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Original Article

Regioselective synthesis and estrogenicity of (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins

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Abstract

Nine new (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins have been synthesized from 2,4,6-trimethoxybenzaldehyde via a short, efficient, and regioselective pathway, together with the unsubstituted analogue (±)-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin. The compounds were tested for estrogenic activity using a yeast-based estrogen screen. Weak estrogenicity was determined for seven members of the series.

Keywords: Phytoestrogens; Neoflavanones; Recombinant yeast estrogen screen

1. Introduction

A wide variety of polyphenolic non-steroidal plant-derived compounds have been shown to exert estrogen-like biological activity. In recent years, the scientific interest in these phytoestrogens has increased dramatically as they may represent alternatives for hormonal replacement therapy in the treatment of postmenopausal symptoms and diseases associated with loss of the endogenous estrogen receptor (ER) ligand 17β-estradiol (**1**, Fig. 1) in women. Many members of the isoflavone, lignan, coumestan, and prenylflavonoid structural classes of phytoestrogens display a significant binding affinity to both ER-α and ER-β [1–8], with 8-prenylnaringenin (**2**) from hops (*Humulus lupulus* L.) exerting the highest *in-vitro* estrogenic activity of all phytoestrogens known to date [6–8].

In view of our aim to prepare polyphenols exhibiting interesting biological, in particular estrogen-like activities, our attention was drawn to the 4-aryl-3,4-dihydrocoumarin (**3**, neoflavanone) system, which shares structural similarities with the flavonoids as well as with the coumestans. The bio-

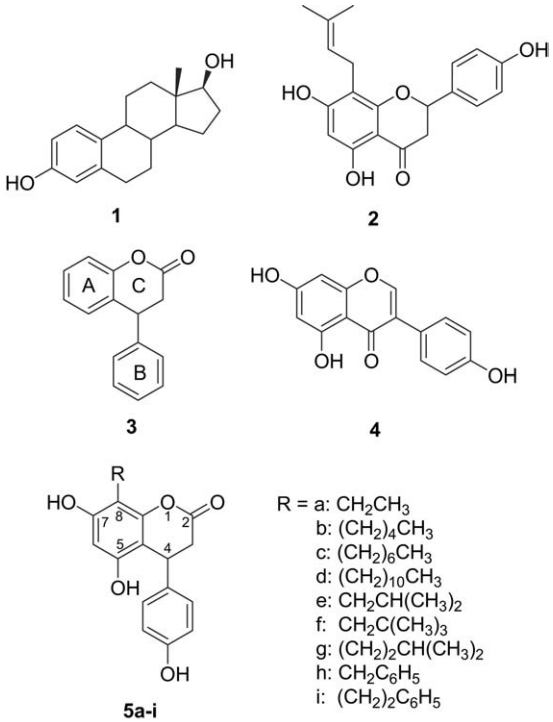

Fig. 1. Structures of compounds **1**–**4**, and **5a**–**i**.

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logical properties of compounds having a 4-aryl-3,4-dihydrocoumarin nucleus have been investigated only scarcely, although the structural entity is found in a number of naturally occurring molecules [9–14] and inhibitory effects on aldose reductase and protein kinases as well as an antiherpetic activity have been reported [13–15]. Interestingly, Nishimura et al. [16] identified a 4-aryl-3,4-dihydrocoumarin derivative having an estrogenic activity comparable to that of the isoflavone genistein (**4**). Thus, it appeared that the former compounds could be interesting targets for further investigations into their ER binding features.

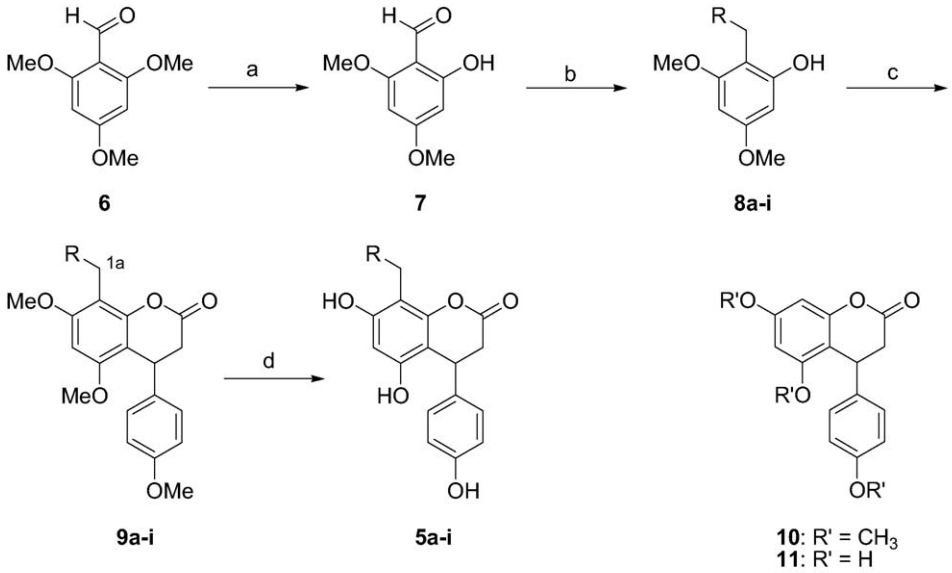
Synthetic endeavors on naturally occurring polyphenolic derivatives have mainly focused on *O*-alkylation. However, *C*-alkylated compounds may express more advantageous biological properties, since free phenolic hydroxyls are pivotal on interaction with specific biological targets [17]. Indeed, a common feature occurring in phytoestrogens is the presence of at least two hydroxyl (or related) groups, positioned at a distinct intramolecular distance, which, albeit few exceptions, is a prerequisite for ER affinity and transcriptional activity [18,19]. In view of the apparently essential role of an alkyl substituent at *C*(8) (naringenin lacking the prenyl substituent at *C*(8) is only weakly active in comparison with 8-prenylnaringenin (**2**) [3,4]), we aimed at introducing regioselectively an alkyl group at *C*(8).

We describe herein a short, efficient, and regioselective synthetic approach to a series of (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins (**5a**–**i**) and the results of a first screening of their estrogenic activity in an ER-α-based yeast estrogen screen.

2. Chemistry

The strategy to build the 4-aryl-3,4-dihydrocoumarin skeleton is based on condensation of a phenol with a cinnamic acid derivative [20,21]. Application to (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins calls for a combination of a *C*-alkylated phloroglucinol derivative and *p*-coumaric acid, while appropriate protection/deprotection protocols of phenolic groups should take care of the regioselectivity. This prerequisite is very important as condensation of *p*-coumaric acid (also methoxylated) with a *C*-alkylated phloroglucinol furnishes inevitably a mixture of two regioisomeric dihydrocoumarins either alkylated at *C*(8) or at *C*(6). Thus, a protection step involving the *p*-hydroxyl, leaving at least one of the *o*-hydroxyls of a *C*-alkylated phloroglucinol unprotected, is mandatory in order to obtain the desired 4-aryl-3,4-dihydrocoumarins alkylated at *C*(8).

Commercially available 2,4,6-trimethoxybenzaldehyde (**6**) was used as a template for the A-ring moiety of the neoflavonoid (Scheme 1). Careful regioselective mono-demethylation in *o*-position of the aldehyde was achieved by using the ortho-directing influence of the acyl substituent [22] in a demethylation induced by boron tribromide as a Lewis acid (optimized conditions: 0.59 equiv, 1.5 h). Evidence for *o*-mono-demethylation followed from ^{1}H NMR spectroscopy, as two different methoxy groups and also two differentiated aromatic protons were observed in the isolated product. The resulting 2-hydroxy-4,6-dimethoxybenzaldehyde (**7**) has the appropriate protection pattern for condensation, while the presence of the aldehyde functionality allows readily mono-alkylation using organometallics (alkyllithium, alkyl-



R = CH_3 (**a**), $(CH_2)_3CH_3$ (**b**), $(CH_2)_5CH_3$ (**c**), $(CH_2)_9CH_3$ (**d**), $CH(CH_3)_2$ (**e**), $C(CH_3)_3$ (**f**), $CH_2CH(CH_3)_2$ (**g**), C_6H_5 (**h**), $CH_2C_6H_5$ (**i**)

a: BBr_3 (0.59 equiv), 1.5 h, CH_2Cl_2, -78°C to rt; b: (i) RLi/RMgBr, Et_2O, -78°C to rt (ii) $HSiEt_3$ (rt), CF_3COOH, CH_2Cl_2, -78°C to rt; c: *p*-methoxycinnamic acid, $BF_3.Et_2O$, rt; d: BBr_3 (6 equiv), 15 h, CH_2Cl_2, -78°C to rt

Scheme 1. Regioselective synthesis of (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins.

magnesium bromide) leading to rather unstable secondary benzylic alcohols. Deoxygenation was effected on treatment with triethylsilane and trifluoroacetic acid [23] whereby *C*-alkylated 3,5-dimethoxyphenols (**8a**–**i**) were obtained (yields > 85%). Subsequent condensation with *p*-methoxycinnamic acid (B-ring moiety of the 4-aryl-3,4-dihydrocoumarin skeleton) in the presence of excess boron trifluoride yielded (±)-8-alkyl-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarins in high yields (**9a**–**i**). Confirmation of the desired position of the alkyl substituent at *C*(8) was established unambiguously by ^{1}H NOE NMR experiments, as irradiation of the aromatic *C*(6) proton signal showed clear signal enhancement of the two adjacent methoxy groups. Demethylation of the protected phenolic groups using boron tribromide furnished the desired (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins (**5a**–**i**). In order to obtain the non-alkylated analogue as a reference, 3,5-dimethoxyphenol was used as the A-ring moiety for condensation with *p*-methoxycinnamic acid yielding (±)-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**10**), which, after demethylation, led to (±)-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**11**). All structures were established by analysis of relevant ^{1}H NMR, ^{13}C NMR, ^{1}H NOE NMR, and MS data, and by comparison with known spectral properties of similar compounds [9,21,24,25].

3. Biological investigation and discussion

A well-known *Saccharomyces cerevisiae*-based estrogen assay [26] (YES) was used to investigate the binding to the ER-α and the subsequent transactivation at an ERE-driven *Lac-Z* reporter gene of the newly synthesized compounds **5a**–**i**. Results are presented in Table 1. 17β-Estradiol (**1**), 8-prenylnaringenin (**2**), and genistein (**4**) were included as positive controls, biphenyl served as a negative control. The yeast assay coupled a high sensitivity (EC_{50} of 17β-estradiol: 16 pM at day 3 of incubation) [27] with a good specificity. We applied biphenyl as a negative control because of the absence of hydroxyls which are known for most compounds to be essential for ER binding [18,19,28]. Biphenyl, in contrast to phenylphenol or biphenol (data not shown), did not elicit a dose–response color formation in the course of the experimental period of 12 days in total, which is in accordance to literature data [29]. In addition, the assay ranked 8-prenylnaringenin and genistein in line with their previously described relative estrogenic activities after 3 days of incubation [3]. The (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins exhibited very weak activities and dose–response curves could only be established after a prolonged incubation time (6–12 days) [30]. In these conditions, EC_{50}-values do no longer allow direct comparison of activities. Therefore, we relied on relative potencies (RPs) (see Section 5.2) to compare the estrogenic activities of the compounds.

The estrogenicities of the (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins were at least 250-fold weaker compared to 8-prenylnaringenin. Remarkably, the short-chain compounds **5a** (ethyl) and **5h** (benzyl) did not elicit a response in the YES and the same observation was made for the non-alkylated analogue **11**. Furthermore, the relative induction efficiencies (RIEs; see Section 5) indicate that the active compounds could not produce a maximum response in the yeast assay, which may suggest that they behave as partial agonists on ER-α. For the linear groups, the *n*-heptyl (**5c**) was at least 10-fold more active than a shorter (*n*-pentyl, **5b**) or a longer chain (*n*-undecyl, **5d**). A bulky substituent (2,2-dimethylpropyl, **5f**) resulted in a decrease in activity with respect to other branched chains (2-methylpropyl, **5e** and 3-methylbutyl, **5g**) and, in fact, the presence of a phenyl in the side chain led to the lowest estrogenic activities (benzyl, **5h**: not active and 2-phenylethyl, **5i**: weakest compound in the series). Although estrogenic activity is low, it is clear that suitable alkylation at *C*(8) can induce binding to the ER, as the unsubstituted compound **11** did not show an estrogenic response.

4. Conclusion

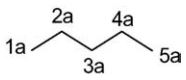
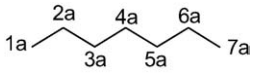
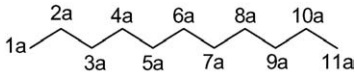
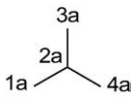
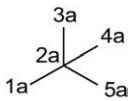
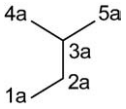
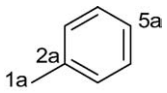
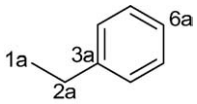
In summary, a versatile, efficient, and regioselective synthetic pathway to (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins was elaborated starting from 2,4,6-trimethoxybenzaldehyde. Although some compounds exhibited an estrogenic activity in the yeast-based estrogen screen, this activity was generally moderate to low.

5. Experimental

5.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were obtained with a Varian Mercury 300 spectrometer (^{1}H NMR: 300 MHz, ^{13}C NMR: 75 MHz). All spectra were recorded in DMSO-d_6. Chemical shifts (δ) are expressed in parts per million (ppm) relative to the residual solvent peak. All signals assigned to hydroxyl groups were exchangeable with D_2O. Exact mass measurements were performed on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Q-TOF 1, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in an *i*-propanol/water (1:1) mixture (negative mode: + 12.5 mM ammonium acetate, positive mode: + 0.1% formic acid) at 5 μl/min. Analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values. Thin layer chromatography was carried out on precoated Alugram® SIL G/UV_{254} silica gel plates (Macherey-Nagel & Co., Düren, Germany) and TLC separations were examined under UV light at 254 nm and revealed by a sulfuric acid–anisaldehyde spray. Column chromatography was carried out on silica (Eco-chrom, ICN Silica 63–200 mesh) from ICN Biomedicals (Eschwege, Germany). Compounds were obtained as amor-

Table 1
EC_{50} values, RPs, and RIEs of 8-alkylated 5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins in an estrogen-sensitive yeast assay ($n > 3$)

Compound	R	EC_{50}, μM[a] (95% CI)[b]	RP[a] (95% CI)[b]	RIE[c] (%)
1		1.6E-5, (5.3E-6–4.7E-5)	1	100
5a		NE[d]		
5b		69 (55–85)	3.3E-7, (8.2E-8–1.3E-6)	92.6 (21.5)
5c		9.0 (7.1–12)	2.4E-6, (6.1E-7–9.3E-6)	57.2 (28.3)
5d		3.6 (1.1–12)	4.4E-7, (8.0E-8–2.4E-6)	69.8 (28.6)
5e		160 (120–210)	1.3E-7, (1.1E-8–1.5E-6)	56.4 (14.7)
5f		89 (78–100)	4.3E-8, (6.2E-9–3.0E-7)	66.5 (16.6)
5g		50 (31–81)	1.4E-7, (1.3E-8–1.6E-6)	84.3 (4.3)
5h		NE		
5i		82 (56–120)	5.1E-8, (4.7E-9–5.5E-7)	62.8 (19.8)
11		NE		
2		0.087 (0.058–0.13)	6.5E-3, (4.5E-3–9.4E-3)	103.3 (3.8)
4		0.51 (0.28–0.94)	4.4E-4, (2.7E-4–7.2E-4)	101.8 (2.3)
Biphenyl		NE		

[a] Geometric mean.
[b] CI, confidence interval.
[c] Arithmetic mean (S.D.).
[d] NE, non-estrogenic.

phous powders or as colorless to light-yellow oils. Technical solvents were purchased from Chemlab (Zedelgem, Belgium), while anhydrous solvents and reagents were obtained from Acros Organics (Geel, Belgium) and Sigma-Aldrich (Bornem, Belgium). Reference compounds 17β-estradiol (> 98% pure), biphenyl (99.5% pure), naringenin (97% pure), and genistein (> 98% pure) were acquired from Sigma-Aldrich. 8-Prenylnaringenin was synthesized according to a literature procedure [4]. All reactions were performed under a nitrogen atmosphere.

*5.1.1. 2-Hydroxy-4,6-dimethoxybenzaldehyde (**7**)*

To a solution of 2,4,6-trimethoxybenzaldehyde (**6**) (10.0 g, 51.0 mmol) in dry CH_2Cl_2 (80 ml) was added dropwise BBr_3 (30.0 ml, 1.0 M in CH_2Cl_2) at –78 °C. The reaction mixture was allowed to warm up to room temperature, stirred for 1.5 h,

cooled to 0 °C, and poured on ice. The organic solvent was removed under reduced pressure and the aqueous suspension was extracted with EtOAc. The combined organic phases were washed with brine and dried over anhydrous $MgSO_4$. Removal of the solvent afforded **7** as a white solid (8.9 g, 96%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.83 and 3.85 (2s, 3H each, C(4)–OCH_3 and C(6)–OCH_3), 6.09 and 6.14 (2d, 1H each d, J = 2.1 Hz, C(3)–H and C(5)–H), 10.00 (s, 1H, C(1)–CHO), 12.38 (s, 1H, C(2)–OH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 56.68, 56.87, 91.48, 93.88, 106.00, 164.13, 165.88, 168.82, 192.15; HRMS calcd for $C_9H_{10}O_4$ 182.0579; [M – H^+] calcd 181.0501; found 181.0490.

5.1.2. General procedure for the preparation of 2-alkyl-3,5-dimethoxyphenols (**8a–i**)

To a stirred solution of **7** in dry Et_2O (5 ml/mmol) was added dropwise at –78 °C alkyllithium or alkylmagnesium bromide (2.2 equiv). The cooling bath was removed and, after completion of the reaction (1.5–2 h as monitored by TLC), the reaction mixture was poured on ice and extracted with EtOAc. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed at room temperature under reduced pressure. Without purification, the residue was dissolved in dry CH_2Cl_2 (4 ml/mmol) and $HSiEt_3$ (2.5 equiv) was added at room temperature together with CF_3COOH (6 equiv) at –78 °C [23]. The reaction mixture was allowed to warm up to room temperature (1 h) and stirred for 30 min. After neutralization with saturated aqueous $NaHCO_3$, the mixture was extracted with Et_2O. The combined organic phases were washed with water, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc, v/v) to yield **8a–i**.

*5.1.2.1. 2-Ethyl-3,5-dimethoxyphenol (**8a**).* Hexane/EtOAc, 17:3. Yield: 93%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, 3H, J = 7.3 Hz, C(2a)–H), 2.41 (q, 2H, J = 7.3 Hz, C(1a)–H), 3.65 and 3.69 (2s, 3H each, C(3)–OCH_3 and C(5)–OCH_3), 6.00 and 6.02 (2d, 1H each, J = 2.4 Hz each, C(4)–H and C(6)–H), 9.03 (s, 1H, C(1)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.93, 16.45, 55.33, 56.08, 90.56, 94.13, 109.43, 156.80, 158.89, 159.33; HRMS calcd for $C_{10}H_{14}O_3$ 182.0943; [M – H^+] calcd 181.0865; found 181.0853.

*5.1.2.2. 2-n-Pentyl-3,5-dimethoxyphenol (**8b**).* Hexane/EtOAc, 9:1. Yield: 91%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.84 (br t, 3H, J = 6.7 Hz, C(5a)–H), 1.19–1.40 (m, 6H, C(2a)–H to C(4a)–H), 2.41 (br t, 2H, J = 7.0 Hz, C(1a)–H), 3.66 and 3.69 (2s, 3H each, C(3)–OCH_3 and C(5)–OCH_3), 6.00 and 6.02 (2d, 1H each, J = 2.4 Hz each, C(4)–H and C(6)–H), 9.13 (s, 1H, C(1)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.68, 22.78, 22.82, 29.46, 32.04, 55.43, 55.99, 90.20, 94.10, 109.51, 156.82, 158.95, 159.36; HRMS calcd for $C_{13}H_{20}O_3$ 224.1412; [M – H^+] calcd 223.1334; found 223.1335.

*5.1.2.3. 2-n-Heptyl-3,5-dimethoxyphenol (**8c**).* Hexane/EtOAc, 19:1. Yield: 88%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (br t, 3H, J = 6.7 Hz, C(7a)–H), 1.21–1.38 (m, 10H, C(2a)–H to C(6a)–H), 2.41 (br t, 2H, J = 7.0 Hz, C(1a)–H), 3.65 and 3.69 (2s, 3H each, C(3)–OCH_3 and C(5)–OCH_3), 6.00 and 6.02 (2d, 1H each, J = 2.4 Hz each, C(4)–H and C(6)–H), 9.14 (s, 1H, C(1)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.69, 22.80, 22.83, 29.38, 29.79, 29.96, 32.06, 55.43, 55.98, 90.18, 94.09, 109.52, 156.82, 158.95, 159.35; HRMS calcd for $C_{15}H_{24}O_3$ 252.1725; [M – H^+] calcd 251.1647; found 251.1655.

*5.1.2.4. 2-n-Undecyl-3,5-dimethoxyphenol (**8d**).* Hexane/EtOAc, 97:3. Yield: 85%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (br t, 3H, J = 6.5 Hz, C(11a)–H), 1.17–1.35 (m, 18H, C(2a)–H to C(10a)–H), 2.41 (br t, 2H, J = 7.0 Hz, C(1a)–H), 3.65 and 3.68 (2s, 3H each, C(3)–OCH_3 and C(5)–OCH_3), 6.00 and 6.02 (2d, 1H each, J = 2.4 Hz each, C(4)–H and C(6)–H), 9.11 (s, 1H, C(1)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.58, 22.78, 22.85, 29.41, 29.68, 29.72, 29.77, 31.99, 55.41, 55.94, 90.21, 94.18, 109.61, 156.82, 158.97, 159.38; HRMS calcd for $C_{19}H_{32}O_3$ 308.2351; [M – H^+] calcd 307.2273; found 307.2273.

*5.1.2.5. 2-(2-Methylpropyl)-3,5-dimethoxyphenol (**8e**).* Hexane/EtOAc, 9:1. Yield: 93%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.81 (d, 6H, J = 6.7 Hz, C(3a)–H and C(4a)–H), 1.78 (br n, 1H, J = 6.8 Hz, C(2a)–H), 2.31 (d, 2H, J = 7.0 Hz, C(1a)–H), 3.67 and 3.69 (2s, 3H each, C(3)–OCH_3 and C(5)–OCH_3), 6.00 and 6.02 (2d, 1H each, J = 2.4 Hz each, C(4)–H and C(6)–H), 9.06 (s, 1H, C(1)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.15 (2C), 28.59, 31.91, 55.45, 55.99, 90.27, 94.18, 109.58, 156.78, 158.50, 159.06; HRMS calcd for $C_{12}H_{18}O_3$ 210.1256; [M – H^+] calcd 209.1178; found 209.1163.

*5.1.2.6. 2-(2,2-Dimethylpropyl)-3,5-dimethoxyphenol (**8f**).* Hexane/EtOAc, 9:1. Yield: 90%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (s, 9H, C(2a)-$(CH_3)_3$), 2.40 (s, 2H, C(1a)–H), 3.66 and 3.67 (2s, 3H each, C(3)–OCH_3 and C(5)–OCH_3), 6.01 and 6.04 (2d, 1H each, J = 2.4 Hz each, C(4)–H and C(6)–H), 9.03 (s, 1H, C(1)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.95 (3C), 33.43, 35.38, 55.82, 56.43, 91.27, 94.36, 108.80, 156.51, 159.15, 159.87; HRMS calcd for $C_{13}H_{20}O_3$ 224.1412; [M – H^+] calcd 223.1334; found 223.1321.

*5.1.2.7. 2-(3-Methylbutyl)-3,5-dimethoxyphenol (**8g**).* Hexane/EtOAc, 9:1. Yield: 91%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88 (d, 6H, J = 6.7 Hz, C(4a)–H and C(5a)–H), 1.24 (br q, 2H, J = 7.0 Hz, C(2a)–H), 1.48 (br n, 1H, J = 7.0 Hz, C(3a)–H), 2.43 (br t, 2H, J = 6.7 Hz, C(1a)–H), 3.66 and 3.70 (2s, 3H each, C(3)–OCH_3 and C(5)–OCH_3), 6.00 and 6.02 (2d, 1H each, J = 2.4 Hz each, C(4)–H and C(6)–H), 9.10 (s, 1H, C(1)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.88, 23.26, 28.25, 39.10, 55.46, 56.06, 90.30, 94.21, 109.78,

156.74, 158.95, 159.35; HRMS calcd for $C_{13}H_{20}O_3$ 224.1412; [M – H^+] calcd 223.1334; found 223.1324.

*5.1.2.8. 2-Benzyl-3,5-dimethoxyphenol (**8h**).* Hexane/EtOAc, 9:1. Yield: 87%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.68 and 3.70 (2s, 3H each, C(3)–OCH_3 and C(5)–OCH_3), 3.77 (s, 2H, C(1a)–H), 6.06 and 6.08 (2d, 1H each, J = 2.4 Hz each, C(4)–H and C(6)–H), 7.04–7.22 (m, 5H, C(3a)–H to C(7a)–H), 9.46 (s, 1H, C(1)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.61, 55.52, 56.09, 90.31, 94.18, 108.20, 125.86, 128.53 (2C), 128.83 (2C), 142.68, 156.95, 159.40, 159.59; HRMS calcd for $C_{15}H_{16}O_3$ 244.1099; [M – H^+] calcd 243.1021; found 243.1010.

*5.1.2.9. 2-(2-Phenylethyl)-3,5-dimethoxyphenol (**8i**).* Hexane/EtOAc, 9:1. Yield: 88%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.59–2.74 (m, 4H, C(1a)–H and C(2a)–H), 3.67 and 3.69 (2s, 3H each, C(3)–OCH_3 and C(5)–OCH_3), 6.03 and 6.05 (2d, 1H each, J = 2.4 Hz each, C(4)–H and C(6)–H), 7.12–7.35 (m, 5H, C(4a)–H to C(8a)–H), 9.27 (s, 1H, C(1)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.26, 35.85, 55.51, 56.14, 90.39, 94.25, 108.84, 126.23, 128.82 (4C), 143.23, 156.87, 159.29, 159.43; HRMS calcd for $C_{16}H_{18}O_3$ 258.1256; [M – H^+] calcd 257.1178; found 257.1182.

*5.1.3. General procedure for the preparation of (±)-8-alkyl-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarins (**9a**–**i**)*

To a mixture of 2-alkyl-3,5-dimethoxyphenol (**8a**–**i**) and *p*-methoxycinnamic acid (1.1 equiv) was added $BF_3{\cdot}Et_2O$ (5 ml/mmol **8a**–**i**) at room temperature and the solution was stirred for 15 h. After cooling to 0 °C, the reaction mixture was poured on ice and repeatedly extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc, v/v) to afford **9a**–**i**.

*5.1.3.1. (±)-8-Ethyl-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9a**).* Hexane/EtOAc, 17:3. Yield: 73%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.03 (t, 3H, J = 7.3 Hz, C(2a)–H), 2.56 (q, 2H, J = 7.3 Hz, C(1a)–H), 2.81 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.14 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.67, 3.75, and 3.84 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 4.46 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.52 (s, 1H, C(6)–H), 6.81 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.93 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.97, 16.34, 33.71, 37.55, 55.69, 56.55, 56.65, 92.84, 106.51, 112.15, 114.76 (2C), 128.28 (2C), 134.36, 150.75, 155.31, 158.08, 158.75, 168.44; HRMS calcd for $C_{20}H_{22}O_5$ 342.1467; [M + H^+] calcd 343.1545; found 343.1531.

*5.1.3.2. (±)-8-n-Pentyl-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9b**).* Hexane/EtOAc, 9:1. Yield: 79%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (br t, 3H, J = 6.7 Hz, C(5a)–H), 1.23–1.50 (m, 6H, C(2a)–H to C(4a)–H), 2.57 (br t, 2H, J = 7.0 Hz, C(1a)–H), 2.84 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.13 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.68, 3.77, and 3.85 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 4.49 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.54 (s, 1H, C(6)–H), 6.82 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.96 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.59, 22.64, 22.74, 29.54, 31.82, 33.71, 37.59, 55.62, 56.46, 56.57, 92.68, 106.37, 110.79, 114.69 (2C), 128.28 (2C), 134.35, 150.98, 155.28, 158.27, 158.76, 168.33; HRMS calcd for $C_{23}H_{28}O_5$ 384.1937; [M + H^+] calcd 385.2015; found 385.2011.

*5.1.3.3. (±)-8-n-Heptyl-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9c**).* Hexane/EtOAc, 9:1. Yield: 73%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (br t, 3H, J = 6.7 Hz, C(7a)–H), 1.19–1.51 (m, 10H, C(2a)–H to C(6a)–H), 2.58 (br t, 2H, J = 7.0 Hz, C(1a)–H), 2.84 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.12 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.68, 3.77, and 3.85 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 4.49 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.54 (s, 1H, C(6)–H), 6.82 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.96 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.62, 22.75, 22.81, 29.25, 29.56, 29.85, 32.04, 33.72, 37.59, 55.59, 56.43, 56.53, 92.65, 106.35, 110.78, 114.65 (2C), 128.27 (2C), 134.35, 150.99, 155.28, 158.27, 158.75, 168.27; HRMS calcd for $C_{25}H_{32}O_5$ 412.2250; [M + H^+] calcd 413.2328; found 413.2322.

*5.1.3.4. (±)-8-n-Undecyl-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9d**).* Hexane/EtOAc, 19:1. Yield: 67%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.84 (br t, 3H, J = 6.5 Hz, C(11a)–H), 1.19–1.44 (m, 18H, C(2a)–H to C(10a)–H), 2.56 (br t, 2H, J = 7.0 Hz, C(1a)–H), 2.83 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.11 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.69, 3.76, and 3.84 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 4.48 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.53 (s, 1H, C(6)–H), 6.82 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.94 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.62, 22.75, 22.82, 29.36, 29.46, 29.68, 29.75, 31.95, 33.68, 37.60, 55.68, 56.52, 56.63, 92.79, 106.41, 110.79, 114.71 (2C), 128.28 (2C), 134.35, 150.97, 155.28, 158.27, 158.75, 168.34; HRMS calcd for $C_{29}H_{40}O_5$ 468.2876; [M + H^+] calcd 469.2954; found 469.2947.

*5.1.3.5. (±)-8-(2-Methylpropyl)-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9e**).* Hexane/EtOAc, 17:3. Yield: 74%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 and 0.86 (2d, 3H each d, J = 6.7 Hz each d, C(3a)–H and C(4a)–H), 1.83 (br n, 1H, J = 6.8 Hz, C(2a)–H), 2.45 (d, 2H, J = 7.0 Hz, C(1a)–H), 2.83 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.14 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.68, 3.78, and 3.85 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 4.48 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.55 (s, 1H, C(6)–H), 6.83 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.94 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ

23.00, 23.02, 28.91, 31.75, 33.68, 37.61, 55.70, 56.53, 56.62, 92.81, 106.40, 109.89, 114.76 (2C), 128.26 (2C), 134.36, 151.20, 155.38, 158.58, 158.77, 168.28; HRMS calcd for $C_{22}H_{26}O_5$ 370.1780; [M + H$^+$] calcd 371.1858; found 371.1856.

*5.1.3.6. (±)-8-(2,2-Dimethylpropyl)-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9f**).* Hexane/EtOAc, 17:3. Yield: 76%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87 (s, 9H, C(2a)-$(CH_3)_3$), 2.49 (d, 1H, J = 12.9 Hz, C(1a)–H_A), 2.53 (d, 1H, J = 12.9 Hz, C(1a)–H_B), 2.81 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.07 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.71, 3.79, and 3.82 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 4.43 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.55 (s, 1H, C(6)–H), 6.64 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.81 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 30.33 (3C), 33.46, 33.82, 35.67, 37.51, 55.63, 56.34, 56.51, 92.66, 106.57, 108.31, 114.99 (2C), 128.24 (2C), 132.55, 151.52, 155.78, 158.33, 158.91, 168.59; HRMS calcd for $C_{23}H_{28}O_5$ 384.1937; [M + H$^+$] calcd 385.2015; found 385.2017.

*5.1.3.7. (±)-8-(3-Methylbutyl)-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9g**).* Hexane/EtOAc, 9:1. Yield: 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91 (d, 6H, J = 6.7 Hz, C(4a)–H and C(5a)–H), 1.32 (br q, 2H, J = 7.0 Hz, C(2a)–H), 1.52 (br n, 1H, J = 6.7 Hz, C(3a)–H), 2.56 (br t, 2H, J = 7.0 Hz, C(1a)–H), 2.80 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.10 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.69, 3.77, and 3.85 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 4.42 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.53 (s, 1H, C(6)–H), 6.64 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.82 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.86, 23.10, 23.15, 28.17, 33.66, 37.63, 39.28, 55.71, 56.52, 56.66, 92.81, 106.68, 111.00, 114.76 (2C), 128.22 (2C), 132.61, 150.86, 155.25, 158.17, 158.47, 168.51; HRMS calcd for $C_{23}H_{28}O_5$ 384.1937; [M + H$^+$] calcd 385.2015; found 385.2006.

*5.1.3.8. (±)-8-Benzyl-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9h**).* Hexane/EtOAc, 9:1. Yield: 78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.85 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.16 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.70, 3.79, and 3.85 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 3.91 (s, 2H, C(1a)–H), 4.50 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.59 (s, 1H, C(6)–H), 6.82 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.95 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 7.11–7.28 (m, 5H, C(3a)–H to C(7a)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.54, 33.71, 37.55, 55.71, 56.61, 56.74, 92.94, 106.59, 109.20, 114.78 (2C), 126.33, 128.27 (2C), 128.65 (2C), 128.89 (2C), 134.26, 141.41, 150.99, 155.93, 158.37, 158.79, 168.23; HRMS calcd for $C_{25}H_{24}O_5$ 404.1624; [M + H$^+$] calcd 405.1702; found 405.1714.

*5.1.3.9. (±)-8-(2-Phenylethyl)-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9i**).* Hexane/EtOAc, 9:1. Yield: 72%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.70–2.89 (m, 5H, C(3)–H_A, C(1a)–H, and C(2a)–H), 2.99 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.69, 3.77, and 3.84 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 4.44 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.54 (s, 1H, C(6)–H), 6.81 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.89 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 7.10–7.29 (m, 5H, C(4a)–H to C(8a)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.95, 33.71, 35.74, 37.53, 55.68, 56.53, 56.72, 92.77, 106.39, 109.73, 114.72 (2C), 126.37, 128.31 (2C), 128.75 (2C), 129.00 (2C), 134.26, 142.25, 150.99, 155.50, 158.36, 158.74, 168.08; HRMS calcd for $C_{26}H_{26}O_5$ 418.1780; [M + H$^+$] calcd 419.1858; found 419.1844.

*5.1.4. General procedure for the preparation of (±)-8-alkyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarins (**5a**–**i**)*

To a solution of (±)-8-alkyl-5,7-dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**9a**–**i**) in dry CH_2Cl_2 (10 ml/mmol) at –78 °C, BBr_3 (6 equiv, 1.0 M in CH_2Cl_2) was added dropwise. The mixture was allowed to warm up to room temperature and was stirred for 15 h. After cooling to 0 °C, the reaction was quenched by pouring on ice. The organic solvent was removed under reduced pressure and the aqueous suspension was repeatedly extracted with EtOAc. The combined organic phases were washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by column chromatography (hexane/EtOAc, v/v) to provide **5a**–**i**.

*5.1.4.1. (±)-8-Ethyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**5a**).* Hexane/EtOAc, 1:1. Yield: 82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.01 (t, 3H, J = 7.3 Hz, C(2a)–H), 2.48 (q, 2H, J = 7.3 Hz, C(1a)–H), 2.74 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.05 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 4.32 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.23 (s, 1H, C(6)–H), 6.62 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.83 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 9.19, 9.31, and 9.36 (3s, 1H each, C(4′)–OH, C(5)–OH, and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.91, 16.42, 33.75, 37.78, 99.08, 104.12, 108.07, 115.87 (2C), 128.30 (2C), 133.23, 151.42, 152.90, 155.87, 156.67, 168.82; HRMS calcd for $C_{17}H_{16}O_5$ 300.0998; [M – H$^+$] calcd 299.0919; found 299.0913. Anal. $C_{17}H_{16}O_5$ (C, H).

*5.1.4.2. (±)-8-n-Pentyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**5b**).* Hexane/EtOAc, 1:1. Yield: 73%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (br t, 3H, J = 6.7 Hz, C(5a)–H), 1.21–1.48 (m, 6H, C(2a)–H to C(4a)–H), 2.48 (br t, 2H, J = 7.0 Hz, C(1a)–H), 2.75 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.07 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 4.33 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.24 (s, 1H, C(6)–H), 6.64 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.84 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 9.27, 9.37, and 9.44 (3s, 1H each, C(4′)–OH, C(5)–OH, and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.70, 22.70, 22.85, 29.61, 31.87, 33.70, 37.85, 99.03, 104.14, 108.03, 115.86 (2C), 128.31 (2C), 133.22, 151.44, 152.92, 155.89, 156.67, 168.86; HRMS calcd

for $C_{20}H_{22}O_5$ 342.1467; [M – H^+] calcd 341.1389; found 341.1382. Anal. $C_{20}H_{22}O_5$ (C, H).

*5.1.4.3. (±)-8-n-Heptyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**5c**).* Hexane/EtOAc, 11:9. Yield: 76%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (br t, 3H, J = 6.7 Hz, C(7a)–H), 1.20–1.48 (m, 10H, C(2a)–H to C(6a)–H), 2.48 (br t, 2H, J = 7.0 Hz, C(1a)–H), 2.76 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.06 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 4.34 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.24 (s, 1H, C(6)–H), 6.64 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.84 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 9.27, 9.36, and 9.43 (3s, 1H each, C(4′)–OH, C(5)–OH, and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.69, 22.78, 22.88, 29.29, 29.57, 29.93, 32.05, 33.72, 37.87, 99.03, 104.13, 108.01, 115.86 (2C), 128.31 (2C), 133.24, 151.45, 152.91, 155.90, 156.67, 168.83; HRMS calcd for $C_{22}H_{26}O_5$ 370.1780; [M – H^+] calcd 369.1702; found 369.1694. Anal. $C_{22}H_{26}O_5$ (C, H).

*5.1.4.4. (±)-8-n-Undecyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**5d**).* Hexane/EtOAc, 3:2. Yield: 68%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (br t, 3H, J = 6.5 Hz, C(11a)–H), 1.19–1.43 (m, 18H, C(2a)–H to C(10a)–H), 2.48 (br t, 2H, J = 7.0 Hz, C(1a)–H), 2.75 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.05 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 4.34 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.24 (s, 1H, C(6)–H), 6.63 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.84 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 9.21, 9.30, and 9.37 (3s, 1H each, C(4′)–OH, C(5)–OH, and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.61, 22.76, 22.86, 29.38, 29.59, 29.69, 29.76, 31.96, 33.75, 37.83, 99.14, 104.22, 108.11, 115.89 (2C), 128.29 (2C), 133.26, 151.45, 152.92, 155.90, 156.68, 168.76; HRMS calcd for $C_{26}H_{34}O_5$ 426.2406; [M – H^+] calcd 425.2328; found 425.2319. Anal. $C_{26}H_{34}O_5$ (C, H).

*5.1.4.5. (±)-8-(2-Methylpropyl)-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**5e**).* Hexane/EtOAc, 1:1. Yield: 78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 and 0.86 (2d, 3H each d, J = 6.7 Hz each d, C(3a)–H and C(4a)–H), 1.82 (br m, 1H, J = 6.8 Hz, C(2a)–H), 2.38 (d, 2H, J = 7.0 Hz, C(1a)–H), 2.76 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.04 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 4.34 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.26 (s, 1H, C(6)–H), 6.63 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.85 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 9.22, 9.29, and 9.40 (3s, 1H each, C(4′)–OH, C(5)–OH, and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.02, 23.05, 28.92, 31.90, 33.70, 37.86, 99.11, 104.15, 107.19, 115.89 (2C), 128.29 (2C), 133.22, 151.67, 153.02, 156.19, 156.68, 168.77; HRMS calcd for $C_{19}H_{20}O_5$ 328.1311; [M – H^+] calcd 327.1232; found 327.1226. Anal. $C_{19}H_{20}O_5$ (C, H).

*5.1.4.6. (±)-8-(2,2-Dimethylpropyl)-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**5f**).* Hexane/EtOAc, 1:1. Yield: 80%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88 (s, 9H, C(2a)-$(CH_3)_3$), 2.43 (d, 1H, J = 12.7 Hz, C(1a)–H_A), 2.49 (d, 1H, J = 12.7 Hz, C(1a)–H_B), 2.77 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.03 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 4.36 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.27 (s, 1H, C(6)–H), 6.63 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.85 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 9.21, 9.26 and 9.43 (3s, 1H each, C(4′)–OH, C(5)–OH and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 30.41 (3C), 33.65, 33.93, 35.78, 37.87, 99.18, 104.22, 108.46, 115.93 (2C), 128.35 (2C), 133.21, 152.05, 153.14, 155.67, 156.74, 168.76; HRMS calcd for $C_{20}H_{22}O_5$ 342.1467; [M – H^+] calcd 341.1389; found 341.1377. Anal. $C_{20}H_{22}O_5$ (C, H).

*5.1.4.7. (±)-8-(3-Methylbutyl)-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**5g**).* Hexane/EtOAc, 1:1. Yield: 73%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (d, 6H, J = 6.7 Hz, C(4a)–H and C(5a)–H), 1.31 (br q, 2H, J = 6.8 Hz, C(2a)–H), 1.50 (br n, 1H, J = 6.7 Hz, C(3a)–H), 2.51 (br t, 2H, J = 6.9 Hz, C(1a)–H), 2.76 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.06 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 4.34 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.24 (s, 1H, C(6)–H), 6.64 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.85 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 9.22, 9.30, and 9.37 (3s, 1H each, C(4′)–OH, C(5)–OH and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.98, 23.19, 23.25, 28.21, 33.71, 37.81, 39.31, 99.10, 104.25, 108.29, 115.89 (2C), 128.30 (2C), 133.24, 151.38, 152.88, 155.82, 156.64, 168.85; HRMS calcd for $C_{20}H_{22}O_5$ 342.1467; [M – H^+] calcd 341.1389; found 341.1383. Anal. $C_{20}H_{22}O_5$ (C, H).

*5.1.4.8. (±)-8-Benzyl-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**5h**).* Hexane/EtOAc, 1:1. Yield: 81%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.77 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.07 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.82 (d, 1H, J = 14.5 Hz, C(1a)–H_A), 3.86 (d, 1H, J = 14.5 Hz, C(1a)–H_B), 4.37 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.30 (s, 1H, C(6)–H), 6.64 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.85 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 7.09–7.26 (m, 5H, C(3a)–H to C(7a)–H), 9.23, 9.45, and 9.49 (3s, 1H each, C(4′)–OH, C(5)–OH, and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.63, 33.73, 37.79, 99.16, 104.38, 106.63, 115.91 (2C), 126.11, 128.29 (2C), 128.75 (4C), 133.13, 142.09, 151.47, 153.65, 156.04, 156.69, 168.69; HRMS calcd for $C_{22}H_{18}O_5$ 362.1154; [M – H^+] calcd 361.1076; found 361.1090. Anal. $C_{22}H_{18}O_5$ (C, H).

*5.1.4.9. (±)-8-(2-Phenylethyl)-5,7-dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**5i**).* Hexane/EtOAc, 1:1. Yield: 77%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.66–2.83 (m, 5H, C(3)–H_A, C(1a)–H, and C(2a)–H), 2.93 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 4.30 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.27 (s, 1H, C(6)–H), 6.63 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.79 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 7.11–7.28 (m, 5H, C(4a)–H to C(8a)–H), 9.28, 9.47, and 9.50 (3s, 1H each, C(4′)–OH, C(5)–OH, and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.05, 33.79, 35.80, 37.75, 99.23, 104.33, 107.15, 115.89 (2C), 126.24, 128.31 (2C), 128.71 (2C), 128.97 (2C), 133.18, 142.66, 151.49, 153.17, 155.97,

156.66, 168.52; HRMS calcd for $C_{23}H_{20}O_5$ 376.1311; [M – H^+] calcd 375.1232; found 375.1229. Anal. $C_{23}H_{20}O_5$ (C, H).

*5.1.5. (±)-5,7-Dimethoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (**10**)*

Synthesis: see general method for preparation of **9a–i**, whereby 3,5-dimethoxyphenol was used instead of **8a–i**. Hexane/EtOAc, 17:3 (v/v). Yield: 81%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.80 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.19 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 3.69, 3.73, and 3.79 (3s, 3H each, C(4′)–OCH_3, C(5)–OCH_3, and C(7)–OCH_3), 4.45 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.41 and 6.44 (2d, 1H each, J = 2.4 Hz each, C(6)–H and C(8)–H), 6.84 (d, 2H, J = 8.8 Hz, C(3′)–H and C(5′)–H), 6.96 (d, 2H, J = 8.8 Hz, C(2′)–H and C(6′)–H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 33.57, 37.78, 55.69, 56.25, 56.59, 94.71, 95.64, 106.63, 114.77 (2C), 128.26 (2C), 134.29, 153.41, 157.73, 158.78, 160.93, 168.28; HRMS calcd for $C_{18}H_{18}O_5$ 314.1154; [M + H^+] calcd 315.1232; found 315.1241.

*5.1.6. (±)-5,7-Dihydroxy-4-(4-hydroxyphenyl)-3,4-dihydrocoumarin (**11**)*

Synthesis: see general method for preparation of **5a–i**. Hexane/EtOAc, 1:1 (v/v). Yield: 79%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.75 (dd, 1H, J = 15.8, 1.8 Hz, C(3)–H_A), 3.12 (dd, 1H, J = 15.8, 6.7 Hz, C(3)–H_B), 4.34 (dd, 1H, J = 6.7, 1.8 Hz, C(4)–H), 6.02 and 6.17 (2d, 1H each, J = 2.4 Hz each, C(6)–H and C(8)–H), 6.65 (d, 2H, J = 8.5 Hz, C(3′)–H and C(5′)–H), 6.86 (d, 2H, J = 8.5 Hz, C(2′)–H and C(6′)–H), 9.26, 9.53, and 9.70 (3s, 1H each, C(4′)–OH, C(5)–OH, and C(7)–OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 33.61, 38.07, 95.36, 99.40, 104.30, 115.94 (2C), 128.28 (2C), 133.19, 153.59, 155.97, 156.73, 158.41, 168.70; HRMS calcd for $C_{15}H_{12}O_5$ 272.0685; [M – H^+] calcd 271.0606; found 271.0613. Anal. $C_{15}H_{12}O_5$ (C, H).

5.2. Recombinant yeast screen

A *S. cerevisiae*-based assay for estrogenicity was kindly provided by Professor J.P. Sumpter (Brunel University, UK). Details of the assay (yeast estrogen screen or YES), including details of the medium components, have been described elsewhere [26]. Briefly, stably transfected yeast cells expressing the human ER-α and containing expression plasmids carrying estrogen-responsive sequences controlling the reporter gene *lac-Z* (encoding for the enzyme β-galactosidase) were incubated in medium together with the test compounds and the chromogenic β-galactosidase substrate chlorophenol red-β-D-galactopyranoside (CPRG). At day 0, 5 ml of growth medium was inoculated with 12.5 μl of a yeast stock (stored at –80 °C) and incubated at 28 °C for 24 h on an orbital shaker (150 rpm) or until an absorbance of 0.8–1.0 at 620 nm was reached. The following day, assay medium was prepared by adding 0.5 ml of the 24 h yeast culture and 0.4 ml of CPRG solution to 50 ml of growth medium (yeast concentration approximately 5×10^5 cells per ml). Chemicals (2 or 1 g/l in ethanol) were serially diluted in absolute ethanol and 10 μl aliquots of each concentration were then transferred in duplicate to separate 96-well optically flat bottom microtiter plates. Ethanol was allowed to evaporate to dryness on the assay plate and 200 μl of assay medium was added. Compounds were incubated for 3 days at 32 °C or longer until a dose–response curve was observed (monitored daily, until 12 days) [30]. All experiments included a standard curve of 17β-estradiol and all serial dilutions of the different compounds were separated by a row of ethanol blanks. Estrogenic activity was determined from the enzymatic hydrolysis of CPRG by monitoring the absorbance at 540 nm (Multiskan RC, ThermoLabsystems, Brussels, Belgium). All data were corrected for turbidity using a second reading at 620 nm. Dose–response curves for β-galactosidase activity were obtained using corrected absorbance units (CAU): CAU = (Abs_{540})compound – [(Abs_{620})compound – (Abs_{620})blank]. Curve analysis was performed using Sigmaplot 8.0 (SPSS Inc., Chicago, IL, USA) using a four parameter logistic regression model. Compounds tested in the YES were dissolved in HPLC-grade absolute ethanol (Merck-Eurolab, Leuven, Belgium) at a concentration of 2 g/l and were kept at 4 °C in amber vials. The RP of a compound is the ratio of the concentration of 17β-estradiol causing the same response as the compound at its half-maximum response and the compound's EC_{50}. The RIE is the ratio between the (max–min) absorbance achieved with the test compound and that of 17β-estradiol (×100). The detection limit is defined as the concentration of 17β-estradiol corresponding to an effect equal to the mean of the corrected absorbances of the blank rows plus three times their standard deviation. A compound was considered to exhibit estrogenic activity, when it caused in at least two separate experiments a dose-dependent (two consecutive concentrations) color formation above the detection limit. Statistical analysis was performed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA).

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