$ Hz, 1H, Ar–CH–C–OH), 3.70 (s, 3H, Ar–O–CH_3), 4.07–4.20 (dd, $J = 3.33$ Hz, $J = 10.00$ Hz, 1H, Ar–O–CH–CH–Ar), 4.28–4.45 (t, $J = 9.74$ Hz, 1H, Ar–O–CH–CH–Ar), 4.98–5.05 (s, 2H, Ar–O–CH_2–Ar), 5.40 (s, 1H, C–OH), 6.53–6.58 (d, $J = 2.31$ Hz, 1H, Ar-H), 6.65–6.75 (dd, $J = 2.31$ Hz, $J = 8.47$ Hz, 1H, Ar-H), 6.80–6.90 (m, 4H, Ar-H), 7.15–7.25 (t, $J = 8.21$ Hz, 1H, Ar-H), 7.30–7.45 (m, 3H, Ar-H), 7.52–7.60 (d, $J = 8.47$ Hz, 1H, Ar-H); MS (ESI) m/e 552 (M^+).

6.26. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-4-[(4-hydroxyphenyl)methyl]-3-(2-methoxyphenyl)-2*H*-1-benzopyran-7-yl ester (6a)

Compound **5a** (3.5 g; 6.3 mmol) was dissolved in ethyl acetate (45 mL), Pd 5%/C (50% water) (1.8 g) was added and the mixture was hydrogenated (40 psi, rt) for 3 h. After filtering, the solution was evaporated under vacuum and diethyl ether was added and then completely evaporated. A white foam of the title compound (2.9 g; 6.3 mmol) was obtained (quantitative yield). The product was used for the subsequent steps without any further purification.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.82–2.97 (d, $J = 14.29$ Hz, 1H, Ar–CH–C–OH), 3.20–3.37 (d, $J = 14.29$ Hz, 1H, Ar–CH–C–OH), 3.61–3.73 (m+s, 4H, Ar–CH–CH_2, Ar–O–CH_3), 3.98–4.38 (m, 2H, Ar–O–CH_2–CH), 5.33 (s, 1H, C–OH), 6.42–6.74 (m, 6H, Ar-H), 6.80–6.90 (m, 1H, Ar-H), 6.93–7.04 (d, $J = 7.53$ Hz, 1H, Ar-H), 7.16–7.30 (m, 1H, Ar-H), 7.52–7.63 (d, $J = 8.57$ Hz, 1H, Ar-H), 7.66–7.76 (d, $J = 6.49$ Hz, 1H, Ar-H), 9.10 (s, 1H, Ar–OH); MS (ESI) m/e 462 (M^+).

6.27. 2,2-Dimethyl-propanoic acid 4-[7-(2,2-dimethyl-1-oxopropoxy)-3,4-dihydro-4-hydroxy-4-[(4-hydroxyphenyl)-methyl]-2*H*-1-benzopyran-3-yl]phenyl ester (6b)

The title compound was prepared in quantitative yield from **5b** by a method similar to that described for **6a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30–1.31 (2s, 18H, 6CH_3), 2.70–2.82 (d, $J = 13.20$ Hz, 1H, Ar–CH–C–OH), 3.11–3.21 (dd, $J = 3.21$ Hz, $J = 10.35$ Hz, 1H, OH–C–CH–Ar), 3.23–3.35 (d, $J = 13.20$ Hz, 1H, Ar–CH–C–OH), 4.07–4.44 (dd+t, $J = 3.21$ Hz, $J = 10.35$ Hz, 2H, Ar–O–CH_2–CH), 5.43 (s, 1H, Ar–CH$_2$–C–OH), 6.50–6.75 (m, 6H, Ar-H), 6.97–7.06 (d, $J = 8.57$ Hz, 2H, Ar-H), 7.27–7.38 (d, $J = 8.57$ Hz, 2H, Ar-H), 7.52–7.62 (d, $J = 8.56$ Hz, 1H, Ar-H), 9.14 (s, 1H, Ar–OH); MS (ESI) m/e 532 (M^+).

6.28. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-4-[(4-hydroxyphenyl)methyl]-3-(4-methoxyphenyl)-2*H*-1-benzopyran-7-yl ester (6c)

The title compound was prepared in quantitative yield from **5c** by a method similar to that described for **6a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.67–2.81 (d, $J = 14.39$ Hz, 1H, Ar–CH–C–OH), 2.99–3.12 (dd, $J = 3.47$ Hz, $J = 9.68$ Hz, 1H, O–CH$_2$–CH–C–OH), 3.24–3.34 (d, $J = 14.39$ Hz, 1H, Ar–CH–C–OH), 3.73 (s, 3H, Ar–O–CH_3), 3.99–4.10 (dd, $J = 3.47$ Hz, $J = 9.68$ Hz, 1H, O–CH–CH–Ar), 4.24–4.39 (t, $J = 10.42$ Hz, 1H, O–CH–CH–Ar), 5.28 (s, 1H, Ar–CH$_2$–C–OH), 6.49–6.58 (m, 3H, Ar-H), 6.63–6.73 (m, 3H, Ar-H), 6.81–6.89 (d, $J = 8.68$ Hz, 2H, Ar-H), 7.17–7.25 (d, $J = 8.68$ Hz, 2H, Ar-H), 7.51–7.60 (d, $J = 8.70$ Hz, 1H, Ar-H); MS (ESI) m/e 462 (M^+).

6.29. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-4-[(4-hydroxyphenyl)methyl]-3-(4-methylphenyl)-2*H*-1-benzopyran-7-yl ester (6d)

The title compound was prepared in quantitative yield from **5d** by a method similar to that described for **6a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_2), 2.27 (s, 3H, Ar–CH_3), 2.66–2.82 (d, $J = 14.46$ Hz, 1H, Ar–CH–C–OH), 3.00–3.14 (dd, $J = 3.43$ Hz, $J = 9.56$ Hz, 1H, Ar–CH–C–OH), 3.16–3.30 (d, $J = 14.46$ Hz, 1H, Ar–CH–C–OH), 3.99–4.12 (dd, $J = 3.43$ Hz, $J = 9.56$ Hz, 1H, Ar–O–CH–CH–C–OH), 4.25–4.42 (t, $J = 10.05$ Hz, 1H, Ar–O–CH–CH–C–OH), 5.29 (s, 1H, C–OH), 6.50–6.73 (2m, 6H, Ar-H), 7.03–7.23 (m, 4H, Ar-H), 7.51–7.60 (d, $J = 8.58$ Hz, 1H, Ar-H), 9.12 (s, 1H, Ar–OH); MS (ESI) m/e 446 (M^+).

6.30. 2,2-Dimethyl-propanoic acid 3-(4-butoxyphenyl)-3,4-dihydro-4-hydroxy-4-[(4-hydroxyphenyl)methyl]-2*H*-1-benzopyran-7-yl ester (6e)

The title compound was prepared in quantitative yield from **5e** by a method similar to that described for **6a**.

^{1}H NMR (200 MHz, DMSO-d_6) 0.87–0.99 (t, $J = 7.22$ Hz, 3H, CH_3), 1.30 (s, 9H, C–[CH_3]$_3$), 1.35–1.53 (m, 2H, CH$_3$–CH_2), 1.58–1.77 (m, 2H, CH$_3$–CH$_2$–CH_2), 2.70–2.80 (d, $J = 14.13$ Hz, 1H, Ar–CH–C–OH), 2.98–3.10 (dd, $J = 3.31$ Hz, $J = 9.62$ Hz, 1H, Ar–O–CH$_2$–CH–C–OH), 3.18–3.30 (d, $J = 14.13$ Hz, Ar–CH–C–OH), 3.85–4.13 (t+m, $J = 6.62$ Hz, 3H, Ar–O–CH_2, Ar–O–CH–C–OH), 4.25–4.40 (t, $J = 10.22$ Hz, 1H, Ar–O–CH–CH–C–OH), 5.28 (s, 1H, C–OH), 6.48–6.60 (m, 3H, Ar-H), 6.65–6.73 (m, 3H, Ar-H), 6.80–6.88 (d, $J = 8.72$ Hz, 2H, Ar-H), 7.15–7.24 (d, $J = 8.72$ Hz, 2H, Ar-H), 7.53–7.60 (d, $J = 8.70$ Hz, 1H, Ar-H), 9.12 (s, 1H, Ar–OH); MS (ESI) m/e 504 (M$^+$).

6.31. 2,2-Dimethyl-propanoic acid 3-(3,4-dimethoxyphenyl)-3,4-dihydro-4-hydroxy-4-[(4-hydroxyphenyl)methyl]-2*H*-1-benzopyran-7-yl ester (6f)

The title compound was prepared in quantitative yield from **5f** by a method similar to that described for **6a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.70–2.82 (d, $J = 14.24$ Hz, 1H, Ar–CH–CH–C–OH), 2.96–3.10 (dd, $J = 3.49$ Hz, $J = 9.58$ Hz, 1H, Ar–O–CH$_2$–CH–C–OH), 3.21–3.32 (d, $J = 14.24$ Hz, 1H, Ar–CH–CH–C–OH), 3.67 (s, 3H, Ar–O–CH_3), 3.73 (s, 3H, Ar–O–CH_3), 4.00–4.12 (m, 1H, Ar–O–CH–CH–C–OH), 4.25–4.42 (t, $J = 10.25$ Hz, 1H, Ar–O–CH–CH–C–OH), 5.27 (s, 1H, C–OH), 6.49–6.58 (m, 3H, Ar-H), 6.61–6.73 (m, 3H, Ar-H), 6.80–6.91 (3H, Ar-H), 7.51–7.60 (d, $J = 8.43$ Hz, 1H, Ar-H), 9.12 (s, 1H, Ar–OH); MS (ESI) m/e 492 (M$^+$).

6.32. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-4-[(4-hydroxyphenyl)methyl]-3-phenyl-2*H*-1-benzopyran-7-yl ester (6g)

The title compound was prepared in quantitative yield from **5g** by a method similar to that described for **6a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.69–2.84 (d, $J = 14.27$ Hz, 1H, Ar–CH–C–OH), 3.05–3.17 (dd, $J = 3.44$ Hz, $J = 9.25$ Hz, 1H, Ar–O–CH$_2$–CH), 3.19–3.36 (d, $J = 14.27$ Hz, 1H, Ar–CH–C–OH), 4.05–4.16 (dd, $J = 3.44$ Hz, $J = 10.83$ Hz, 1H, Ar–O–CH–CH–Ar), 4.28–4.43 (t, $J = 10.83$ Hz, Ar–O–CH–CH–Ar), 5.35 (s, 1H, C–OH), 6.48–6.76 (m, 6H, Ar-H), 7.18–7.36 (br, 5H, Ar-H), 7.50–7.60 (d, $J = 8.72$ Hz, 1H, Ar-H), 9.15 (s, 1H, Ar–OH); MS (ESI) m/e 432 (M$^+$).

6.33. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-4-[(4-hydroxyphenyl)methyl]-3-(3-methoxyphenyl)-2*H*-1-benzopyran-7-yl ester (6h)

The title compound was prepared in quantitative yield from **5h** by a method similar to that described for **6a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 2.72–2.86 (d, $J = 14.38$ Hz, 1H, Ar–CH–C–OH), 3.00–3.15 (dd, $J = 3.23$ Hz, $J = 9.10$ Hz, 1H, Ar–CH–CH$_2$–O), 3.19–3.35 (d, $J = 14.38$ Hz, 1H, Ar–CH–C–OH), 3.70 (s, 3H, Ar–O–CH_3), 4.05–4.18 (dd, $J = 3.23$ Hz, $J = 10.80$ Hz, 1H, Ar–O–CH–CH–Ar), 4.28–4.44 (t, $J = 9.70$ Hz, 1H, Ar–O–CH–CH–Ar), 5.32 (s, 1H, C–OH), 6.50–6.60 (m, 3H, Ar-H), 6.65–6.75 (m, 3H, Ar-H), 6.80–6.90 (m, 3H, Ar-H), 7.13–7.26 (t, $J = 7.93$ Hz, 1H, Ar-H), 7.51–7.60 (d, $J = 8.80$ Hz, 1H, Ar-H), 9.13 (s, 1H, Ar–OH); MS (ESI) m/e 462 (M$^+$).

6.34. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(2-methoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]methyl]-2*H*-1-benzopyran-7-yl ester (7a)

To a solution of **6a** (2.9 g; 6.3 mmol) in acetone (30 mL), K_2CO_3 (1.3 g; 9.4 mmol) was added. The solution was taken to reflux and *N*-chloroethyl-piperidine (1.12 g of free base, previously extracted from the corresponding hydrochloride salt; 7.6 mmol) was added. The reaction was completed in 4 h; the salt was filtered off and the solution was evaporated to dryness. The residue was dissolved in ethyl acetate and extracted with a 5% CH_3COOH aqueous solution. The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by flash chromatography (methylene chloride/methanol = from 99/1 to 90/10) obtaining the title compound (2.17 g; 3.78 mmol) as a yellow oil (yield = 60%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.28 (s, 9H, 3CH_3), 1.35–1.73 (m, 6H, 3CH_2), 2.76–2.90 (m, 4H, N–(CH_2)$_2$), 2.92–3.10 (m, 2H, N–CH_2), 3.28–3.42 (d, $J = 13.84$ Hz, 1H, Ar–CH–CH$_2$), 3.57–3.76 (m+s, 5H, Ar–CH_2–C–OH, Ar–O–CH_3), 3.97–4.40 (m, 4H, Ar–O–CH_2–CH, Ar–O–CH_2–CH$_2$), 6.47–6.53 (d, $J = 2.30$ Hz, 1H, Ar-H), 6.60–7.04 (m, 7H, Ar-H), 7.16–7.30 (m, 1H, Ar-H), 7.54–7.63 (d, $J = 8.60$ Hz, 1H, Ar-H), 7.68–7.80 (dd, $J = 1.47$ Hz, $J = 7.55$ Hz, 1H, Ar-H); MS (ESI) m/e 573 (M$^+$).

6.35. 2,2-Dimethyl-propanoic acid 4-[7-(2,2-dimethyl-1-oxopropoxy)-3,4-dihydro-4-hydroxy-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-3-yl]phenyl ester (7b)

The title compound was prepared from **6b** by a method similar to that described for **7a** (yield = 50%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 18H, 6CH_3), 1.38–1.80 (m, 6H, 3CH_2), 2.77–2.89 (d, $J = 14.58$ Hz, 1H, Ar–CH–C–OH), 2.90–3.03 (br, 4H, N–[CH_2]$_2$), 3.07–3.23 (m, 3H, N–CH_2, OH–C–CH–Ar), 3.26–3.41 (d, $J = 14.58$ Hz, 1H, Ar–CH), 4.08–4.45 (m+t, $J = 5.13$ Hz, 4H, Ar–O–CH_2–CH$_2$, Ar–OCH_2), 5.44–5.61 (br, 1H, Ar–CH$_2$–C–OH), 6.52–6.58 (d, $J = 2.44$ Hz, 1H, Ar-H), 6.65–6.89 (m+d, $J = 5.94$ Hz, 5H, Ar-H), 6.95–7.05 (d, $J = 8.64$ Hz, 2H, Ar-H), 7.26–7.37 (d, $J = 8.64$ Hz, 2H, Ar-H), 7.53–7.62 (d, $J = 8.38$ Hz, 1H, Ar-H); MS (ESI) m/e 643 (M$^+$).

6.36. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(4-methoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (7c)

The title compound was prepared from **6c** by a method similar to that described for **7a** (yield = 54%).

^{1}H NMR (200 MHz, DMSO-d_6+CF_3COOH-d_1) 1.30 (s, 9H, 3CH_3), 1.32–1.57 (m, 6H, 3CH_2), 2.32–2.45 (t, J = 4.56 Hz, 4H, N–[CH_2]$_2$), 2.54–2.64 (t, J = 6.08 Hz, 2H, N–CH_2), 2.72–2.87 (d, J = 13.93 Hz, Ar–CH–C–OH), 2.99–3.10 (dd, J = 3.30 Hz, J = 9.62 Hz, 1H, Ar–CH–CH_2–O), 3.17–3.31 (d, J = 13.93 Hz, 1H, Ar–CH–C–OH), 3.70 (s, 3H, Ar–O–CH_3), 3.88–4.10 (t, J = 5.82 Hz, 3H, Ar–O–CH_2–CH_2–N, O–CH–CH–C–OH), 4.19–4.37 (t, J = 10.64 Hz, 1H, O–CH–CH–C–OH), 6.45–6.53 (d, J = 2.54 Hz, 1H, Ar-*H*), 6.60–6.90 (m, 7H, Ar-*H*), 7.08–7.22 (d, J = 8.87 Hz, 2H, Ar-*H*), 7.45–7.57 (d, J = 8.61 Hz, 1H, Ar-*H*); MS (ESI) m/e 573 (M^+).

6.37. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(4-methylphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (7d)

The title compound was prepared from **6d** by a method similar to that described for **7a** (yield = 50%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.29 (9H, 3CH_3), 1.33–1.65 (m, 6H, 3CH_2), 2.27 (s, 3H, Ar–CH_3), 2.54–2.71 (m, 4H, N–[CH_2]$_2$), 2.73–2.91 (m, 3H, Ar–CH–C–OH, N–CH_2), 3.00–3.13 (dd, J = 3.54 Hz, J = 9.72 Hz, 1H, Ar–O–CH_2–CH–C–OH), 3.23–3.37 (d, J = 14.15 Hz, Ar–CH–C–OH), 4.00–4.16 (m, 3H, Ar–O–CH–CH–C–OH, Ar–O–CH_2–CH_2–N), 4.27–4.43 (t, J = 10.32 Hz, Ar–O–CH–CH–C–OH), 5.25–5.48 (br, 1H, C–O*H*), 6.51–6.54 (d, J = 2.36 Hz, 1H, Ar-*H*), 6.65–6.72 (dd, J = 2.36 Hz, J = 8.84 Hz, 1H, Ar-*H*), 6.73–6.84 (m, 4H, Ar-*H*), 7.00–7.20 (m, 4H, Ar-*H*), 7.52–7.61 (d, J = 8.84 Hz, 1H, Ar-*H*); MS (ESI) m/e 557 (M^+).

6.38. 2,2-Dimethyl-propanoic acid 3-(4-butoxyphenyl)-3,4-dihydro-4-hydroxy-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]methyl]-2*H*-1-benzopyran-7-yl ester (7e)

The title compound was prepared from **6e** by a method similar to that described for **7a** (yield = 58%).

^{1}H NMR (200 MHz, DMSO-d_6) 0.87–0.99 (t, J = 7.39 Hz, 3H, CH_3), 1.30 (s, 9H, C–[CH_3]$_3$), 1.33–1.76 (m, 10H, CH_3–[CH_2]$_2$, 3CH_2), 2.57–2.72 (m, 4H, 2CH_2–N), 2.76–2.90 (m, 3H, CH_2–N, Ar–CH–C–OH), 2.98–3.11 (m, 1H, Ar–CH–C–OH), 3.22–3.36 (m, 1H, Ar–CH–C–OH), 3.87–3.99 (t, J = 6.39 Hz, 2H, Ar–O–CH_2–CH_2–N), 4.02–4.15 (m, 3H, Ar–O–CH_2, Ar–O–CH–CH–C–OH), 4.25–4.41 (t, J = 10.09 Hz, 1H, Ar–O–CH–CH–C–OH), 5.26–5.42 (br, 1H, C–O*H*), 6.51–6.54 (d, J = 2.21 Hz, 1H, Ar-*H*), 6.65–6.95 (m, 7H, Ar-*H*), 7.15–7.25 (d, J = 8.62 Hz, 2H, Ar-*H*), 7.52–7.60 (d, J = 8.61 Hz, 1H, Ar-*H*); MS (ESI) m/e 615 (M^+).

6.39. 2,2-Dimethyl-propanoic acid 3-(3,4-dimethoxyphenyl)-3,4-dihydro-4-hydroxy-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]methyl]-2*H*-1-benzopyran-7-yl ester (compound 7f)

The title compound was prepared from **6f** by a method similar to that described for **7a** (yield = 62%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 1.38–1.71 (m, 6H, 3CH_2), 2.71–2.83 (m, 4H, N–[CH_2]$_2$), 2.85–3.10 (m, 4H, N–CH_2, Ar–CH–CH–C–OH, Ar–O–CH_2–CH–C–OH), 3.27–3.40 (d, J = 13.89 Hz, 1H, Ar–CH–C–OH), 3.68 (s, 3H, Ar–O–CH_3), 3.73 (s, 3H, Ar–O–CH_3), 4.03–4.21 (m, 3H, Ar–O–CH–CH–C–OH, Ar–O–CH_2–CH_2–N), 4.28–4.45 (t, J = 10.14 Hz, 1H, Ar–O–CH–CH–C–OH), 6.52–6.55 (d, J = 2.21 Hz, 1H, Ar-*H*), 6.65–6.73 (dd, J = 2.21 Hz, J = 8.37 Hz, 1H, Ar-*H*), 6.75–6.89 (m, 7H, Ar-*H*), 7.53–7.61 (d, J = 8.37 Hz, 1H, Ar-*H*); MS (ESI) m/e 603 (M^+).

6.40. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-phenyl-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (7g)

The title compound was prepared from **6g** by a method similar to that described for **7a** (yield = 50%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.21–1.57 (s+m, 15H, 3CH_3, 3CH_2), 2.33–2.45 (t, J = 5.90 Hz, 4H, N–[CH_2]$_2$), 2.54–2.65 (t, J = 5.90 Hz, 2H, N–CH_2), 2.75–2.88 (d, J = 14.34 Hz, 1H, Ar–CH–C–OH), 3.05–3.17 (dd, J = 3.38 Hz, J = 9.28 Hz, 1H, Ar–O–CH_2–CH), 3.23–3.36 (d, J = 14.34 Hz, 1H, Ar–CH–C–OH), 3.91–4.03 (t, J = 5.90 Hz, 2H, Ar–O–CH_2–CH_2), 4.06–4.18 (dd, J = 3.37 Hz, J = 10.69 Hz, 1H, Ar–O–CH–CH–Ar), 4.28–4.46 (t, J = 10.69 Hz, 1H, Ar–O–CH–CH–Ar), 5.39 (s, 1H, C–O*H*), 6.50–6.57 (d, J = 2.25 Hz, 1H, Ar-*H*), 6.10–6.87 (m, 5H, Ar-*H*), 7.18–7.33 (br, 5H, Ar-*H*), 7.52–7.60 (d, J = 8.44 Hz, 1H, Ar-*H*); MS (ESI) m/e 543 (M^+).

6.41. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(3-methoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]methyl]-2*H*-1-benzopyran-7-yl ester (7h)

The title compound was prepared from **6h** by a method similar to that described for **7a** (yield = 65%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 1.34–1.70 (m, 6H, 3CH_2), 2.62–2.77 (m, 4H, N–(CH_2)$_2$), 2.80–2.95 (m, 3H, Ar–CH–C–OH, N–CH_2), 3.05–3.16 (dd, J = 3.34 Hz, J = 9.24 Hz, 1H, Ar–CH–CH_2–O), 3.27–3.40 (d, J = 14.10 Hz, 1H, Ar–CH–C–OH), 3.70 (s, 3H, Ar–OCH_3), 4.05–4.20 (m, 3H, Ar–O–CH–CH–Ar, Ar–O–CH_2–CH_2–N), 4.30–4.46 (t, J = 9.24 Hz, 1H, Ar–O–CH–CH–Ar), 6.53–6.57 (d, J = 2.31 Hz, 1H, Ar-*H*), 6.65–6.95 (m, 8H, Ar-*H*), 7.11–7.26 (t, J = 8.21 Hz, 1H, Ar-*H*), 7.50–7.62 (d, J = 8.72 Hz, 1H, Ar-*H*); MS (ESI) m/e 573 (M^+).

6.42. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(4-methoxyphenyl)-4-[[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (7i)

The title compound was prepared from **6c** and *N*-chloroethyl-pyrrolidine by a method similar to that described for **7a** (yield = 36%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 1.80–1.93 (m, 4H, 2CH_2), 1.73–1.90 (t, J = 14.29 Hz, 1H, Ar–CH–C–OH), 2.97–3.42 (2m, 8H, Ar–CH–C–OH, Ar–CH–CH_2–O, N(CH_2)$_3$), 3.73 (s, 3H, Ar–O–CH_3), 4.00–4.11 (dd, J = 3.46 Hz, J = 10.61 Hz, 1H, Ar–O–CH–CH–Ar), 4.13–4.41 (m, 3H, Ar–O–CH–CH–Ar, Ar–O–CH–CH_2), 5.37 (s, 1H, C–OH), 6.48–6.56 (d, J = 2.17 Hz, 1H, Ar-H), 6.64–6.73 (dd, J = 2.17 Hz, J = 8.44 Hz, 1H, Ar-H), 6.75–6.95 (m, 6H, Ar-H), 7.16–7.25 (d, J = 8.87 Hz, 2H, Ar-H), 7.53–7.61 (d, J = 8.44 Hz, 1H, Ar-H); MS (ESI) m/e 559 (M^+).

6.43. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-phenyl-4-[[4-[3-(1-piperidinyl)propoxy]phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (7j)

The title compound was prepared from **6g** and *N*-chloropropyl-piperidine by a method similar to that described for **7a** (yield = 40%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 1.31–1.58 (m, 6H, 3CH_2), 1.69–1.89 (m, 2H, O–CH_2–CH_2–CH_2–N^+), 2.22–2.39 (m, 6H, N^+–[CH_2]$_3$), 2.74–2.89 (d, J = 14.07 Hz, 1H, Ar–CH–C–OH), 3.06–3.17 (dd, J = 3.37 Hz, J = 9.51 Hz, 1H, OH–C–CH–Ar), 3.22–3.38 (d, J = 14.07 Hz1H, Ar–CH–C–OH), 3.84–3.97 (t, J = 6.34 Hz, 2H, Ar–O–CH_2–CH_2), 4.05–4.19 (dd, J = 3.37 Hz, J = 9.51 Hz, 1H, Ar–O–CH–CH–Ar), 4.29–4.46 (t, J = 10.50 Hz, 1H, Ar–O–CH–CH–Ar), 6.51–6.56 (d, J = 2.38 Hz, 1H, Ar-H), 6.65–6.85 (m, 5H, Ar-H), 7.17–7.34 (br, 5H, Ar-H), 7.51–7.60 (d, J = 8.52 Hz, 1H, Ar-H); MS (ESI) m/e 557 (M^+).

6.44. 2,2-Dimethyl-propanoic acid 3,4-dihydro-4-hydroxy-3-(4-methoxyphenyl)-4-[[4-[3-(1-piperidinyl)propoxy]phenyl]-methyl]-2*H*-1-benzopyran-7-yl ester (7k)

The title compound was prepared from **6c** and *N*-chloropropyl-piperidine by a method similar to that described for **7a** (yield = 30%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.22–1.56 (s+m, 15H, 3CH_3, 3CH_2), 1.70–1.90 (m, 2H, Ar–O–CH_2–CH_2), 2.22–2.40 (m, 6H, N–(CH_2)$_3$), 2.72–2.87 (d, J = 14.16 Hz, 1H, Ar–CH–C–OH), 2.98–3.12 (dd, J = 3.46 Hz, J = 9.75 Hz, 1H, Ar–CH–CH_2–O), 3.20–3.39 (d, J = 14.16 Hz, 1H, Ar–CH–C–OH), 3.73 (s, 3H, Ar–O–CH_3), 3.83–3.97 (t, J = 6.29 Hz, 2H, Ar–O–CH_2–CH_2), 4.00–4.13 (dd, J = 2.83 Hz, J = 10.07 Hz, 1H, Ar–O–CH–CH–Ar), 4.24–4.43 (t, J = 10.07 Hz, 1H, Ar–O–CH–CH–Ar), 5.34 (s, 1H, C–OH), 6.48–6.50 (d, J = 2.20 Hz, 1H, Ar-H), 6.33–6.93 (m, 7H, Ar-H), 7.15–7.27 (d, J = 8.81 Hz, 2H, Ar-H), 7.50–7.62 (d, J = 8.80 Hz, 1H, Ar-H); MS (ESI) m/e 587 (M^+).

6.45. 2,2-Dimethyl-propanoic acid 4-[[4-[2-(hexahydro-1*H*-azepin-1-yl)ethoxy]phenyl]methyl]-3,4-dihydro-4-hydroxy-3-(4-methoxyphenyl)-2*H*-1-benzopyran-7-yl ester (7l)

The title compound was prepared from **6c** and 2-(hexamethyleneimino)ethyl chloride by a method similar to that described for **7a** (yield = 38%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (s, 9H, 3CH_3), 1.48–1.82 (br, 6H, 3CH_2), 2.75–2.88 (d, J = 14.40 Hz, 1H, Ar–CH–C–OH), 2.98–3.10 (dd, J = 3.27 Hz, J = 9.60 Hz, 1H, Ar–CH–CH_2–O), 3.22–3.46 (br d, J = 14.40 Hz, 7H, Ar–CH–C–OH, N–[CH_2]$_3$), 3.73 (s, 3H, Ar–O–CH_3), 4.00–4.12 (dd, br, J = 3.27 Hz, J = 10.25 Hz, 3H, Ar–O–CH–CH, Ar–O–CH_2–CH_2), 4.25–4.40 (d, J = 10.25 Hz, 1H, Ar–O–CH–CH), 5.36 (s, 1H, C–OH), 6.50–6.55 (d, J = 2.18 Hz, Ar-H), 6.65–6.90 (m, 7H, Ar-H), 7.16–7.26 (d, J = 8.72 Hz, 2H, Ar-H), 7.52–7.62 (d, J = 8.50 Hz, 1H, Ar-H); MS (ESI) m/e 587 (M^+).

6.46. 3,4-Dihydro-3-(2-methoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8a)

To a solution of **7a** (3.6 g; 6.3 mmol) in methanol (100 mL), K_2CO_3 (1.4 g; 10.1 mmol) was added and the mixture was stirred at rt for 3 h. The volume of the solution was then reduced under vacuum to 10 mL and the solution was added dropwise to water (150 mL) under stirring.

The mixture was left at +4 °C overnight and a white amorphous solid precipitated. The solid was filtered, washed with water (10 mL), and purified by flash chromatography (methylene chloride/methanol = from 99/1 to 90/10) obtaining 2.8 g (5.7 mmol) of the title compound (yield = 90%) as a yellow oil.

^{1}H NMR (200 MHz, DMSO-d_6) 1.27–1.56 (m, 6H, 3CH_2), 2.31–2.45 (t, J = 5.38 Hz, 4H, N–(CH_2)$_2$), 2.53–2.63 (t, J = 6.01 Hz, 2H, N–CH_2), 2.76–2.90 (d, J = 13.92 Hz, 1H, Ar–CH–C–OH), 3.20–3.35 (d, J = 13.92 Hz, 1H, Ar–CH–COH), 3.50–3.70 (m+s, 4H, Ar–CH–CH_2–O, Ar–O–CH_3), 3.82–4.03 (m, 3H, Ar–O–CH_2–CH_2, Ar–O–CH–CH), 4.10–4.27 (t, J = 10.12 Hz, 1H, Ar–O–CH–CH), 4.90–5.25 (br, 1H, C–OH), 6.00–6.15 (d, J = 2.22 Hz, 1H, Ar-H), 6.32–6.42 (dd, J = 2.22 Hz, J = 8.54 Hz, 1H, Ar-H), 6.60–6.76 (m, 4H, Ar-H), 6.78–7.00 (m, 2H, Ar-H), 7.15–7.25 (m, 1H, Ar-H), 7.30–7.40 (d, J = 8.85 Hz, 1H, Ar-H), 7.72–7.82 (dd, J = 1.58 Hz, J = 7.59 Hz, 1H, Ar-H); MS (ESI) m/e 489 (M^+).

6.47. 3,4-Dihydro-3-(4-hydroxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8b)

The title compound was prepared in 60% yield from **7b** by a method similar to that described for **8a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.25–1.62 (m, 6H, 3CH_2), 2.29–2.45 (m, 4H, 2CH_2), 2.53–2.63 (t, $J = 6.90$ Hz, 2H, CH_2–N), 2.64–2.78 (d, $J = 14.19$ Hz, 1H, Ar–C*H*–C–OH), 2.83–2.97 (dd, $J = 2.97$ Hz, $J = 10.89$ Hz, 1H, OH–C–C*H*–Ar), 3.13–3.34 (d, $J = 14.19$ Hz, 1H, Ar–C*H*), 3.80–4.06 (dd+t, $J = 2.97$ Hz, $J = 10.89$ Hz, $J = 11.88$ Hz, 3H, Ar–O–CH_2, Ar–O–C*H*–CH–Ar), 4.16–4.34 (t, $J = 11.88$ Hz, 1H, Ar–O–C*H*–CH–Ar), 4.82–5.12 (br, 1H, Ar–CH$_2$–C–O*H*), 6.09–6.18 (d, $J = 1.98$ Hz, 1H, Ar-*H*), 6.35–6.47 (dd, $J = 1.98$ Hz, $J = 8.25$ Hz, 1H, Ar-*H*), 6.59–6.81 (m, 6H, Ar-*H*), 7.00–7.13 (d, $J = 8.57$ Hz, 2H, Ar-*H*), 7.30–7.42 (d, $J = 8.25$ Hz, 1H, Ar-*H*), 8.73–9.77 (br, 2H, Ar–O*H*); MS (ESI) m/e 475 (M^+).

6.48. 3,4-Dihydro-3-(4-methoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8c)

The title compound was prepared from **7c** by a method similar to that described for **8a** (yield = 70%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.25–1.56 (m, 6H, 3CH_2), 2.31–2.44 (t, $J = 4.54$ Hz, 4H, N–[CH_2]$_2$), 2.53–2.63 (t, $J = 5.67$ Hz, 2H, N–CH_2), 2.64–2.76 (d, $J = 14.19$ Hz, 1H, Ar–C*H*–C–OH), 2.89–3.01 (dd, $J = 3.41$ Hz, $J = 10.79$ Hz, 1H, OH–C–C*H*–Ar), 3.17–3.31 (d, $J = 14.19$ Hz, 1H, Ar–C*H*–C–OH), 3.73 (s, 3H, Ar–O–CH_3), 3.83–4.02 (d+m, $J = 3.41$ Hz, 3H, Ar–O–C*H*–C–OH, Ar–O–CH_2–CH$_2$–N), 4.18–4.34 (t, $J = 10.79$ Hz, 1H, Ar–O–C*H*–CH–C–OH), 4.78–5.30 (br, 1H, Ar–CH$_2$–C–O*H*), 6.10–6.20 (d, $J = 2.55$ Hz, 1H, Ar-*H*), 6.35–6.45 (dd, $J = 2.55$ Hz, $J = 8.52$ Hz, 1H, Ar-*H*), 6.65–6.79 (m, 4H, Ar-*H*), 6.80–6.89 (d, $J = 8.80$ Hz, 2H, Ar-*H*), 7.14–7.25 (d, $J = 8.80$ Hz, 2H, Ar-*H*), 7.30–7.40 (d, $J = 8.52$ Hz, 1H, Ar-*H*); MS (ESI) m/e 489 (M^+).

6.49. 3,4-Dihydro-3-(4-methylphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8d)

The title compound was prepared in 80% yield from **7d** by a method similar to that described for **8a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.25–1.56 (m, 6H, 3CH_2), 2.27 (s, 3H, Ar–CH_3), 2.32–2.44 (t, $J = 5.34$ Hz, 4H, N–[CH_2]$_2$), 2.54–2.63 (t, $J = 5.91$ Hz, 2H, N–CH_2), 2.64–2.76 (d, $J = 14.35$ Hz, 1H, Ar–C*H*–C–OH), 2.90–3.02 (dd, $J = 3.09$ Hz, $J = 10.13$ Hz, 1H, Ar–O–CH$_2$–C*H*–C–OH), 3.17–3.30 (d, $J = 10.13$ Hz, Ar–C*H*–C–OH), 3.82–4.06 (m+t, $J = 5.91$ Hz, 3H, ArO–CH_2–CH$_2$–N, Ar–O–C*H*–CH–C–OH), 4.18–4.37 (t, $J = 10.40$ Hz, 1H, Ar–O–C*H*–CH–C–OH), 6.09–6.18 (d, $J = 2.25$ Hz, 1H, Ar-*H*), 6.34–6.46 (dd, $J = 2.25$ Hz, $J = 8.44$ Hz, 1H, Ar-*H*), 6.64–6.80 (m, 4H, Ar-*H*), 6.99–7.23 (m, 4H, Ar-*H*), 7.29–7.41 (d, $J = 8.44$ Hz, 1H, Ar-*H*); MS (ESI) m/e 473 (M^+).

6.50. 3-(4-Butoxyphenyl)-3,4-dihydro-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8e)

The title compound was prepared from **7e** by a method similar to that described for **8a** (yield = 30%).

^{1}H NMR (200 MHz, DMSO-d_6) 0.87–0.98 (t, $J = 7.55$ Hz, 3H, CH_3), 1.27–1.55 (m, 8H, 4CH_2), 1.60–1.78 (m, 2H, CH_2), 2.34–2.44 (t, $J = 4.84$ Hz, 4H, 2CH_2–N), 2.54–2.64 (t, $J = 6.04$ Hz, 2H, CH_2–N), 2.65–2.75 (d, $J = 14.20$ Hz, 1H, Ar–C*H*–C–OH), 2.88–3.00 (dd, $J = 3.32$ Hz, $J = 10.27$ Hz, 1H, Ar–O–CH$_2$–C*H*), 3.18–3.31 (d, $J = 14.20$ Hz, Ar–C*H*–C–OH), 3.82–4.03 (m, 5H, Ar–O–CH_2–CH$_2$–N, Ar–O–CH_2–[CH$_2$]$_2$, Ar–O–C*H*–CH–C–OH), 4.18–4.33 (t, $J = 10.58$ Hz, 1H, Ar–C*H*–CH–C–OH), 5.04 (s, 1H, C–O*H*), 6.11–6.16 (d, $J = 2.42$ Hz, 1H, Ar-*H*), 6.36–6.45 (dd, $J = 2.42$ Hz, $J = 8.46$ Hz, 1H, Ar-*H*), 6.65–6.88 (m+d, $J = 8.77$ Hz, 6H, Ar-*H*), 7.13–7.23 (d, $J = 8.77$ Hz, 2H, Ar-*H*), 7.31–7.40 (d, $J = 8.46$ Hz, 1H, Ar-*H*), 9.34 (s, 1H, Ar-*H*); MS (ESI) m/e 531 (M^+).

6.51. 3-(3,4-Dimethoxyphenyl)-3,4-dihydro-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8f)

The title compound was prepared from **7f** by a method similar to that described for **8a** (yield = 90%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.25–1.57 (m, 6H, 3CH_2), 2.33–2.44 (t, $J = 5.50$ Hz, 4H, N–[CH_2]), 2.54–2.64 (t, $J = 5.80$ Hz, 2H, N–CH_2O), 2.66–2.78 (d, $J = 14.04$ Hz, 1H, Ar–C*H*–C–OH), 2.87–2.98 (dd, $J = 306$ Hz, $J = 10.68$ Hz, Ar–O–CH$_2$–C*H*–C–OH), 2.20–2.35 (d, $J = 14.04$ Hz, 1H, Ar–C*H*–C–OH), 3.68 (s, 3H, Ar–O–CH_3), 3.73 (s, 3H, Ar–CH_3), 3.84–4.02 (m+t, $J = 5.80$ Hz, 3H, Ar–O–C*H*–CH–C–OH, Ar–O–CH_2–CH$_2$–N), 4.20–4.36 (t, $J = 10.68$ Hz, 1H, Ar–O–C*H*–CH–C–OH), 4.89–5.17 (br, 1H, C–O*H*), 6.11–6.17 (d, $J = 2.44$ Hz, 1H, Ar-*H*), 6.36–6.46 (dd, $J = 2.44$ Hz, $J = 8.54$ Hz, 1H, Ar-*H*), 6.67–6.90 (2m, 7H, Ar-*H*), 7.31–7.41 (d, $J = 8.54$ Hz, 1H, Ar-*H*); MS (ESI) m/e 519 (M^+).

6.52. 3,4-Dihydro-3-phenyl-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8g)

The title compound was prepared in 90% yield from **7g** by a method similar to that described for **8a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.28–1.58 (m, 6H, 3CH_2), 2.31–2.43 (t, $J = 5.60$ Hz, 4H, N–[CH_2]$_2$), 2.54–2.68 (t, $J = 5.82$ Hz, 2H, N–CH_2), 2.70–2.82 (d, $J = 9.72$ Hz, 1H, Ar–C*H*–C–OH), 2.95–3.15 (dd, $J = 2.71$ Hz, $J = 4.34$ Hz, 1H, Ar–C*H*–CH$_2$–O), 3.18–3.34 (d, $J = 9.72$ Hz, 1H, Ar–C*H*–C–OH), 3.45–4.10 (m, 3H, Ar–O–C*H*–CH, Ar–O–CH_2–CH$_2$), 4.18–4.42 (t, $J = 8.34$ Hz, 1H, Ar–OC*H*–CH), 3.60–4.80 (very br, O*H*), 6.05–6.15 (d, $J = 2.22$ Hz, 1H, Ar-*H*), 6.35–6.50 (dd, $J = 2.22$ Hz, $J = 8.45$ Hz, 1H, Ar-*H*), 6.55–6.80 (q, AA′BB′ system, 4H, Ar-*H*), 7.15–7.35 (br, 6H, Ar-*H*); MS (ESI) m/e 459 (M^+).

6.53. 3,4-Dihydro-3-(3-methoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8h)

The title compound was prepared from **7h** by a method similar to that described for **8a** (yield = 90%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.25–1.60 (m, 6H, 3CH_2), 2.32–2.45 (t, $J = 5.45$ Hz, 4H, N–9(CH_2)$_2$), 2.54–2.64 (t, $J = 5.73$ Hz, 2H, N–CH_2), 2.67–2.80 (d, $J = 14.33$ Hz, 1H, Ar–CH–C–OH), 2.92–3.05 (dd, $J = 2.87$ Hz, $J = 10.31$ Hz, 1H, Ar–CH–CH$_2$–O), 3.20–3.33 (d, $J = 14.33$ Hz, 1H, Ar–CH–C–OH), 3.70 (s, 3H, Ar–O–CH_3), 3.88–4.04 (m, 3H, Ar–O–CH–CH–Ar, Ar–O–CH_2–CH$_2$–N), 4.20–4.36 (t, $J = 10.31$ Hz, 1H, Ar–O–CH–CH–Ar), 4.95–5.20 (br, 1H, C–OH), 6.10–6.20 (d, $J = 2.30$ Hz, 1H, Ar-H), 6.35–6.45 (dd, $J = 2.30$ Hz, $J = 8.60$ Hz, 1H, Ar-H), 6.63–6.91 (m, 7H, Ar-H), 7.12–7.24 (t, $J = 8.02$ Hz, 1H, Ar-H), 7.30–7.40 (d, $J = 8.60$ Hz, 1H, Ar-H); MS (ESI) m/e 489 (M$^+$).

6.54. 3,4-Dihydro-3-(4-methoxyphenyl)-4-[[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8i)

The title compound was prepared in 85% yield from **7i** by a method similar to that described for **8a**.

^{1}H NMR (200 MHz, DMSO-d_6) 1.55–1.80 (m, 4H, 2CH_2), 2.60–2.80 (m, 1H, Ar–CH–C–OH), 2.88–3.01 (m, 1H, Ar–O–CH$_2$–CH–Ar), 3.14–3.35 (m, 1H, Ar–CH–C–OH), 3.73 (s, 3H, Ar–OCH_3), 3.87–4.05 (m, 3H, Ar–O–CH_2–CH$_2$, Ar–O–CH–CH–Ar), 4.17–4.36 (t, $J = 10.22$ Hz, 1H, Ar–O–CH–CH–Ar), 5.05 (s, 1H, C–OH), 6.10–6.18 (d, $J = 2.34$ Hz, 1H, Ar-H), 6.35–6.46 (dd, $J = 2.34$ Hz, $J = 8.46$ Hz, 1H, Ar-H), 6.63–6.90 (m, 6H, Ar-H), 7.13–7.25 (d, $J = 8.76$ Hz, 2H, Ar-H), 7.30–7.40 (d, $J = 8.46$ Hz, 1H, Ar-H), 9.34 (s, 1H, Ar–OH); MS (ESI) m/e 475 (M$^+$).

6.55. 3,4-Dihydro-3-phenyl-4-[[4-[3-(1-piperidinyl)propoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8j)

The title compound was prepared from **7j** by a method similar to that described for **8a** (yield = 40%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.26–1.57 (m, 6H, 3CH_2), 1.70–1.90 (m, 2H, O–CH$_2$–CH_2–CH$_2$–N), 2.22–2.40 (m, 6H, N–[CH_2]$_3$), 2.63–2.95 (d, $J = 14.14$ Hz, 1H, Ar–CH–C–OH), 2.95–3.08 (dd, $J = 3.41$ Hz, $J = 9.69$ Hz, 1H, Ar–O–CH$_2$–CH–Ar), 3.17–3.34 (d, $J = 14.14$ Hz, 1H, Ar–CH–C–OH), 3.83–4.03 (m, 3H, Ar–O–CH–CH–Ar, Ar–O–CH_2–CH$_2$), 4.21–4.39 (t, $J = 10.22$ Hz, Ar–O–CH–CH–Ar), 5.11 (s, 1H, Ar–CH$_2$–COH), 6.13–6.18 (d, $J = 2.62$ Hz, 1H, Ar-H), 6.37–6.46 (dd, $J = 2.62$ Hz, $J = 8.39$ Hz, 1H, Ar-H), 6.65–6.80 (m, 4H, Ar-H), 7.20–7.30 (s, 5H, Ar-H), 7.32–7.39 (d, $J = 8.39$ Hz, 1H, Ar-H), 9.35 (s, 1H, Ar–OH); MS (ESI) m/e 473 (M$^+$).

6.56. 3,4-Dihydro-3-(4-methoxyphenyl)-4-[[4-[3-(1-piperidinyl)propoxy]phenyl]methyl]-2*H*-1-benzopyran-4,7-diol (8k)

The title compound was prepared in 85% yield from **7k** by a method similar to that described for **8a**.

^{1}H NMR (200 MHz, CH$_3$OH-d_4) 1.38–1.60 (m, 6H, 3CH_2), 1.85–2.04 (m, 2H, Ar–O–CH$_2$–CH_2–CH$_2$), 2.37–2.58 (m, 6H, N–(CH_2)$_3$), 2.77–2.90 (d, $J = 14.18$ Hz, 1H, Ar–CH–C–OH), 3.00–3.12 (dd, $J = 10.11$ Hz, $J = 3.49$ Hz, 1H, Ar–CH–CH$_2$–O), 3.40 (s, 1H, Ar–CH–C–OH), 3.77 (s, 3H, Ar–O–CH_3), 3.90–4.02 (m, 3H, Ar–O–CH_2–CH$_2$, Ar–CH–CH–O), 4.20–4.35 (t, $J = 10.48$ Hz, 1H, Ar–CH–CH–O), 6.21–6.25 (d, $J = 2.58$ Hz, 1H, Ar-H), 6.42–6.50 (dd, $J = 2.58$ Hz, $J = 8.64$ Hz, 1H, Ar-H), 6.65–6.90 (m, 6H, Ar-H), 7.15–7.23 (d, $J = 8.83$ Hz, 2H, Ar-H), 7.33–7.40 (d, $J = 8.64$ Hz, 1H, Ar-H); MS (ESI) m/e 503 (M$^+$).

6.57. 4-[[4-[2-(Hexahydro-1*H*-azepin-1-yl)ethoxy]phenyl]-methyl]-3,4-dihydro-3-(4-methoxyphenyl)-2*H*-1-benzopyran-4,7-diol (8l)

The title compound was prepared in 90% yield from **7l** by a method similar to that described for **8a**.

^{1}H NMR (200 MHz, CH$_3$OH-d_4) 1.46–1.64 (br, 8H, 4CH_2), 2.60–2.70 (m, 4H, N–(CH_2)$_2$), 2.72–2.85 (m, 3H, N–CH_2, Ar–CH–C–OH), 2.90–3.01 (dd, $J = 3.41$ Hz, $J = 10.23$ Hz, 1H, Ar–O–CH$_2$–CH–C–OH), 3.16–3.33 (d, $J = 13.98$ Hz, 1H, Ar–CH–C–OH), 3.73 (s, 3H, Ar–O–CH_3), 3.83–4.00 (m, 3H, Ar–O–CH–CH, Ar–O–CH_2–CH$_2$), 4.18–4.35 (t, $J = 10.23$ Hz, 1H, Ar–O–CH–CH), 4.90–5.18 (br, 1H, C–OH), 6.11–6.18 (d, $J = 2.38$ Hz, 1H, Ar-H), 6.36–6.46 (dd, $J = 2.38$ Hz, $J = 8.52$ Hz, 1H, Ar-H), 6.65–6.90 (m+d, $J = 8.53$ Hz, 6H, Ar-H), 7.15–7.25 (d, $J = 8.86$ Hz, 2H, Ar-H), 7.30–7.40 (d, $J = 8.52$ Hz, 1H, Ar-H), 9.00–10.00 (br, 1H, Ar–OH); MS (ESI) m/e 503 (M$^+$).

6.58. 3-(2-Methoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9a)

Compound **8a** (2.8 g; 5.7 mmol) was dissolved in boiling acetonitrile (20 mL), and HCl 37% (0.5 mL) was added. The solution was stirred at reflux for 1 h, after which the volume was reduced under a nitrogen flux; the solution was kept at +4 °C for several hours and the title compound crystallized. The white solid was filtered and washed with acetonitrile and diethyl ether, obtaining 300 mg of the title compound (mp 134–136 °C; yield = 10%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.20–1.90 (m, 6H, 3CH_2), 2.80–3.09 (m, 2H, N$^+$–CH_2), 3.34–3.68 (m, 6H, N$^+$–(CH_2)$_2$, Ar–CH_2–C=C), 3.80 (s, 3H, Ar–O–CH_3), 4.25–4.40 (t, $J = 4.92$ Hz, 2H, Ar–O–CH_2–CH$_2$), 4.66–4.82 (br, 2H, Ar–O–CH_2–C=C), 6.18–6.27 (m, 2H, Ar-H), 6.77–6.98 (m, 4H, Ar-H), 7.01–7.20 (m, 4H, Ar-H), 7.23–7.38 (m, 1H, Ar-H); MS (ESI) m/e 471 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{30}H_{34}NO_4$ (MH$^+$) 472.2488, found 472.2482.

6.59. 3-(4-Hydroxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9b)

The title compound was prepared from **8b** by a method similar to that described for **9a**. The reaction was con-

ducted in ethanol and the product was precipitated by diethyl ether (mp 153–164 °C dec; yield = 70%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.20–1.90 (2m, 6H, 3CH_2), 2.77–3.12 (m, 2H, N–CH_2), 3.33–3.54 (m, 4H, N–[CH_2]$_2$), 3.72 (s, 2H, Ar–CH_2), 4.24–4.41 (t, J = 5.92 Hz, 2H, Ar–O–CH_2), 4.85 (s, 2H, Ar–O–CH_2–C≡C), 6.16–6.26 (d+br, J = 2.37 Hz, 2H, Ar-*H*), 6.69–6.80 (d, J = 8.59 Hz, 2H, Ar-*H*), 6.81–6.92 (m, 3H, Ar-*H*), 7.03–7.17 (dd, J = 2.66 Hz, J = 8.59 Hz, 4H, Ar-*H*), 9.51 and 9.54 (2s, 2H, Ar–O*H*), 10.12–10.50 (br, 1H, N$^+$–*H*); MS (ESI) m/e 457 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{29}H_{32}NO_4$ (MH$^+$) 458.2331, found 458.2330.

6.60. 3-(4-Methoxy)phenyl-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9c)

The title compound was prepared from **8c** by a method similar to that described for **9a** (mp 199–205 °C dec; yield = 85%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.20–1.96 (2m, 6H, 3CH_2), 2.80–3.11 (m, 2H, N$^+$–CH_2), 3.34–3.55 (m, 4H, N$^+$–[CH_2]$_2$), 3.69–3.75 (br+s, 5H, Ar–O–CH_3, Ar–CH_2–C≡C), 4.29–4.40 (t, J = 4.95 Hz, 2H, Ar–O–CH_2–CH$_2$–N$^+$), 4.88 (s, 2H, Ar–O–CH_2–C≡C), 6.18–6.28 (d+m, J = 2.32 Hz, 2H, Ar-*H*), 6.80–6.98 (2d, J = 8.72 Hz, 5H, Ar-*H*), 7.04–7.28 (2d, J = 8.72 Hz, 4H, Ar-*H*), 9.58 (s, 1H, Ar–O*H*), 10.52–10.78 (br, 1H, N$^+$–*H*); MS (ESI) m/e 471 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{30}H_{34}NO_4$ (MH$^+$) 472.2488, found 472.2487.

6.61. 3-(4-Methylphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9d)

The title compound was prepared in 10% yield from **8d** by a method similar to that described for **9a**. The solution was then evaporated and the product crystallized from a mixture of ethanol and diethyl ether (mp 175–182 °C dec; yield = 10%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.21–1.87 (2m, 6H, 3CH_2), 2.29 (s, 3H, Ar–CH_3), 2.83–3.11 (m, 2H, N$^+$–CH_2), 3.34–3.55 (m, 4H, N$^+$–[CH_2]$_2$), 3.72 (s, 2H, Ar–CH_2–C≡C), 4.28–4.38 (t, J = 5.24 Hz, 2H, Ar–CH_2–CH$_2$–N$^+$), 4.89 (s, 2H, Ar–O–CH_2–C≡C), 6.16–6.27 (d+m, J = 2.46 Hz, 2H, Ar-*H*), 6.80–6.93 (2d, J = 2.46 Hz, J = 8.63 Hz, 3H, Ar-*H*), 7.05–7.13 (d, J = 8.63 Hz, 2H, Ar-*H*), 7.18 (s, 4H, Ar-*H*), 9.54 (s, 1H, Ar-*H*), 10.05–10.30 (br, 1H, N$^+$–*H*); MS (ESI) m/e 455 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{30}H_{34}NO_3$ (MH$^+$) 456.2539, found 456.2526.

6.62. 3-(4-*n*-Butoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]-phenyl]methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9e)

The title compound was prepared in ethanol from **8e** by a method similar to that described for **9a** (mp 129–134 °C dec; yield = 50%).

^{1}H NMR (200 MHz, DMSO-d_6) 0.83–0.98 (t, J = 7.31 Hz, 3H, CH_3–[CH$_2$]$_3$–O), 1.27–1.88 (2m, 10H, 3CH_2'CH$_3$–[CH_2]$_2$–CH$_2$–O), 2.82–3.09 (m, 2H, N$^+$–CH_2), 3.35–3.54 (m, 4H, N$^+$–[CH_2]$_2$), 3.65–3.79 (br, 2H, Ar–CH_2–C≡C), 3.87–4.02 (t, J = 6.35 Hz, 2H, Ar–OCH_2–[CH$_2$]$_2$–CH$_3$), 4.24–4.40 (t, J = 4.77 Hz, 2H, Ar–O–CH_2–CH$_2$–N$^+$), 4.88 (s, 2H, Ar–O–CH_2–C≡C), 6.17–6.27 (d+m, J = 2.22 Hz, 2H, Ar-*H*), 6.81–6.97 (m, 5H, Ar-*H*), 7.04–7.28 (2d, J = 8.90 Hz, J = 8.58 Hz, 4H, Ar-*H*), 9.53 (s, 1H, Ar–O*H*), 10.06 (br, 1H, N$^+$–*H*); MS (ESI) m/e 513 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{33}H_{40}NO_4$ (MH$^+$) 514.2957, found 514.2949.

6.63. 3-(3,4-Dimethoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9f)

The title compound was prepared from **8f** by a method similar to that described for **9a** (mp 209–214 °C dec; yield = 50%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.19–191 (2m, 6H, 3CH_2), 2.80–3.09 (m, 2H, N$^+$–CH_2), 3.34–3.53 (m, 4H, N$^+$–[CH_2]$_2$), 3.60 (s, 3H, Ar–O–CH_3), 3.69–3.78 (br, 5H, Ar–O–CH_3, Ar–CH_2–C≡C), 4.27–4.38 (t, J = 5.29 Hz, 2H, Ar–O–CH_2–CH$_2$–N$^+$), 4.91 (s, 2H, Ar–O–CH_2–C≡C), 6.18–6.29 (d+br, J = 2.49 Hz, 2H, Ar-*H*), 6.78–7.00 (m, 6H, Ar-*H*), 7.05–7.18 (d, J = 8.41 Hz, 2H, Ar-*H*), 9.55 (s, 1H, Ar–O*H*), 10.22–10.48 (br, 1H, N$^+$–*H*); MS (ESI) m/e 501 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{31}H_{36}NO_5$ (MH$^+$) 502.2593, found 502.2595.

6.64. 3-Phenyl-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]-methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9g)

The title compound was first prepared as the free base in a 70% yield from **8g** by a method similar to that described for **9a**, although the reaction was conducted in more diluted conditions. The free base of **9g** was isolated by adding a 7-fold excess of K_2CO_3 when the reaction was finished (by HPLC analysis). After 10 min stirring, the salt was filtered off and the solution was evaporated to dryness. The residue was treated with acetone, obtaining a light yellow solid which was filtered. The free base of **9g** was then salified with HCl (1:1 ratio) in ethyl acetate (500 vol), obtaining the title compound crystallizing as a white solid (mp 175–180 °C dec; yield = 90%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.25–1.90 (2m, 6H, 3CH_2), 2.80–3.10 (m, 2H, N$^+$–CH_2), 3.32–3.55 (m, 4H, N$^+$–[CH_2]$_2$), 3.72 (s, 2H, Ar–CH_2–C≡C), 4.24–4.42 (t, J = 5.18 Hz, 2H, Ar–O–CH_2–CH$_2$–N$^+$), 4.91 (s, 2H, Ar–O–CH_2–C≡C), 6.17–6.31 (m, 2H, Ar-*H*), 6.81–6.96 (m, 3H, Ar-*H*), 7.04–7.17 (d, J = 8.63 Hz, 2H, Ar-*H*), 7.23–7.47 (m, 5H, Ar-*H*), 9.58 (s, 1H, Ar–O*H*), 10.18–10.48 (br, 1H, N$^+$–*H*); MS (ESI) m/e 441 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{29}H_{32}NO_3$ (MH$^+$) 442.2382, found 442.2364.

6.65. 3-(3-Methoxyphenyl)-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9h)

The title compound was prepared from **8h** by a method similar to that described for **9a**. The product was precipitated by diethyl ether (mp 104–112 °C; yield = 30%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.20–1.90 (m, 6H, 3CH_2), 2.77–3.14 (m, 2H, N$^+$–CH_2), 3.34–3.55 (m, 4H, (CH_2)$_2$), 3.61–3.81 (s+br, 5H, Ar–CH_2–C≡C, Ar–O–CH_3), 4.24–4.41 (t, J = 4.59 Hz, 2H, Ar–O–CH_2–CH$_2$), 4.90 (s, 2H, Ar–O–CH_2–C≡C), 6.18–6.28 (m, 2H, Ar-*H*), 6.76–6.96 (m, 5H, Ar-*H*), 7.04–7.37 (m, 4H, Ar-*H*), 9.57 (s, 1H, Ar–O*H*), 9.98–10.41 (br, 1H, N$^+$–*H*); MS (ESI) m/e 471 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{30}H_{34}NO_4$ (MH$^+$) 472.2488, found 472.2490.

6.66. 3-(4-Methoxyphenyl)-4-[[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9i)

The title compound was prepared in ethanol from **8i** by a method similar to that described for **9a** (mp 231–232 °C dec; yield = 55%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.75–2.09 (br, 4H, 2CH_2), 2.95–3.20 (br, 2H, N$^+$–CH_2), 3.41–3.63 (t, J = 5.09 Hz, 4H, N$^+$–(CH_2)$_2$), 3.67–3.80 (br+s, 5H, Ar–CH_2–C≡C, Ar–O–CH_3), 4.20–4.30 (t, J = 4.79 Hz, 2H, Ar–O–CH_2–CH$_2$), 4.88 (s, 2H, Ar–O–CH_2–C≡C), 6.18–6.28 (m, 2H, Ar-*H*), 6.80–7.00 (m, 5H, Ar-*H*), 7.05–7.15 (d, J = 8.68 Hz, 2H, Ar-*H*), 7.18–7.30 (d, J = 8.68 Hz, 2H, Ar-*H*), 9.52 (s, 1H, Ar–O*H*), 10.35–10.56 (br, 1H, N$^+$–*H*); MS (ESI) m/e 457 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{29}H_{32}NO_4$ (MH$^+$) 458.2331, found 458.2334.

6.67. 3-Phenyl-4-[[4-[3-(1-piperidinyl)propoxy]phenyl]methyl]-2*H*-1-benzopyran-7-ol (9j)

The title compound was first prepared as the free base in a 45% yield from **8j** by a method similar to that described for **9a**, although the reaction was conducted in more diluted conditions than those described for **9a**. The free base of the product was isolated by adding a 7-fold excess of K_2CO_3 when the reaction was finished (by HPLC analysis). After 10 min stirring, the salt was filtered off and the solid was washed with water to completely eliminate the inorganic salts. The white solid was dried under vacuum (mp 107–109 °C dec; yield = 45%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.34–1.79 (m, 6H, 3CH_2), 1.89–2.14 (br, 2H, O–CH$_2$–CH_2–CH$_2$–N$^+$), 2.57–3.12 (br, 6H, N$^+$–[CH_2]$_3$), 3.70 (s, 2H, Ar–CH_2–C≡C), 3.88–4.03 (t, J = 6.04 Hz, 2H, Ar–O≡CH_2–CH$_2$), 4.91 (s, 2H, Ar–O–CH_2–C≡C), 6.18–6.28 (m, 2H, Ar-*H*), 6.76–6.85 (d, J = 8.72 Hz, 2H, Ar-H), 6.86–6.94 (d, J = 8.38 Hz, 1H, Ar-*H*), 7.01–7.12 (d, J = 8.72 Hz, 2H, Ar-*H*), 7.24–7.44 (m, 5H, Ar-*H*); MS (ESI) m/e 455 (M$^+$); HRMS (ES$^+$) m/e calcd for $C_{30}H_{34}NO_3$ (MH$^+$) 456.2539, found 456.2527.

6.68. 3-(4-Methoxyphenyl)-4-[[4-[3-(1-piperidinyl)propoxy]phenyl]methyl]-2*H*-1-benzopyran-7-ol hydrochloride (9k)

The title compound was prepared in ethanol from **8k** by a method similar to that described for **9a** (mp 164–169 °C dec; yield = 40%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.23–1.87 (2m, 6H, 3CH_2), 2.03–2.25 (m, 2H, Ar–O–CH$_2$–CH_2), 2.72–2.96 (m, 2H, N$^+$–CH_2–CH$_2$), 3.02–3.20 (m, 2H, N$^+$–CH$_2$), 3.34–3.50 (m, 2H, N$^+$–CH_2), 3.70–3.78 (br+s, 5H, Ar–OCH_3, Ar–CH_2–C≡C), 3.90–4.04 (t, J = 5.95 Hz, 2H, Ar–O–CH_2–CH$_2$), 4.88 (s, 2H, Ar–O–CH_2–C≡C), 6.17–6.28 (m, 2H, Ar-*H*), 6.75–6.98 (m, 5H, Ar-*H*), 7.02–7.12 (d, J = 8.64 Hz, 2H, Ar-*H*), 7.18–7.28 (d, J = 8.63 Hz, 2H, Ar-*H*), 9.55 (s, 1H, Ar–O*H*), 10.18–10.48 (br, 1H, N$^+$–*H*); MS (ESI) m/e 485 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{31}H_{36}NO_4$ (MH$^+$) 486.2644, found 486.2632.

6.69. 4-[[4-[2-(Hexahydro-1*H*-azepin-1-yl)ethoxy]phenyl]methyl]-3-(4-methoxyphenyl)-2*H*-1-benzopyran-7-ol hydrochloride (9l)

The title compound was prepared from **8l** by a method similar to that described for **9a**. The product was isolated by crystallization from ethanol (mp 129–137 °C; yield = 30%).

^{1}H NMR (200 MHz, DMSO-d_6) 1.50–1.95 (2m, 8H, 4CH_2), 3.02–3.58 (m, 6H, N$^+$–(CH_2)$_3$), 3.65–3.82 (br+s, 5H, Ar–CH–C≡C, Ar–O–CH_3), 4.25–4.42 (t, J = 4.87 Hz, 2H, Ar–O–CH_2–CH$_2$), 4.88 (s, 2H, Ar–O–CH_2–C≡C), 6.18–6.28 (d+m, J = 2.24 Hz, 2H, Ar-*H*), 6.80–6.98 (m, 5H, Ar-*H*), 7.04–7.15 (d, J = 8.61 Hz, 2H, Ar-*H*), 7.19–7.29 (d, J = 8.61 Hz, 2H, Ar-*H*), 9.55 (s, 1H, Ar–O*H*), 10.40–10.65 (br, 1H, N$^+$–*H*); MS (ESI) m/e 485 (M$^+$ free base); HRMS (ES$^+$) m/e calcd for $C_{31}H_{36}NO_4$ (MH$^+$) 486.2644, found 486.2651.

6.70. 3-Phenyl-4-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]-7-methoxy-2*H*-1-benzopyran methansulfonate (10a)

The title compound was prepared from **9g** as free base. The free base of compound **9g** (1.2 g; 2.7 mmol) was dissolved in DMF (5 mL). NaH 80% (154 mg; 5.4 mmol) and methyl iodide (3.6 mmol; 22.4 mL of a 0.16 M solution in DMF) were added and the mixture was stirred at rt for 2 h. After quenching with water (20 mL) the product was purified by flash chromatography (CH_2Cl_2/CH_3OH from 80/20 to 90/10). The residue was dissolved in acetone and an equimolar amount of methansulfonic acid was added. The title compound was precipitated as the methansulfonate salt by adding diethyl ether (mp 122–124 °C dec; yield = 35%).

^{1}H NMR (200 MHz, CH_3OH-d_4) 1.23–1.91 (2m, 6H, 3CH_2), 2.33 (s, 3H, CH_3–SO_3), 2.85–3.12 (m, 2H, N^+–CH_2), 3.38–3.58 (m, 4H, N^+–[CH_2]$_2$), 3.69–3.75 (2s, 5H, Ar–O–CH_3, Ar–CH_2–C≡C), 4.21–4.34 (t, $J = 4.62$ Hz, 2H, Ar–O–CH_2–CH_2–N^+), 4.96 (s, 2H, Ar–O–CH_2–C≡C), 6.33–6.48 (d+m, $J = 244$ Hz, 2H, Ar-*H*), 6.82–6.94 (d, $J = 8.72$ Hz, 2H, Ar-*H*), 6.96–7.05 (d, $J = 8.47$ Hz, 1H, Ar-*H*), 7.06–7.17 (d, $J = 8.72$ Hz, 2H, Ar-*H*), 7.24–7.48 (m, 5H, Ar-*H*), 9.10–10.48 (br, 1H, N^+–*H*); MS (ESI) *m/e* 455 (M^+ free base); HRMS (ES^+) *m/e* calcd for $C_{30}H_{34}NO_3$ (MH^+) 456.2539, found 456.2537.

6.71. Pharmacology

6.71.1. Human ERα and ERβ binding. ERα and β binding analysis was performed as previously described.[23] Briefly, the standard assay was performed in a volume of 100 μL, containing a final concentration of 0.5 nM [^{3}H]-17β-estradiol (PerkinElmer Life Science, Boston, MA), increasing concentration of unlabeled test compounds (0.01–100 nM), 5 μL of diluted (1:100 in binding buffer) human recombinant ERα or β (insect Sf9 cells) and 95 μL of binding buffer (10 mM Tris pH 7.5, 10% glycerol, 1 mM dithiothreithol, 1 mg/mL bovine serum albumin). The K_i of [^{3}H]-17β-estradiol at ERα and ERβ were 0.018 and 0.015 nM, respectively. Incubation was carried out at room temperature for 3 h. After incubation, 100 μL of 50% hydroxyapatite slurry (equilibrated in 50 mM Tris pH 7.4, 1 mM EDTA) was added to each tube and vortexed three times over 15 min. One milliliter of wash buffer (40 mM Tris pH 7.4, 1 mM EGTA, 1 mM EDTA, and 100 mM KCl) was added to each reaction and was centrifuged at 10,000*g* for 5 min, and the supernatant was aspirated. The wash step was repeated two more times and then the hydroxyapatite pellet was resuspended in 400 μL of ethanol, transferred to a scintillation vial, and counted. Nonspecific binding was defined as that which occurred in the presence of 1 μM diethylstilbestrol and represented 10–15% of the total binding. K_i values were calculated from the equation of Cheng and Prusoff[30] using the observed half maximal inhibition concentration (IC_{50}) of the tested compound, the concentration of radioligand used in the assay, and the dissociation constant value of the ligand. The data were also fitted by an iterative program (RECEPT) for nonlinear regression analysis[31] both to a one-site and to a two-site model. The one-site model was then chosen when it yielded the best correlation coefficient and when the improvement of goodness-of-fit for the two-site model was not statistically significant ($P < 0.05$) according to the *F*-test on the sums of squared errors.

6.71.2. Immature female rat study. Female Sprague-Dawley rats (21 days old), weighing approximately 40–50 g (Charles River Italica, Calco, Italy) were treated by oral gavage with vehicle (0.5% methylcellulose, 3 mL/kg), test compounds (0.001–10 mg/kg day), or EE2 at 0.05 mg/kg day for 3 days. The compounds under investigation were also administered 15 min before the EE2 gavage, used as estrogenic stimulus to increase uterine weight. Nonestrogenic controls were given vehicle alone. After the final dose, the animals fasted overnight. The rats were autopsied 24 h after the final dose. At autopsy, the uterine wet weight was determined, and uterine weight/body weight ratios (UWR) were calculated for each animal. The inhibition percentage of the estrogen-induced response was then calculated by the following formula: % inhibition $= 100 \times [(UWR_{EE2} - UWR_{test\ agent})/(UWR_{EE2} \times UWR_{control})]$.

6.72. Molecular modeling

Molecular modeling studies were performed with Sybyl 6.8,[32] running on a Silicon Graphics O2 workstation. The geometry of the ligands was optimized in vacuo using the MMFF94s force field,[33] with the Powell method[34] to an energy gradient of 0.05 kcal/mol Å using MMFF94 charges, with the dielectric constant set at 1. The same force field was applied to proteins and complexes, with energy minimization performed to a gradient of 0.10 kcal/mol Å. ERα and ERβ molecular models were built starting from the crystal coordinates of receptor-ligand complexes found in the PDB.[21] The BIOPOLYMER module of Sybyl was employed to add the missing side chains and hydrogen atoms, and for other protein-building processes. Ligand structures within the complexes were refined by the standard commands of Sybyl.

The model of human ERα was built starting from the coordinates of the complex ERα/OHT (PDB code: 3ERT[35]). After the addition of missing side chains and hydrogen atoms, the model was submitted to three cycles of energy minimization: at first, only the position of the hydrogen atoms was optimized, and then the optimization process was extended to the protein side chains and to the ligand, and, finally, to the whole complex.

The model of human ERβ was built starting from the coordinates of the complex between rat ERβ and raloxifene (PDB code: 1QKN[15]). Hydrogen atoms and missing side chains were added and then the last 25 amino acids of the ligand-binding domain (473–497, partially unresolved in 1QKN) were replaced by the corresponding amino acids taken from our ERα refined structure. The replaced sequence included His475 (corresponding to His524 in ERα), the loop connecting His475 to helix 12 and helix 12. On the basis of a homology modeling approach, the alignment performed by ClustalX[36] between rat and human ERβ amino acid sequences was employed to build the human model of ERβ, by replacing the appropriate amino acids. The ligand raloxifene was replaced by the 4-hydroxytamoxifen (OHT) structure, taken from the refined model of ERα; OHT was fitted in the ligand-binding domain of ERβ by superposition of its stiryl portion to that of raloxifene. The resulting complex, ERβ-OHT, was submitted to a cycle of energy minimizations as described above for ERα. The amino acid numeration of ERβ, which we referred to in the text, is that of the 1QKN crystal structure, as reported by Pike et al.[15] Both the refined models of ERα and ERβ were analyzed with

the program Procheck v. 3.5.4[37] and resulted acceptable from a biophysical point of view, with no bad contacts and proper values of phi, psi, and chi angles.

The minimum-energy conformations of the ERα and ERβ receptors were employed for docking studies, after deletion of the ligand OHT atoms. Docking studies were performed using the *Dock* command of Sybyl 6.8, on benzopyran derivatives whose basic side chain was considered in its protonated state. The minimization of the interaction energy was performed by keeping the protein structure fixed and that of the ligand flexible, applying the MMFF94s force field, to a gradient of 0.10 kcal/mol Å.

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