Synthesis and Cytotoxic Activity of a New Group of Heterocyclic Analogues of the Combretastatins

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Received: 15 April 2014; in revised form: 3 June 2014 / Accepted: 5 June 2014 / Published: 11 June 2014

Abstract: A series of new analogs of combretastatin A-4 (CA-4, 1) with the A or B-ring replaced by a 3-oxo-2,3-dihydrofurocoumarin or a furocoumarin residue have been designed and synthesized by employing a cross-coupling approach. All the compounds were evaluated for their cytotoxic activity with respect to model cancer cell lines (CEM-13, MT-4, U-937) using conventional MTT assays. Structure-activity relationship analysis reveals that compounds 2, 3, 6–8 in which the (Z)-styryl substituent was connected to the 2-position of the 3-oxo-2,3-dihydrofurocoumarin core, demonstrated increased potency compared to 3-(Z)-styrylfurocoumarins 4, 5, 9–11. The methoxy-, hydroxyl- and formyl-substitution on the aromatic ring of the (Z)-styryl moiety seems to play an important role in this class of compounds. Compounds 2 and 3 showed the best potency against the CEM-13 cell lines, with CTD_{50} values ranging from 4.9 to 5.1 μM. In comparison with CA-4, all synthesized compounds presented moderate cytotoxic activity to the T-cellular human leucosis cells MT-4 and lymphoblastoid leukemia cells CEM-13, but most of them were active in the human monocyte cell lines U-937.

Keywords: furocoumarins; oreoselone; psoralen; Sonogashira coupling; semi-hydrogenation; combretastatins; cytotoxicity
1. Introduction

Combretastatin A-4 (CA-4, 1, Figure 1), isolated from the bark of South African tree *Combretum caffrum* [1] is one of the well-known natural tubulin-binding molecules affecting microtubule dynamics by binding to the colchicine site [2]. CA-4 (1) shows strong cytotoxic activity against a wide variety of human cancer cell lines, including those that are multidrug resistant [2]. A water soluble disodium phosphate derivative of CA-4 (CA-4P, fosbretabulin) has shown promising results in human cancer clinical trials [3,4], thus stimulating significant interest in a variety of CA-4 analogues [5].

**Figure 1.** Structures of combretastatin A-4 (1) and furocoumarin analogs of combretastatins 2–13.

![Image of structures](image)

Different analogs of combretastatins are obtained, including heterocombretastatins containing heterocyclic fragments as rings A or B [6–12]. A considerable cytotoxicity and antitubulin activity was revealed for heterocombretastatins containing a (4-methoxypyridin-3-yl)- or a (1-methyl-2-oxo-1,2-dihydropyridin-4-yl) ring B [6]. Compounds with benzo[b]furan ring B [9,10] or A [10], attached to the 6 position, demonstrated high antitumor activity in *in vitro* and *in vivo* models. In more recent works, replacement of the B-ring of CA-4 (1) with a (benzofuran-2-yl) moiety was also examined and the activity of the benzofuranyl derivatives was evaluated [11,12].

Herein we describe the first synthesis of combretastatin A-4 analogues 2,3 and 4,5 by replacement of the A or B aromatic ring with linear dihydrofurocoumarins or furocoumarins (psoralens) (Figure 1). The (Z)-styryl moiety was attached to 2- or 3-position of the heterocyclic molecule. We also include other examples of (Z)-arylvinylfurocoumarins 6–11 for obtaining structure-cytotoxicity relationships.

Linear furocoumarins are employed in Psoralen + UVA (PUVA) therapy for the treatment of autoimmune or hyper-proliferative skin diseases, including psoriasis and vitiligo. Activated by UV-A
light furocoumarins induce many biological effects, such as photocycloadditions to DNA, immune system modulation, reactions with proteins, RNA and lipids [13–15]. In last decades many new potential therapeutic applications for furocoumarins are found. For instance, some psoralen derivatives were found to induce erythroid differentiation in different cellular models [16,17]. Moreover it was shown that annelation of a cyclopentane, cyclohexane, benzene, or pyridazine ring to the furan ring in the psoralen skeleton changed the phototoxicity and, in some cases led to a marked increase in the photo-antiproliferative activity [18]. There was therefore value in a targeted preparation and investigation of novel furocoumarins, containing a (Z)-styryl substituent in the furan ring of the molecules. Compounds 12 and 13, containing an additional N-methylpiperazinyl substituent in the furocoumarin scaffold were also prepared and investigated, since modification in the 9-position has a great influence on the properties of psoralenes as enzymatic inhibition and human keratinocyte proliferation [13].

2. Results and Discussion

2.1. Chemical Synthesis

The synthetic route followed for the synthesis of the desired novel combretastatin A-4 analogs 2–3, and 6–8 is outlined in Scheme 1. Previously we found that the plant coumarin oreoselone (14) by reaction with p-toluenesulfonyl chloride gave 2-(tosyl)oreoselone (15), which showed a high activity in Pd-catalyzed desulfonative cross-coupling reactions [19]. Herein, oreoselone (14) was converted into 2-(arylethynyl)furocoumarins 16–20 by the copper-free cross-coupling reaction with arylalkynes 21a–e in the presence of p-toluenesulfonyl chloride. After the purification by column chromatography compounds 16–20 were isolated in 44%–62% yield. 2-(Propan-2-ylidene)-7H-furo[3,2-g]chromene-3,7(2H)-dione (22) [20] was also isolated with 10%–25% yield, presumably after elimination of p-toluenesulfonic acid from the in situ formed 2-(tosyl)oreoselone (15). Alkynes 16 and 20 were also prepared by the cross-coupling reaction of previously synthesized 2-(tosyl)oreoselone (15) with arylalkynes 21a,e in a THF solution in the presence of a base and a catalytic amounts of trans-dichlorobis(triphenylphosphine)palladium(II) (yield 59%–66%). Additionally, compound 22 was also isolated in the yield 5%–7%. To obtain heterocombretastatins containing a furocoumarin residue, we have studied the reduction of 2-(arylethynyl)furocoumarins 16–20. Several protocols (alkyne hydroboration [21], hydrolysis of a preformed Ti(II)-diarylkyne complexes [22], or Lindlar’s semi-hydrogenation [23]) are employed for the selective transformation of diarylalkynes into cis alkenes in the synthesis of CA-4 1. Treatment of alkynes 16, 18 with Ti(O-iPr)4 (2 equiv) and n-BuLi (4 equiv.) at −78 °C in THF and then increasing the temperature to 50 °C and keeping the reaction mixture at this temperature for 2 h (for hydrolysis of the formed complexes) afforded 2-(Z)-(styryl)furocoumarins 2, 6 (yield 40%–42%). 2-(Z)-(Styryl)furocoumarins 3, 7 and 8 were obtained by partial hydrogenation of alkynes 17, 19 and 20 (isolated in 32%, 24% and 37% yield, respectively, after column chromatography) using Lindlar’s catalyst (Scheme 1).
Scheme 1. Synthesis of 2-(Z)-styrylfurocoumarins 2, 3, 6, 7, 8.

Reagents and Reaction Conditions: (a): oreoselone (14), TsCl, Pd(PPh₃)₂Cl₂ (5 mol %), THF, reflux, 6 h, then alkyne 21, Et₃N, THF, reflux, 7–8 h; (b): 2-tosyloreselone (15), alkynes 21a–e, Pd(PPh₃)₂Cl₂ (10 mol %), Et₃N, THF, reflux, 8 h; (c) Ti(O-iPr)₄–n-BuLi, THF, −78 °С, then 50 °С, 2 h; (d): H₂, 1 atm, Lindlar’s catalyst, EtOH, rt, 20–30 h.

3-(Z)-(Styryl)furocoumarins 9, 10, 11, 12 and 13 (Scheme 2) are obtained from 2-isopropyl-3-(trifluoromethanesulfonyloxy)psoralene (23) [24] or 2-isopropyl-9-(4-methylpiperazin-1-ylmethyl)-3-(trifluoromethanesulfonyloxy)psoralene (24) [25], by approaches described early for preparation of compounds 4, 5 [26]. The reaction of triflates 23 with arylalkynes 21c–e in a benzene (WARNING—this solvent is a known carcinogenic substance) solution in the presence of a catalytic amounts of trans-dichlorobis(triphenylphosphine)palladium(II), copper(I) iodide, and triethylamine led to the corresponding 3-(aryalkyne)furocoumarins 25–27 in the yield 68%–73% after the column chromatography. By using the mentioned cross-coupling conditions for reacting of psoralen derivatives 24 with the alkynes 21d,e, compounds 28, 29 are obtained (yield 42%–58%). For preparation of compound 26 we employed another approach. At first, by the Sonogashira coupling of oreoselone triflate 23 with (trimethylsilyl)acetylene 30 we obtained 3-(trimethylsilyl)-2-isopropylpsoralene (31) (yield 58%). Desilylation of compound 31 produced the corresponding 3-ethynyl-2-isopropylpsoralen (32) (66% yield). Hydrolysis of in situ formed Ti(II)-alkyne complex of alkyne 25 gave the 3-(Z)-(2,3,4-trimethoxystyryl)furocoumarin (9) (yield 60%). Partial reduction of alkynes 26–29 using Lindlar’s catalyst resulted in the isolation of 3-(Z)-styrylsubstituted furocoumarins 10, 11, 12, or 13 (yield 40%–60%) (Scheme 2).
Scheme 2. Synthesis of 3-(Z)-styrylfurocoumarins 9–13.

Reagents and Reaction Conditions: (a): triflate 23, or 24, Pd(PPh₃)₂Cl₂ (4 mol %), CuI (2 mol %), Et₃N, alkynes 21c–e, PhH, reflux, 8–10 h; (b): triflate 23, alkyn 30, Pd(PPh₃)₂Cl₂ (4 mol %), CuI (2 mol %), Et₃N, PhH, reflux, 10 h; (c): CsF, MeOH, benzyltrimethylammonium chloride, rt; (d): Pd(PPh₃)₂Cl₂ (4 mol %), CuI (2 mol %), Et₃N, bromide 33, PhH, reflux, 10 h; (e): Ti(O-t-Bu)Li, THF, −78 °C, then 50 °C, 2 h; (f): H₂, 1 atm, Lindlar’s catalyst, EtOH, rt, 30 h.

The structure of the synthesized compounds was established based on the combination of IR, UV, and NMR spectral data. The IR spectra of compounds 16–20, 25, 26, 27, 28, 29, 31 and 32 is characterized by the presence of the absorption band of the alkyne linker group at 2046–2191 cm⁻¹. The ¹H and ¹³C-NMR spectra of all synthesized compounds agree well with their structure and contain one set of characteristic signals of psoralen (3-oxo-2,3-dihydrofurocoumarin) skeleton and the corresponding substituent. The spin-spin coupling constants between alkanyl proton signals H-1a and H-1b (J 8.8–9.5 Hz) indicate the formation of compounds 2–13 with the (Z)-configuration of the double bond.
2.2. Cytotoxicity Studies

The cytotoxic activity of the synthesized series of 2-(Z)-styryldihydrofurocoumarins 2, 3, 6–8, 3-(Z)-styrlyfurocoumarins 4, 5, 9–13, the parent oreoselone (14) and combretastatin A-4 (1) was determined by measuring the concentration inhibiting human tumor cell viability by 50% (CTD50). The CTD50 was determined using the conventional MTT assay, which allows to estimate the number of survived cells spectrophotometrically [27]. The results are presented in Table 1. At first glance, it is evident that there is a difference in activity between the parent compound 14 and (Z)-styrly modified furocoumarins 2–13.

| Compound | Cytotoxicity (CTD50, μM) against Cell Line a |
|----------|-------------------------------------------|
|          | CEM-13  | MT-4  | U-937 |
| 2        | 4.9 ± 0.2 | 13.4 ± 4.5 | 12.1 ± 2.0 |
| 3        | 5.1 ± 0.9 | 10.1 ± 2.1 | 18.0 ± 2.9 |
| 4        | 28.6 ± 2.1 | 22.4 ± 2.6 | 41.3 ± 6.8 |
| 5        | 29.0 ± 3.2 | 36.1 ± 4.6 | 32.1 ± 3.5 |
| 6        | 22.1 ± 2.3 | 28.6 ± 3.7 | 33.1 ± 3.9 |
| 7        | 9.3 ± 2.6 | 9.2 ± 0.5 | 12.0 ± 0.8 |
| 8        | 14.1 ± 1.6 | 12.1 ± 3.2 | 13.3 ± 2.1 |
| 9        | 95.0 ± 3.4 | 24.5 ± 3.2 | >100  |
| 10       | 49.1 ± 4.5 | 13 ± 2.8  | 29.0 ± 3.1 |
| 11       | 26.7 ± 2.9 | 45.1 ± 1.9 | 28.0 ± 4.2 |
| 12       | 20.2 ± 1.6 | 5.2 ± 0.8  | 9.1 ± 1.2  |
| 13       | 9.0 ± 2.6 | 8.1 ± 0.7  | 12.1 ± 2.3  |
| (14)     | >100     | 70.9 ± 3.6 | 65.2 ± 3.9 |
| CA-4     | 0.8 ± 0.03 | 0.1 ± 0.012 | >100 b |

a The cell were continuously treated with compounds for 72 h; b CA-4 provide inhibition of 47% in concentration 0.1–100 μM.

All synthesized compounds 2–13 exhibited cytotoxic activity in respect to model cancer cell lines and were more potent than furocoumarin 14. Furocoumarins 4, 5, 9, 10 and 11, in which the (Z)-styrly substituent was located in the 3-position, demonstrated decrease of potency compared to 2-(Z)-styrly-3-oxodihydrofurocoumarins 2, 3, 6, 7 and 8. Compounds 2, 3 possessed the best activity on the lymphoblastoid leukemia cells CEM-13. The phenolic substituent in (Z)-styrly moiety seems to have an important role in this class of compounds; indeed 3,4-dihydroxyphenyl substituent in compounds 7, 10, 12 demonstrated increase of potency against MT-4 cell lines. Compounds having a 2,3,4-trimethoxyphenyl moiety (compounds 6, 9) show weak cytotoxic activity, while several derivatives containing a 3,4,5-trimethoxyphenyl residue (compounds 2, 4) shown improved activity. The latter effect is unsurprising, since it is well established that the replacement of A-ring in combretastatins [28,29] and phenstatin [30] is highly detrimental for the activity of the compounds. In the 3,9-disubstituted furocoumarins 12, 13 cytotoxicity does not seem to greatly influence by the substituent in the aromatic ring, however, the (4-methylpiperazin-1-ylmethyl) substitution on the 9th-position in 3-styrlyfurocoumarin core gave increase in cytotoxic activity of compounds (compare 10 and 12 or 11 and 13), especially, on the human monocyte-lines U-937. The activity of all synthesized compounds on the lymphoblastoid
cell lines was lower than the activity of CA-4 1, however, compounds 2,7,12 and 13 shown potency in respect to human monocyte-lines U-937 in comparison with CA-4.

3. Experimental Section

3.1. General

NMR spectra were acquired on Bruker AV-400 (1H: 400.13 MHz, 13C: 100.78 MHz) or Bruker AV-600 (1H: 600.30 MHz, 13C: 150.95 MHz) (Bruker BioSpin GmbH, Rheinstetten, Germany) instruments, using tetramethylsilane (TMS) as an internal standart. In the description of the 1H and 13C-NMR spectra, the furocoumarin skeleton atoms numeration system given in structure 14 was used. The IR spectra were recorded by means of the KBr pellet technique on a Bruker Vector-22 spectrometer. The UV spectra were obtained on an HP 8453 UV-Vis spectrometer (Hewlett-Packard, Waldbronn, Germany). The melting points were determined on a Stuart SMF-38 melting point apparatus (Bibby Scientific, Staffordshire, UK) and are uncorrected. Elemental analysis was carried out on a Carlo-Erba 1106 Elemental analysis instrument (Carlo-Erba, Milan, Italy). Spectral and analytical investigations were carried out at Collective Chemical Service center of Siberian Branch of the Russian Academy of Sciences.

Reaction products were isolated by column chromatography on silica gel 60 (0.063–0.200 mm, Merck KGaA, Darmstadt, Germany) and eluted with chloroform and chloroform-ethanol (100:1; to 25:1). The reaction progress and the purity of the obtained compounds were monitored by TLC on Silufol UV-254 plates (Kavaler, Czech Republic, CHCl₃-EtOH, 25:1; detection under UV light or by treatment with iodine vapor).

Chemicals used—TsCl, Ti(O-iPr)₄, n-BuLi, CuI, PPh₃, Lindlar’s catalysts and (trimethylsilyl)acetylene 30—were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Alfa Aesar (GmbH, Karlsruhe, Germany). Dichlorobis(triphenylphosphine)palladium(II) was obtained as described in [31]. 1-Ethynyl-3,4,5-trimethoxybenzene (21a) [32], 1-ethynyl-3-hydroxy-4-trimethoxybenzene (21b) [33], 1-ethyl-2,3,4-trimethoxybenzene (21c) [34], 1-ethyl-3,4-dihydroxybenzene (21d) [35], 1-ethyl-2-hydroxy-3-methoxy-5-formylbenzene (21e) [36], 3,4-dihydroxybromobenzene (33) [37] and combretastatin A-4 (1) [22] are known compounds and were prepared by the reported methods. Solvents (THF, benzene, MeOH) and Et₃N were purified by standard methods and distilled in a stream of argon just before use.

3.2. Synthesis and Spectral Data

2-Isopropyl-2-[(3,4,5-trimethoxyphenyl)ethynyl]-2H-furo[3,2-g]chromene-3,7-dione (16). (a) To a solution of oreoselone (14, 488 mg, 2 mmol) in THF (7 mL) under argon was added TsCl (470 mg, 2.5 mmol) and Pd(PPh₃)₂Cl₂ (7 mg, 0.1 mmol). The mixture was heated at 60 °C for 6 h (TLC), then 1-ethyl-3,4,5-trimethoxybenzene (21a, 770 mg, 4 mmol) and Et₃N (0.84 mL, 6 mmol) was added. The reaction mixture was heated under stirring another 7 h. After cooling, 3 mL of water was added and the mixture was extracted with methylene chloride (5 × 4 mL). The combined extract was washed with water, dried over MgSO₄ and filtered. The solvent was removed under reduced pressure, and the residue was subjected to column chromatography to isolate 538 mg (62%) of 16 and
40 mg (12%) of 22. Compound 16 was recrystallized from diethyl ether, m.p. 101–102 °C. IR (KBr, ν, cm⁻¹): 2974, 2927, 2879, 2852, 2150, 1732, 1664, 1626, 1531, 1481, 1411, 1390, 1353, 1286, 1253, 1126, 1095, 1039, 970, 948, 916, 866, 827, 734, 661. ¹H-NMR (600 MHz, CDCl₃, δ₀H): 0.98 (d, J = 6.9 Hz, 3H, CH₃), 1.28 (d, J = 6.9 Hz, 3H, CH₃), 2.46 (m, 1H, CH-(CH₃)₂), 3.84 (s, 9H, 3 × OCH₃), 6.34 (d, J = 9.6 Hz, 1H, H-6), 6.96 (s, 1H, H-9), 7.04 (s, 2H, H-2',6'), 7.68 (d, J = 9.6 Hz, 1H, H-5), 7.87 (s, 1H, H-4). ¹³C-NMR (150 MHz, CDCl₃, δC): 16.5 (CH₃), 17.4 (CH₃), 36.4 (CH), 56.0 (3C-CH₃), 72.1 (C-1a), 82.6 (C-1b), 99 (C-2), 101.4 (C-9), 106.5 (C-2' and C-6'), 115.2 (C-6'), 115.6, 115.5 (C-3a,4a), 119.4 (C-1'), 125.8 (C-4'), 131.5 (C-5'), 143.0 (C-5), 153.4 (C-8a), 155.8 (C-3' and C-5'), 158.6 (C-7), 169.4 (C-9a), 193.8 (C-3). UV (EtOH) λmax (lg ε): 257 (4.56), 297 (4.3), 307 (4.27), 348 (4.11) nm. Anal. Caled for C₂₅H₂₂O₇: C, 69.12; H, 5.10; found C, 69.29; H, 5.02. (b) A mixture of 2-(p-toluenesulfonyl)oreoselone (15, 398 mg, 1 mmol), 1-ethyl-3,4,5-trimethoxybenzene (21a, 385 mg, 2 mmol), Et₃N (0.42 mL, 3 mmol), and Pd(PPh₃)₂Cl₂ (7 mg, 0.1 mmol) in 5 mL of anhydrous THF was heated for 7 h under reflux in argon. The mixture was evaporated, the residue containing 10 mL of water and extracted with methylene chloride (4 × 5 mL). The combined extract was dried over MgSO₄, filtered and evaporated. The residue was subjected to column chromatography on silica gel to isolate 287 mg (66%) of compound 16 and 12 mg (5%) of compound 22.

2-[(3-Hydroxy-4-methoxyphenyl)ethyl]-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (17). This compound (374 mg, 48%) was prepared as a yellow oil from oreoselone (14, 488 mg, 2 mmol) and 1-ethyl-3-hydroxy-4-methoxybenzene (21b (592 mg, 4 mmol) using the procedure (a) described for 16. IR (KBr, ν, cm⁻¹): 3467, 3086, 3062, 3030, 2976, 2923, 2850, 2191, 1751, 1660, 1625, 1600, 1494, 1419, 1353, 1267, 1207, 1149, 1110, 1066, 1028, 977, 935, 806, 752, 696. ¹H-NMR (400 MHz, CDCl₃, δH): 1.09 (d, J = 6.9 Hz, 3H, CH₃), 1.20 (d, J = 6.9 Hz, 3H, CH₃), 3.03 (m, 1H, CH-(CH₃)₂), 3.79 (s, 3H, OCH₃), 6.05 (s, 1H, OH), 6.40 (d, J = 9.6 Hz, 1H, H-6), 6.92 (d, J = 7.8 Hz, 1H, H-5'), 6.97 (dd, J = 7.8 and 1.8 Hz, 1H, H-6'), 6.98 (s, 1H, H-9), 6.99 (d, J = 1.8 Hz, 1H, H-2'), 7.66 (d, J = 9.6 Hz, 1H, H-5), 7.85 (s, 1H, H-4). ¹³C-NMR (100 MHz, CDCl₃, δC): 16.2 (CH₃), 17.4 (CH₃), 30.1 (CH), 54.1 (CH₃), 73.5 (C-1a), 82.4 (C-1b), 96.1 (C-2), 100.1 (C-9), 112.4 (C-6), 113.1 (C-5'), 113.4 (C-3a), 115.2 (C-4a), 118.1 (C-1'), 120.5 (C-2'), 123.1 (C-6'), 125.7 (C-4), 142.3 (C-5), 147.9 (C-3'), 149.1 (C-4'), 156.6 (C-8a), 158.1 (C-7), 172.6 (C-9a), 195.5 (C-3). UV (EtOH) λmax (lg ε): 258 (4.52), 295 (3.96), 307 (3.88), 351 (3.93) nm. Anal. Caled for C₂₃H₁₈O₆: C, 70.76; H, 4.65; found C, 70.39; H, 4.92.

2-Isopropyl-2-[(2,3,4-trimethoxyphenyl)ethyl]-2H-furo[3,2-g]chromene-3,7-dione (18). Compound 18 (560 mg, 65%) was prepared from oreoselone (14, 488 mg, 2 mmol) and 1-ethyl-2,3,4-trimethoxybenzene (21c, 770 mg, 4 mmol) using the procedure (a) described for 16. Compound 22 (30 mg, 10%) was also isolated. Compound 18, m.p. 101–102 °C (ether). IR (KBr, ν, cm⁻¹): 3066, 2976, 2939, 2861, 2189, 1737, 1679, 1625, 1487, 1423, 1388, 1352, 1286, 1224, 1141, 1122, 1093, 1022, 983, 943, 866, 825, 790, 734, 691. ¹H-NMR (400 MHz, CDCl₃, δH): 0.91 (d, J = 6.9 Hz, 3H, CH₃), 1.31 (d, J = 6.9 Hz, 3H, CH₃), 2.50 (m, 1H, CH-(CH₃)₂), 3.82 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 3.97 (s, 3H, OCH₃), 6.34 (d, J = 9.6 Hz, 1H, H-6), 6.70 (d, J = 8.2 Hz, 1H, H-5'), 6.99 (s, 1H, H-9), 7.52 (d, 1H, J = 8.2 Hz, H-6'), 7.72 (d, 1H, J = 9.6 Hz, H-5), 7.90 (1H, s, H-4). ¹³C-NMR (100 MHz, CDCl₃, δC): 16.6 (CH₃), 17.5 (CH₃), 36.4 (CH), 56.0 (CH₃), 60.8 (CH₃), 62.2 (CH₃), 71.6 (C-1a), 82.3 (C-1b), 98.9 (C-
2-[(3,4-Dihydroxyphenyl)ethylidene]-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (19). Compound 19 (330 mg, 44%) was prepared from oreoselone (14, 488 mg, 2 mmol) and 1-ethyl-2,3,4-trimethoxybenzene (21d, 540 mg, 4 mmol) using the procedure (a) described for 16. Compound 22 (82 mg, 25%) was also isolated. Compound 19, m.p. 84–86 °C (ether). IR (KBr, ν, cm⁻¹): 3340, 3320, 3087, 3071, 2974, 2929, 2879, 2852, 2114, 1732, 1664, 1625, 1595, 1531, 1390, 1353, 1286, 1253, 1126, 1095, 1039, 948, 866, 827, 735. ¹H-NMR (400 MHz, CDCl₃, δH): 0.96 (d, J = 7.0 Hz, 3H, CH₃), 1.37 (d, J = 7.0 Hz, 3H, CH₃), 2.55 [m, 1H, CH(CH₃)₂], 6.40 (d, J = 9.6 Hz, 1H, H-6), 7.05 (s, 1H, H-9), 7.46 (d, J 1.8 Hz, 1H, H-2'), 7.52 (dd, J = 8.0 and 1.8 Hz, 1H, H-6'), 7.66 (d, J = 8.0 Hz, 1H, H-5'), 7.73 (d, J = 9.6 Hz, 1H, H-5), 7.93 (s, 1H, H-4). ¹³C-NMR (100 MHz, CDCl₃): 15.6 (CH₃), 16.0 (CH₃), 31.1 (CH), 73.9 (C-1a), 81.9 (C-1b), 98.6 (C-2), 101.4 (C-9), 115.7 (C-3a), 116.0 (C-6), 116.7 (C-4a), 117.8 (C-1'), 120.2 (C-5') 122.7 (C-2'), 124.1 (C-4), 126.1 (C-6'), 143.3 (C-5), 153.1 (C-3'), 158.9 (C-2'), 161.1 (C-7), 169.5 (C-9a), 192.6 (C-3). UV (EtOH) λₘₐₓ (lge): 257 (4.42), 297 (4.17), 307 (4.14), 348 (3.97) nm. Anal. Calcd for C₂₅H₂₂O₇: C, 69.12; H, 5.10; found C, 69.49; H, 5.12.

2-[(5-Formyl-3-hydroxy-3-methoxyphenyl)ethylidene]-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (20). Compound 20 (493 mg, 59%) was prepared from oreoselone (14, 488 mg, 2 mmol) and 1-ethyl-5-formyl-2-hydroxy-3-methoxybenzene (21e, 705 mg, 4 mmol) using the procedure (a) described for 16, or from 2-(p-toluenesulfonyl)oreoselone (15, 398 mg, 1 mmol) and 1-ethyl-5-formyl-2-hydroxy-3-methoxybenzene (21e, 353 mg, 2 mmol) using the procedure (b) described for 16. Yield 60%. Compound 22 was also isolated [62 mg, procedure (a) and 23 mg, procedure (b)]. Compound 20, m.p. 91–93 °C (ether). IR (KBr, ν, cm⁻¹): 3456, 3155, 3065, 2978, 2939, 2881, 2839, 2112, 1738, 1680, 1626, 1582, 1554, 1487, 1423, 1389, 1352, 1286, 1260, 1250, 1225, 1201, 1141, 1122, 1094, 1043, 1022, 984, 970, 943, 916, 866, 826, 790, 760, 735, 690, 6800. ¹H-NMR (400 MHz, CDCl₃, δH): 0.91 (d, J = 6.9 Hz, 3H, CH₃), 1.29 (d, J = 6.9 Hz, 3H, CH₃), 2.48 [m, 1H, CH(CH₃)₂], 3.77 (s, 3H, OCH₃), 6.32 (d, J = 9.8 Hz, 1H, H-6), 6.96 (s, 1H, H-9), 7.43 (br s, 1H, H-4'), 7.50 (br s, 1H, OH), 7.60 (br s, 1H, H-6'), 7.69 (d, J = 9.8Hz, 1H, H-5), 7.88 (s, 1H, H-4), 9.91 (s, 1H, CHO). ¹³C-NMR (100 MHz, CDCl₃, δC): 15.2 (CH₃), 15.6 (CH₃), 33.7 (CH), 55.4 (OCH₃), 71.3 (C-1a), 80.2 (C-1b), 98.3 (C-2), 101.1 (C-9), 115.4 (C-3a), 115.7 (C-6), 116.4 (C-4'), 118.3 (C-4a), 119.5 (C-1'), 121.5 (C-6'), 125.7 (C-4), 133.6 (C-5'), 143.1 (C-5), 156.6 (C-3'), 158.9 (C-2'), 161.6 (C-7), 165.1 (C-8a), 171.5 (C-9a), 191.6 (CHO), 192.3 (C-3). UV (EtOH) λₘₐₓ (lge): 253 (4.21), 328 (3.45), 350 (3.15) nm. Anal. Calcd for C₂₂H₁₈O₆: C, 70.21; H, 4.29; found C, 70.49; H, 4.20.

(Z)-2-Isopropyl-2-(3,4,5-trimethoxystyryl)-2H-furo[3,2-g]chromene-3,7-dione (2). A solution of n-BuLi (0.08 mL, 1.04 mmol) was added dropwise to a solution of 2-isopropyl-2-[(3,4,5-trimethoxyphenyl)ethylidene]-2H-furo[3,2-g]chromene-3,7-dione (16, 100 mg, 0.23 mmol) and tetraisopropoxytitanium (140 mg, 0.52 mmol) in anhydrous THF (3 mL) at −78 °C. The stirring was continued for 10 min at the same temperature. The reaction mixture was warmed to room temperature.
and heated at 50 °C for 2 h. After cooling, the reaction was quenched with a saturated solution of NH₄Cl (3 mL), water (3 mL), and extracted with dichloromethane (3 × 4 mL), the combined organic layers was washed with water, dried over anhydrous MgSO₄, and filtered. The solvent was evaporated, and the residue was subjected to column chromatography on silica gel (chloroform and chloroform–ethanol 100:1 as eluent) to afford compound 2 (42 mg, 42% yield) as a yellow powder, m.p. 91–92 °C (ether).

IR (KBr, ν, cm⁻¹): 3047, 2974, 2935, 1736, 1701, 1628, 1585, 1474, 1390, 1356, 1286, 1226, 1195, 1120, 1034, 1011, 972, 900, 866, 840, 827, 756, 740, 700, 681. ¹H-NMR (400 MHz, CDCl₃, δH): 0.88 (d, J = 6.9 Hz, 3H, CH₃), 1.11 (d, J = 6.9 Hz, 3H, CH₃), 3.20 [m, 1H, CH−(CH₃)₂], 3.83 (s, 9H, 3 × OCH₃), 6.23 (d, J = 9.2 Hz, 1H, H-1a), 6.36 (d, J = 9.7 Hz, 1H, H-6), 6.74 (d, J = 9.2 Hz, 1H, H-1b), 6.94 (s, 1H, H-9), 7.10 (br s, 2H, H-2',6'), 7.67 (d, J = 9.7 Hz, 1H, H-5), 7.90 (s, 1H, H-4). ¹³C-NMR (100 MHz, CDCl₃, δC): 16.1 (CH₃), 17.0 (CH₃), 29.2 (CH), 55.6 (3×OCH₃), 98.3 (C-2), 101.0 (C-9), 106.1 (C-2',6'), 114.8 (C-3a,4a), 115.1, 115.2 (C-6), 125.4 (C-4), 119.0 (C-1'), 126.8 (C-1a), 131.1 (C-1b), 131.8 (C-4'), 142.6 (C-5), 153.0 (C-8a), 155.3 (C-3',5'), 158.2 (C-7), 168.9 (C-9a), 192.4 (C-3). UV (EtOH) λmax (lge): 255 (4.44), 296 (4.07), 310 (4.01), 344 (sh), 354 (3.9) nm. Anal. Calcd for C₂₃H₂₂O₇: C, 68.80; H, 5.54; found C, 68.69; H, 5.17.

(Z)-2-(3-Hydroxy-4-methoxystyryl)-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (3). Lindlar’s catalyst (5 mg, 2 mol %) was added to a solution of 2-isopropyl-2-[3-(hydroxy-4-methoxy-phenyl)ethynyl]-2H-furo[3,2-g]chromene-3,7-dione (17, 100 mg, 0.26 mmol) in dry ethanol (6 mL). The system was filled with hydrogen, and the reaction mixture was stirred in a hydrogen flow for 20 h and then concentrated under reduced pressure. Column chromatography on silica gel afforded compound 3 (31 mg, 32% yield) as a yellow oil. IR (KBr, ν, cm⁻¹): 3520, 3488, 3398, 2960, 2925, 2852, 1750, 1726, 1724, 1630, 1576, 1510, 1480, 1463, 1356, 1335, 1300, 1250, 1176, 1157, 1143, 1105, 1032, 978, 935, 901, 885, 831, 813, 760, 744, 700, 671. ¹H-NMR δH (600 MHz, CDCl₃, δH): 0.87 [d, J = 6.9 Hz, 3H, CH₃], 1.15 [d, J = 6.9 Hz, 3H, CH₃], 2.37 [m, 1H, CH−(CH₃)₂], 3.76 (s, 3H, OCH₃), 6.26 (d, J = 9.5 Hz, 1H, H-1b), 6.33 (d, J = 9.7 Hz, 1H, H-6), 6.92 (d, J = 7.8 Hz, 1H, H-5’), 6.79 (d, J = 9.5 Hz, 1H, H-1b), 6.89 (dd, J = 8.0 and 1.8 Hz, 1H, H-6’), 7.00 (s, 1H,H-9), 7.06 (d, J = 8.0 Hz, 1H, H-5’), 7.25 (d, J = 1.8 Hz, 1H, H-2’), 7.68 (d, J = 9.7 Hz, 1H, H-5’), 7.78 (s, 1H, H-4). ¹³C-NMR (150 MHz, CDCl₃, δC): 15.6 (CH₃), 18.6 (CH₃), 31.2 (CH), 55.3 (OCH₃), 92.9 (C-2), 100.9 (C-9), 113.6 (C-6), 114.4 (C-5’), 114.8 (C-3a), 116.4 (C-4a), 119.3 (C-2’), 121.7 (C-6’), 124.3 (C-4), 125.2 (C-1a), 129.2 (C-1b), 134.9 (C-1’), 143.5 (C-5), 149.2 (C-3’), 150.3 (C-4’), 159.4 (C-8a), 160.1 (C-7), 173.9 (C-9a),199.4 (C-3). UV (EtOH) λmax (lge): 253 (4.41), 296 (4.08), 308 (4.06), 341 (sh), 353 (4.1) nm, Anal. Calcd for C₂₂H₂₀O₆: C, 70.40; H, 5.14; found C, 70.78; H, 4.92.

(Z)-2-Isopropyl-2-(2,3,4-trimethoxystyryl)-2H-furo[3,2-g]chromene-3,7-dione (6). Compound 6 (40 mg) was prepared from 2-isopropyl-2-[(2,3,4-trimethoxyphenyl)ethynyl]-2H-furo[3,2-g]chromene-3,7-dione (18, 100 mg, 0.23 mmol) using the procedure described for 2. Yield 40%, m.p. 112–114 °C (ether). IR (KBr, ν, cm⁻¹): 3047, 2974, 1736, 1701, 1628, 1585, 1473, 1391, 1355, 1286, 1220, 1196, 1121, 1034, 1011, 972, 900, 866, 850, 827, 800, 756, 740, 680. ¹H-NMR (400 MHz, CDCl₃, δH): 0.98 (d, J = 6.9 Hz, 3H, CH₃), 1.19 (3H, J = 6.9 Hz, d, CH₃), 3.28 [m, 1H, CH−(CH₃)₂], 3.91 (s, 3H, OCH₃), 3.92 (s, 3H, OCH₃), 3.94 (s, 3H, OCH₃), 6.23 (d, J = 9.3 Hz, 1H, H-1a), 6.45 (d, J = 9.6 Hz, 1H, H-6), 6.67 (d, J = 8.3 Hz, 1H, H-5’), 6.81 (d, J = 9.3 Hz, 1H, H-1b), 7.18 (d, J = 8.3 Hz, 1H, H-6’), 7.97 (d, J = 9.6 Hz,
1H, H-5), 8.19 (s, 1H, H-4). $^{13}$C-NMR (100 MHz, CDCl$_3$, $\delta_C$): 16.5 (CH$_3$), 17.4 (CH$_3$), 31.6 (CH), 55.9 (OCH$_3$), 62.1 (OCH$_3$), 60.7 (OCH$_3$), 99.5 (C-2), 101.4 (C-9), 107.2 (C-5'), 115.2 (C-6), 115.5, 115.6 (C-3a, 4a), 123.9 (C-6'), 123.0 (C-1'), 125.9 (C-4), 126.9 (C-1a), 133.1 (C-1b), 141.3 (C-5), 144.2 (C-3'), 156.6 (C-2'), 158.7 (C-4'), 159.0 (C-8a), 160.9 (C-7), 170.7 (C-9a), 190.5 (C-3). UV (EtOH) $\lambda_{max}$ (lg$e$): 255 (4.32), 296 (3.94), 310 (3.88), 344 (sh), 354 (3.78) nm. Anal. Calcd for C$_{25}$H$_{24}$O$_7$: C, 68.80; H, 5.54; found C, 69.09; H, 5.42.

(Z)-2-[(3,4-Dihydroxystyril)-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (7). Compound 7 (24 mg) was prepared by partial reduction of 2-[(3,4-dihydroxyphenyl)ethynyl]-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (19, 100 mg, 0.26 mmol) using the procedure described for 3 (reaction time 30 h). Yield 24%, m.p. 68–71 °C (ether). IR (KBr, $\nu$, cm$^{-1}$): 3470, 2979, 2922, 2851, 1751, 1661, 1626, 1601, 1598, 1495, 1420, 1354, 1327, 1267, 1207, 1150, 1111, 1086, 1028, 1002, 978, 935, 914, 823, 800, 752, 696. $^1$H-NMR (400 MHz, CDCl$_3$, $\delta_H$): 0.85 (d, $J = 6.9$ Hz, 3H, CH$_3$), 0.91 [d, $J = 6.9$ Hz, 3H, CH$_3$], 2.99 [m, 1H, CH$_2$(CH$_3$_)]$_2$, 6.21 (d, $J = 9.5$ Hz, 1H, H-1a), 6.37 (d, $J = 9.4$ Hz, 1H, H-6), 6.37 (d, $J = 8.3$ Hz, 1H, H-5$'$), 6.81 (d, $J = 9.5$ Hz, 1H, H-1b), 6.89 (s, 1H, H-9), 7.36 (d, $J = 1.8$ Hz, 1H, H-2$'$), 7.49 (dd, $J = 8.0$ and 1.8 Hz, 1H, H-6$'$), 7.65 (d, $J = 8.0$ Hz, 1H, H-5$'$), 7.91 (d, $J = 9.6$ Hz, 1H, H-5), 8.03 (s, 1H, H-4). $^{13}$C-NMR (100 MHz, CDCl$_3$, $\delta_C$): 15.1 (CH$_3$), 15.5 (CH$_3$), 30.5 (CH), 98.5 (C-2), 100.9 (C-9), 115.2 (C-3a), 115.5 (C-6), 116.2 (C-5'), 117.2 (C-4a), 119.7 (C-1'), 122.2 (C-2'), 123.6 (C-6'), 125.5 (C-4), 127.1 (C-1a), 132.4 (C-1b), 142.8 (C-5), 157.9 (C-3'), 158.8 (C-4'), 159.9 (C-8a), 161.4 (C-7), 171.3 (C-9a), 190.3 (C-3). UV (EtOH) $\lambda_{max}$ (lg$e$): 256 (4.76), 292 (4.33), 349 (4.13) nm. Anal. Calcd for C$_{22}$H$_{18}$O$_6$: C, 69.83; H, 4.79; found C, 70.10; H, 4.31.

(Z)-2-(5-Formyl-3-hydroxy-3-methoxystyril)-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (8). Compound 8 (37 mg) was prepared from 2-[(5-formyl-3-hydroxy-3-methoxyphenyl)ethynyl]-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (20, 100 mg, 0.24 mmol) using the procedure described for 3. Yield 37%, a yellow oil. IR (KBr, $\nu$, cm$^{-1}$): 3435, 3063, 3042, 2960, 2925, 2853, 2808, 1739, 1726, 1714, 1629, 1600, 1576, 1510, 1500, 1464, 1440, 1394, 1355, 1334, 1300, 1250, 1230, 1176, 1157, 1144, 1105, 1032, 978, 935, 915, 893, 831, 814, 761, 744, 720, 668. $^1$H-NMR (400 MHz, CDCl$_3$, $\delta_H$): 0.97 (d, $J = 6.9$ Hz, 3H, CH$_3$), 1.07 [d, $J = 6.9$ Hz, 3H, CH$_3$], 3.15 [m, 1H, CH$_2$(CH$_3$_)], 3.92 (s, 3H, OCH$_3$), 6.23 (d, $J = 9.3$ Hz, 1H, H-1a), 6.37 (1H, $J = 9.8$ Hz, d, H-6), 6.41 (d, $J = 1.8$ Hz, 1H, H-6$'$), 6.83 (d, $J = 9.3$ Hz, 1H, H-1b), 7.05 (s, 1H, H-9), 7.69 (d, 1H, $J = 9.6$ Hz, H-5), 7.78 (1H, $J = 1.8$ Hz, d, H-4$'$), 7.85 (s, 1H, H-4), 8.07 (1H, $J = 8.8$ Hz, d, H-1b), 9.98 (s, 1H, CHO). $^{13}$C-NMR (100 MHz, CDCl$_3$, $\delta_C$): 15.6 (CH$_3$), 15.9 (CH$_3$), 34.0 (CH), 55.7 (OCH$_3$), 99.5 (C-2), 100.6 (C-9), 115.6 (C-6), 116.0 (C-3a), 116.7 (C-4a), 118.7 (C-4'), 121.8 (C-6'), 119.8 (C-1'), 126.0 (C-4), 127.5 (C-1a), 132.6 (C-1b), 133.9 (C-5'), 144.8 (C-5), 156.9 (C-3'), 158.9 (C-2'), 161.9 (C-8a), 165.3 (C-7), 171.8 (C-9a), 191.9 (CHO), 194.6 (C-3). UV (EtOH) $\lambda_{max}$ (lg$e$): 256 (4.41), 291 (3.97), 349 (3.78) nm. Anal. Calcd for C$_{24}$H$_{20}$O$_7$: C, 68.57; H, 4.80; found C, 68.24; H, 4.68.

2-Isopropyl-3-[(2,3,4-trimethoxyphenyl)ethynyl]-7H-furo[3,2-g]chromene-7-one (25). To a solution of 2-isopropyl-3-(trifluoromethanesulfonyloxy)psoralene (23, 150 mg, 0.4 mmol) and 1-ethylthiophosphine (21e, 85 mg, 0.44 mmol) in benzene (5 mL) was added Cul (1.5 mg, 2 mol%), Pd(PPh$_3$)$_2$Cl$_2$ (11 mg, 4 mol%), and Et$_3$N (0.076 mL, 0.55 mmol; 1.4 equiv) under argon. The reaction
mixture was stirred at 80 °C for 8 h (TLC). The mixture was cooled and 5 mL of water was added. The separated water layer was extracted with methylene chloride (5 × 4 mL). The combined organic extracts was washed with water, dried over MgSO₄, filtered, and concentrated under reduced pressure.

The residue was subjected to column chromatography on silica gel. Eluting with chloroform and crystallization from diethyl ether gave 122 mg (73%) of compound 25. M.p. 121–122 °C (ether). IR (KBr, v, cm⁻¹): 3051, 2979, 2927, 2854, 2472, 2118, 1732, 1622, 1603, 1580, 1514, 1470, 1386, 1363, 1321, 1277, 1252, 1213, 1194, 1167, 1140, 1115, 1096, 1068, 1043, 980, 959, 935, 916, 870, 850, 820, 770, 750, 741, 702, 677. ¹H-NMR (400 MHz, CDCl₃, δH): 1.35 (d, J = 7.0 Hz, 3H, CH₃), 1.37 [d, J = 7.0 Hz, 3H, CH₃], 3.25 [m, 1H, CH-(CH₃)₂], 3.86 (s, 3H, OCH₃), 3.92 (s, 3H, OCH₃), 4.01 (s, 3H, OCH₃), 6.40 (d, J = 9.8 Hz, 1H, H-6), 6.72 (d, J = 8.2 Hz, 1H, H-5'), 6.98 (s, 1H, H-9), 7.59 (d, J = 8.2 Hz, 1H, H-6'), 7.79 (s, 1H, H-4), 7.80 (d, 1H, J = 9.8 Hz, H-5). ¹³C-NMR (100 MHz, CDCl₃, δC): 20.3 (CH₃), 20.5 (CH₃), 25.9 (OCH₃), 60.9 (OCH₃), 62.3 (OCH₃), 86.5 (C-1a), 94.0 (C-1b), 95.5 (C-3), 97.5 (C-9), 107.3 (C-5'), 111.1 (C-6), 116.6 (C-3a), 118.9 (C-1'), 124.2 (C-6'), 126.5 (C-4), 141.6 (C-3'), 143.5 (C-5), 152.0 (C-7). UV (EtOH) λmax (lgε): 249 (3.65), 306 (3.75), 327 (3.73), 351 (sh), 355(2.85) nm. Anal. Caled for C₂₂H₁₆O₅: C, 71.76; H, 5.30; found C, 71.49; H, 5.12.

3-[(3,4-Dihydroxyphenyl)ethynyl]-2-isopropyl-7H-furo[3,2-g]chromene-7-one (26). Compound 26 was prepared by two methods. Reaction of psoralene derivative 23 (150 mg, 0.44 mmol) with 1-ethyl-3,4-dihydroxybenzene (21d, 60 mg, 0.44 mmol) by the procedure described for 25 (method a) gave 98 mg (68%) of compound 26. (b) To a solution of 3-ethyl-2-isopropylpsoralene (32, 100 mg, 0.4 mmol) in benzene (5 mL) was added 3,4-dihydroxybromobenzene (33, 83 mg, 0.44 mmol), 1.5 mg (2 mol %) of CuI, 11 mg (4 mol %) of Pd(PPh₃)₂Cl₂, and 0.076 mL (1.4 equiv, 0.55 mmol) of Et₃N in benzene (0.4 mmol) in benzene (5 mL) was added 3,4-dihydroxybromobenzene (33, 83 mg, 0.44 mmol) using the procedure described for 25 (method a) gave 98 mg (68%) of compound 26. (b) To a solution of 3-ethynyl-2-isopropylpsoralene (32, 100 mg, 0.4 mmol) in benzene (5 mL) was added 3,4-dihydroxybromobenzene (33, 83 mg, 0.44 mmol), 1.5 mg (2 mol %) of CuI, 11 mg (4 mol %) of Pd(PPh₃)₂Cl₂, and 0.076 mL (1.4 equiv, 0.55 mmol) of Et₃N under argon. The mixture was stirred at 80 °C for 10 h (TLC). Then the solution was cooled, 3 mL of water was added and reaction mixture was extracted with methylene chloride (5 × 4 mL). The combined extract was washed with water, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel. Eluting with chloroform and crystallization from diethyl ether gave 60 mg (42%) of compound 26. M.p. 104–105 °C. IR (KBr, v, cm⁻¹): 3450, 3327, 3051, 2980, 2880, 2556, 2472, 2096, 1732, 1716, 1697, 1620, 1590, 1550, 1514, 1431, 1364, 1278, 1253, 1213, 1191, 1160, 1140, 1095, 1041, 980, 959, 935, 916, 870, 850, 820, 770, 754, 740, 685, 650. ¹H-NMR (400 MHz, CDCl₃, δH): 1.38 (d, J = 7.0 Hz, 3H, CH₃), 1.40 (d, J = 7.0 Hz, 3H, CH₃), 3.30 [m, 1H, CH-(CH₃)₂], 4.09 (br.s, 2H, OH), 6.32 (d, J = 9.7 Hz, 1H, H-6), 7.07 (s, 1H, H-9), 7.26 (d, J = 2.0 Hz, 1H, H-2'), 7.32 (dd, J = 8.0 and 2.0 Hz, 1H, H-6'), 7.46 (d, J = 8.0 Hz, 1H, H-5'), 7.77 (d, J = 9.7 Hz, 1H, H-5), 7.80 (s, 1H, H-4). ¹³C-NMR (100 MHz, CDCl₃, δC): 20.0 (CH₃), 20.1 (CH₃), 25.8 (CH₃), 86.4 (C-1a), 94.0 (C-1b), 100.3 (C-3), 102.8 (C-9), 112.3 (C-4a), 115.2 (C-6), 115.3 (C-3a), 116.5 (C-1'), 120.2 (C-5'), 121.2 (C-4), 124.5 (C-2'), 126.2 (C-2'), 144.2 (C-5), 145.9 (C-3'), 147.4 (C-4'), 152.1 (C-9a), 155.3 (C-8a), 160.4 (C-2), 161.3 (C-7). UV (EtOH) λmax (lgε): 252 (3.64), 309 (3.94), 339 (3.43), 355 (sh) nm. Anal. Caled for C₂₅H₂₂O₆: C, 73.33; H, 4.48; found C, 73.30; H, 4.55.

3-[(5-Formyl-3-hydroxy-3-methoxyphenyl)ethynyl]-2-isopropyl-7H-furo[3,2-g] chromene-7-one (27). Compound 27 (112 mg) was prepared from psoralene derivative 23 (150 mg, 0.44 mmol) and 1-ethyl-5-formyl-2-hydroxy-3-methoxybenzene (21e, 75 mg, 0.44 mmol) using the procedure
described for 25. Yield 70%, m.p. 110–112 °C (ether). IR (KBr, ν, cm⁻¹): 3430, 3325, 2989, 2880, 2713, 2472, 2046, 1944, 1782, 1732, 1716, 1697, 1620, 1602, 1514, 1469, 1431, 1363, 1315, 1278, 1253, 1220, 1191, 1139, 1095, 1041, 1020, 980, 904, 860, 848, 769, 754, 695, 650, 600. ¹H-NMR (400 MHz, CDCl₃, δH): 1.39 (d, J = 7.0 Hz, 3H, CH₃), 1.42 (d, J = 7.0 Hz, 3H, CH₃), 3.29 [m, 1H, CH(CH₃)₂], 3.98 (s, 3H, OCH₃), 6.36 (d, J = 9.8 Hz, 1H, H-6), 7.09 (s, 1H, H-9), 7.44 (br.s, 1H, H-4'), 7.50 (br.s, 1H, OH), 7.60 (br.s, 1H, H-6'), 7.75 (d, J = 9.8 Hz, 1H, H-5'), 7.82 (s, 1H, H-4), 10.01 (s, 1H, CHO). ¹³C-NMR (100 MHz, CDCl₃, δC): 20.5 (CH₃), 20.6 (CH₃), 26.2 (CH), 60.6 (OCH₃), 85.6 (C-1a), 91.0 (C-1b), 99.5 (C-3), 104.4 (C-9), 115.8 (C-3a), 116.3 (C-6), 116.9 (C-4'), 118.9 (C-4a), 119.8 (C-1'), 120.6 (C-6'), 128.7 (C-4'), 132.2 (C-5'), 143.9 (C-9), 150.7 (C-3'), 152.5 (C-8a), 153.1 (C-9a), 154.6 (C-2'), 159.2 (C-2), 160.7 (C-7), 191.8 (CHO). UV (EtOH) λₘₐₓ (lge): 246 (3.92), 305 (sh), 329 (3.63), 355 (2.81) nm. Anal. Calcd for C₂₄H₁₈O₆: C, 71.64; H, 4.51; found C, 71.30; H, 4.58.

3-[(3,4-Dihydroxyphenyl)ethynyl]-2-isopropyl-9-[4-(4-methylpiperazin-1-yl)methyl]-7H-furo[3,2-g]-chromene-7-one (28). Compound 28 (79 mg) was prepared from 2-isopropyl-9-[(4-methylpiperazin-1-yl)methyl]-7-oxo-7H-furo[3,2-g]chromen-3-yl trifluoromethanesulfonate (24, 195 mg, 0.4 mmol) and 1-ethyl-3,4-dihydroxybenzene (11d, 60 mg, 0.44 mmol) using the procedure described for 25. Yield 42%, yellow powder, m.p. 118–120 °C (ether). IR (KBr, ν, cm⁻¹): 3437, 2967, 2922, 2851, 2111, 1732, 1635, 1628, 1585, 1511, 1496, 1465, 1431, 1388, 1348, 1319, 1250, 1213, 1198, 1140, 1114, 1095, 1047, 957, 870, 820, 737, 700, 626, 602. ¹H-NMR (400 MHz, CDCl₃, δH): 1.33 (d, J = 7.0 Hz, 3H, CH₃), 1.36 (3H, d, J = 7.0 Hz, CH₃), 2.32 (s, 3H, NCH₃), 3.28 (m, 4H, H-3′,5′), 2.68 (m, 4H, H-2′,6′), 3.25 [m, 1H, CH(CH₃)₂], 4.46 (d, 1H, J = 9.8 Hz, 1H, CH₂), 4.58 (d, 1H, J = 9.8 Hz, 1H, CH₂), 6.27 (d, J = 9.8 Hz, 1H, H-6), 6.52 (dd, J = 8.0 and 2.0 Hz, 1H, H-6'), 6.64 (d, J = 2.0 Hz, 1H, H-2'), 6.79 (d, J = 8.0 Hz, 1H, H-5'), 7.59 (d, J = 9.8 Hz, 1H, H-5), 7.74 (s, 1H, H-4), 8.00 (br.s, 2H, OH). ¹³C-NMR (100 MHz, CDCl₃, δC): 20.1 (CH₃), 20.3 (CH₃), 25.9 (CH), 42.7 (NCH₃), 48.5 (CH₂N), 51.6 (C-2′,6′), 53.4 (C-3′,5′), 85.3 (C-1a), 90.7 (C-1b), 99.2 (C-3), 104.4 (C-9), 115.0 (C-1′), 115.6 (C-3a), 116.0 (C-6), 116.6 (C-4a), 117.6 (C-5′), 122.0 (C-2′), 128.4 (C-4), 130.8 (C-6′), 143.5 (C-5), 147.1 (C-2′), 148.7 (C-4′), 152.2 (C-8a), 153.0 (C-9a), 156.9 (C-2), 160.4 (C-7). UV (EtOH) λₘₐₓ (lge): 250 (3.76), 284 (2.65), 327 (3.20), 346 (2.78) nm. Anal. Calcd for C₂₈H₂₈N₂O₅: C, 71.17; H, 5.97; N, 5.93; found C, 70.91; H, 6.02; N, 5.63.

3-[(5-Formyl-3-hydroxy-3-methoxyphenyl)ethynyl]-2-isopropyl-9-[(4-methylpiperazin-1-yl)methyl]-7H-furo[3,2-g]chromen-7-one (29). Compound 29 (119 mg) was prepared from 2-isopropyl-9-[(4-methylpiperazin-1-yl)methyl]-7-oxo-7H-furo[3,2-g]chromen-3-yl trifluoromethanesulfonate (24, 195 mg, 0.4 mmol) and 1-ethyl-3,4-dihydroxybenzene (21e, 5 mg, 0.44 mmol) using the procedure described for 25. Yield 58%, m.p. 104–105 °C (ether). IR (KBr, ν, cm⁻¹): 3402, 3117, 3080, 2958, 2850, 2783, 2711, 2611, 2172, 2133, 1726, 1693, 1610, 1593, 1537, 1464, 1427, 1402, 1367, 1331, 1304, 1252, 1229, 1213, 1180, 1142, 1107, 1065, 1030, 993, 968, 908, 876, 845, 752, 741, 715, 667, 633, 621. ¹H-NMR (400 MHz, CDCl₃, δH): 1.36 (d, J = 7.0 Hz, 3H, CH₃), 1.39 (d, J = 7.0 Hz, 3H, CH₃), 2.38 (s, 3H, NCH₃), 2.42 (m, 4H, H-3′,5′), 2.65 (m, 4H, H-2′,6′), 3.25 [m, 1H, CH(CH₃)₂], 4.03 (s, 3H, OCH₃), 4.41 (d, J = 9.8 Hz, 1H, CH₂), 4.48 (d, J = 9.8 Hz, 1H, CH₂), 6.41 (d, J = 9.8 Hz, 1H, H-6), 6.89 (br.s, 1H, H-4′), 7.41 (br.s, 1H, OH), 7.44 (br.s, 1H, H-6′), 7.57 (s, 1H, H-4), 7.79 (d, J = 9.8 Hz, 1H, H-5), 9.99 (s, 1H, CHO). ¹³C-NMR (100 MHz, CDCl₃, δC): 20.2 (CH₃), 20.5 (CH₃),
25.9 (CH), 42.5 (NCH₃), 48.2 (CH₂N), 51.5 (C-2",6"), 52.9 (C-3",5"), 58.3 (OCH₃), 85.2 (C-1a), 93.5 (C-1b), 100.5 (C-9), 104.2 (C-3), 115.5 (C-6), 115.7 (C-3a), 116.6 (C-4), 118.9 (C-4a), 119.8 (C-1'), 120.5 (C-6'), 128.4 (C-4'), 132.1 (C-5'), 143.5 (C-5), 150.6 (C-8a), 152.2 (C-9a), 153.4 (C-3'), 154.7 (C-2'), 156.9 (C-2), 160.4 (C-7), 189.3 (CHO). UV (EtOH) λ_max (lge): 253 (3.81), 289 (2.52), 328 (3.11), 350 (3.08) nm. Anal. Calcd for C₃₀H₃₀N₂O₆: C, 70.02; H, 5.88; N, 5.44; found C, 69.79; H, 5.85; N, 5.16.

(Z)-2-Isopropyl-3-(2,3,4-trimethoxysteryl)-2H-furo[3,2-g]chromene-3,7-dione (9). Compound 9 (60 mg) was prepared from 2-isopropyl-3-[(2,3,4-trimethoxyphenyl)ethynyl]-7H-furo[3,2-g]chromene-7-one (25, 100 mg, 0.24 mmol) using the procedure described for 2. Yield 60%, m.p. 94–97 °C (ether). IR (KBr, cm⁻¹): 3062, 3049, 2980, 2950, 1732, 1700, 1636, 1589, 1495, 1465, 1433, 1389, 1346, 1288, 1259, 1203, 1169, 1140, 1094, 1047, 1029, 1009, 962, 937, 903, 870, 831, 820, 781, 754, 700, 678, 650, 623, 602. ¹H-NMR (600 MHz, CDCl₃, δ₀H): 1.26 (d, J = 6.9 Hz, 3H, CH₃), 1.28 [d, J = 6.9 Hz, 3H, CH₃], 3.16 [m, 1H, CH-(CH₃)₂], 3.87 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 3.90 (s, 3H, OCH₃), 6.23 (d, J = 9.8 Hz, 1H, H-6), 6.40 (d, J = 8.8 Hz, 1H, H-1a), 6.71 (d, J = 8.8 Hz, 1H, H-1b), 6.83 (d, J = 8.2 Hz, H-5'), 6.90 (s, 1H, H-9), 7.19 (d, J = 8.2 Hz, 1H, H-6'), 7.77 (d, J = 9.8 Hz, 1H, H-5), 7.94 (s, 1H, H-4). ¹³C-NMR (150 MHz, CDCl₃, δC): 20.1 (CH₃), 20.3 (CH₃), 25.9 (CH), 55.3 (OCH₃), 58.7 (OCH₃), 60.6 (OCH₃), 95.9 (C-3), 98.4 (C-9), 108.5 (C-3a), 114.0 (C-6), 115.5, 115.6 (C-5',4a), 123.0 (C-1'), 124.2 (C-6'), 125.9 (C-4), 130.0 (C-1a), 133.4 (C-1b), 141.5 (C-3'), 143.5 (C-5), 152.2 (C-8a), 153.0 (C-2'), 153.8 (C-4'), 154.7 (C-9a), 157.5 (C-2), 160.4 (C-7). UV (EtOH) λ_max (lge): 240 (4.25), 252 (4.14), 296 (3.82), 352 (3.49) nm. Anal. Calcd for C₂₅H₂₅N₂O₆: C, 71.41; H, 5.75; found C, 71.09; H, 5.68.

(Z)-3-[(3,4-Dihydroxysteryl)-2-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (10). Compound 10 (42 mg) was prepared from 3-[3,4-dihydroxyphenyl]ethyl]n-2-isopropyl-7H-furo[3,2-g]chromene-7-one (26, 100 mg, 0.27 mmol) using the procedure described for 3. Yield 42%, a yellow oil. IR (KBr, cm⁻¹): 3450, 3050, 2980, 1732, 1717, 1670, 1610, 1603, 1514, 1470, 1431, 1412, 1364, 1279, 1254, 1192, 1140, 1110, 1086, 1041, 990, 975, 928, 910, 860, 849, 820, 770, 754, 740, 648, 627, 601. ¹H-NMR (400 MHz, CDCl₃, δ₀H): 1.36 (d, J = 6.9 Hz, 3H, (CH₃), 1.37 (d, J = 6.9 Hz, 3H, (CH₃), 3.25 [m, 1H, CH-(CH₃)₂], 3.78 (br.s, 2H, OH), 6.30 (d, J = 9.8 Hz, 1H, H-6), 6.39 (d, J = 9.0 Hz, 1H, H-1a), 6.65 (d, J = 9.0 Hz, 1H, H-1b), 6.80 (d, J = 8.0 Hz, 1H, H-5'), 6.89 (s, 1H, H-9), 7.36 (d, J = 1.6 Hz, 1H, H-2'), 7.42 (dd, J = 8.0 and 1.6 Hz, 1H, H-6'), 7.79 (d, J = 9.8 Hz, 1H, H-5), 7.80 (s, 1H, H-4). ¹³C-NMR (100 MHz, CDCl₃, δC): 20.1 (CH₃), 20.3 (CH₃), 24.4 (CH), 96.0 (C-3), 98.4 (C-9), 106.6 (C-3a), 114.0 (C-6), 114.9, 115.1 (C-1',5'), 115.5 (C-2'), 117.2 (C-4a), 117.6 (C-6'), 125.6 (C-4), 130.9 (C-1a), 133.8 (C-1b), 143.5 (C-5), 146.5 (C-3'), 147.9 (C-4'), 152.2 (C-8a), 153.0 (C-9a), 156.4 (C-2), 160.3 (C-7). UV (EtOH) λ_max (lge): 252 (4.47), 287 (4.02), 350 (3.62) nm. Anal. Calcd for C₂₂H₁₉O₅: C, 72.92; H, 5.01; found C, 72.78; H, 4.88.

(Z)-3-[5-Formyl-3-hydroxy-3-methoxysteryl]-3-isopropyl-2H-furo[3,2-g]chromene-3,7-dione (11). Compound 11 (61 mg) was prepared from 3-[5-formyl-3-hydroxy-3-methoxyphenyl]ethyl]-2-isopropyl-7H-furo[3,2-g]chromene-7-one (27, 100 mg, 0.24 mmol) using the procedure described for 3. Yield 60%, m.p. 104–105 °C (ether). IR (KBr, cm⁻¹): 3435, 3063, 3042, 2960, 2925, 2853, 2808,
1H-NMR (400 MHz, CDCl3, δH): 1.43 (d, J = 6.9 Hz, 3H, CH3), 1.46 (d, J = 6.9 Hz, 3H, CH3), 3.34 [m, 1H, CH-(CH3)2], 3.98 (s, 3H, OCH3), 6.39 (d, J = 9.8 Hz, 1H, H-6), 6.41 (d, J = 1.8 Hz, 1H, H-6’), 6.48 (d, J = 8.8 Hz, 1H, H-1a), 6.82 (d, J = 8.8 Hz, 1H, H-1b), 7.05 (s, 1H, H-9), 7.10 (br.s, 1H, OH), 7.59 (d, J = 1.8 Hz, 1H, H-4’), 7.64 (s, 1H, H-4), 7.86 (d, J = 9.8 Hz, 1H, H-5), 10.03 (1H, s, CHO). 13C-NMR (100 MHz, CDCl3, δ): 20.1 (CH3), 20.3 (CH3), 25.8 (CH), 55.9 (OCH3), 99.1 (C-3), 104.1 (C-9), 115.4 (C-3a), 115.9 (C-6), 116.5 (C-4’), 117.1 (C-4a), 118.6 (C-1’), 121.4 (C-6’), 126.8 (C-4), 128.3 (C-1b), 132.1 (C-1a), 132.6 (C-5’), 143.4 (C-5), 149.9 (C-3’), 152.1 (C-8a), 152.9 (C-9a), 154.4 (C-2’), 156.8 (C-2), 160.3 (C-7), 188.2 (CHO). UV (EtOH) λmax (lge): 252 (4.26), 284 (3.65), 346 (3.53) nm. Anal. Caled for C24H20N2O5: C, 70.92; H, 4.88; found C, 71.28; H, 4.98.

(Z)-3-[(3,4-Dihydroxystryryl)-2-isopropyl-9-[(4-methylpiperazin-1-yl)methyl]-2H-furo[3,2-g]-chromene-3,7-dione (12). The compound 12 (49 mg) was prepared from 3-[(3,4-dihydroxyphenyl)ethynyl]-2-isopropyl-9-[(4-methylpiperazin-1-yl)methyl]-7H-furo[3,2-g]-chromene-7-one (28, 123 mg, 0.26 mmol) using the procedure described for 3. Yield 40%, a yellow oil. IR (KBr, ν, cm⁻¹): 3433, 3060, 3049, 2955, 2922, 2851, 1732, 1660, 1628, 1580, 1496, 1467, 1431, 1389, 1349, 1310, 1286, 1250, 1213, 1198, 1140, 1115, 1071, 1047, 988, 957, 905, 870, 820, 790, 768, 752, 736, 725, 708, 690, 675, 650. 1H-NMR (600 MHz, CDCl3, δH): 1.35 (d, J = 7.0 Hz, 3H, CH3), 1.37 (d, J = 7.0 Hz, 3H, CH3), 2.28 (s, 3H, CH3), 2.48 (m, 4H, H-3″,5″), 2.89 (m, 4H, H-2″,6″), 3.25 [m, 1H, CH-(CH3)2], 4.57 (d, J = 9.8 Hz, 1H, CH2), 4.62 (d, J = 9.8 Hz, 1H, CH2), 6.28 (d, J = 9.7 Hz, 1H, H-6), 6.37 (d, J = 9.0 Hz, 1H, H-1a), 6.77 (d, J = 8.3 Hz, 1H, H-5′), 6.95 (d, J = 9.0 Hz, 1H, H-1b), 7.38 (d, J = 1.8 Hz, 1H, H-2’), 7.49 (dd, J = 8.0 and 1.8 Hz, 1H, H-6′), 7.65 (d, J = 8.0 Hz, 1H, H-5′), 7.78 (s, 1H, H-4), 7.91 (d, 1H, J = 9.7 Hz, H-5), 8.17 (br.s, 2H, OH). 13C-NMR (100 MHz, CDCl3, δC): 19.9 (CH3), 20.2 (CH3), 25.6 (CH), 42.4 (CH3), 48.2 (CH2), 52.8 (C-3″,5″), 51.3 (C-2″,6″), 98.9 (C-3), 103.9 (C-9), 115.2 (C-3a), 116.3 (C-6), 117.0 (C-5′), 117.4 (C-4a), 120.0 (C-1′), 126.1 (C-2′), 127.8 (C-6′), 128.1 (C-4), 130.6, 131.6 (C-1a,1b), 143.2 (C-5′), 147.1 (C-3′), 148.1 (C-4′), 151.9 (C-9a), 152.7 (C-8a), 157.7 (C-2′), 160.0 (C-7). UV (EtOH) λmax (lge): 252 (4.42), 275(sh), 288 (3.91), 306 (3.84), 322 (sh), 354 (3.28) nm. Anal. Caled for C28H30N2O5: C, 70.87; H, 6.37; N, 5.90; found C, 71.02; H, 6.33; N, 5.81.

(Z)-2-(5-Formyl-3-hydroxy-3-methoxostryryl)-3-isopropyl-9-[(4-methylpiperazin-1-yl)methyl]-2H-furo[3,2-g]chromene-3,7-dione (13). Compound 13 (56 mg) was prepared from 3-[(5-formyl-3-hydroxy-3-methoxostryryl)ethynyl]-2-isopropyl-9-[(4-methylpiperazin-1-yl)methyl]-7H-furo[3,2-g]chromene-7-one (29, 133 mg, 0.26 mmol) using the procedure described for 3. Yield 42%, m.p. 100–102 °C (ether). IR (KBr, ν, cm⁻¹): 3402, 3200, 3061, 3049, 2968, 2954, 2922, 2874, 2818, 1732, 1705, 1628, 1580, 1510, 1497, 1466, 1431, 1410, 1389, 1348, 1300, 1287, 1250, 1214, 1198, 1140, 1115, 1070, 1047, 920, 920, 870, 820, 780, 752, 737, 720, 700, 660. 1H-NMR (400 MHz, CDCl3, δH): 1.34 (d, J = 6.9 Hz, 3H, (CH3), 1.39 (d, J = 6.9 Hz, 3H, (CH3), 2.28 (s, 3H, CH3), 2.41 (m, 4H, H-3″,5″), 2.65 (m, 4H, H-2″,6″), 3.24 [m, 1H, CH-(CH3)2], 4.03 [s, 3H, OCH3], 4.48 (d, J = 9.8 Hz, 1H, CH2), 4.52 (d, J = 9.8 Hz, 1H, CH2), 6.30 (d, J = 9.8 Hz, 1H, H-6), 6.38 (d, J = 9.1 Hz, 1H, H-1a), 6.45 (d, J = 1.8 Hz, 1H, H-6′), 6.95 (d, J = 9.1 Hz, 1H, H-1b), 7.48 (d, J = 1.8 Hz, 1H, H-4′), 7.68 (s, 1H, H-4), 7.77 (d, J = 9.8 Hz, 1H, H-5), 9.95 (br.s, 1H, CHO). 13C-NMR (100 MHz, CDCl3,
δC): 19.9 (CH3), 20.1 (CH3), 25.8 (CH), 42.5 (CH3), 48.3 (CH2), 51.4 (C-2",6"), 52.8 (C-3",5"), 58.3 (OCH3), 102.6 (C-3), 104.0 (C-9), 115.4 (C-3a), 115.8 (C-6), 116.4 (C-4a), 118.8 (C-4'), 119.9 (C-1'), 120.0 (C-6'),125.2 (C-4'), 128.6 (C-1a),131.9 (C-1b), 133.1 (C-5'), 145.1 (C-5), 145.6 (C-3'), 151.3 (C-8a), 152.0 (C-9a), 154.6 (C-2'), 156.8 (C-2), 158.9 (C-7), 189.2 (CHO). UV (EtOH) λmax (lgε): 252 (4.59), 290 (3.82), 336 (sh), 353(3.42) nm. Anal. Calcd for C30H32N2O6: C, 69.75; H, 6.24; N, 5.42; found C, 69.52; H, 6.38; N, 5.35.

2-Isopropyl-3-[(trimethylsilyl)ethynyl]-7H-furo[3,2-g]chromen-7-one (31). To a solution of oreoselone triflake 23 (200 mg, 0.55 mmol) and (trimethylsilyl)acetylene (30, 73 mg, 0.75 mmol) in benzene (5 mL) was added CuI (1.5 mg, 2 mol %), Pd(PPh3)2Cl2 (11 mg, 4 mol %), and Et3N (0.076 mL, 0.55 mmol; 1.1 equiv) under argon. The reaction mixture was stirred at 80 °C for 10 h (TLC). The mixture was cooled, and 5 mL of water was added. The separated water layer was extracted with methylene chloride (5 × 4 mL). The combined organic extracts was washed with water, dried over MgSO4, filtered, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel. Eluting with chloroform and crystallization from diethyl ether gave 90 mg (58%) of compound 31, m.p. 94–96 °C. IR (KBr, ν, cm⁻¹): 3435, 2962, 2925, 2152, 1730, 1625, 1577, 1485, 1431, 1388, 1355, 1284, 1249, 1211, 1197, 1139, 1101, 1068, 1047, 914, 869, 754, 688. 1H-NMR (600 MHz, CDCl3, δH): 0.16 [9H, s, (CH3)2Si], 1.35 (d, J = 7 Hz, 3H, CH3), 1.38 (d, J = 7 Hz, 3H, CH3), 3.25 [1H, m, CH(CH3)2], 6.40 (d, J = 9.6 Hz, 1H, H-6), 7.40 (s, 1H, H-9), 7.57 (s, 1H, H-4), 7.79 (d, J = 9.6 Hz, 1H, H-5). 13C-NMR (150 MHz, CDCl3, δC): 9.5 (3×CH3), 20.1 (CH3), 20.3 (CH3), 27.3 (CH), 89.2 (C-1a), 94.8 (C-1b), 96.2 (C-3), 98.6 (C-9), 106.9 (C-3a), 114.3 (C-6), 115.7 (C-4a), 115.8 (C-4), 116.8 (C-3), 142.7 (C-5), 152.4 (C-8a), 153.2 (C-9a), 159.0 (C-2), 160.8 (C-7). UV (EtOH) λmax (lgε): 222 (4.36), 251 (4.07), 294 (2.74), 338 (2.64) nm. Anal. Calcd for C19H20O3Si: C, 70.34; H, 6.21; Si 8.66; found C, 69.98; H, 5.99; Si, 8.35.

3-Ethynyl-2-isopropyl-7H-furo[3,2-g]chromen-7-one (32). To a solution of compound 31 (100 mg, 0.3 mmol) in methanol (5 mL) were added CsF (230 mg, 1.5 mmol) and benzyltrimethylammonium chloride (28 mg, 0.15 mmol). The mixture was stirred at rt for 10 h in under argon (TLC). Then 10 mL of water was added and the mixture was extracted with methylene chloride (5 × 4 mL). The combined extract was washed with water, dried over MgSO4, filtered, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel. Eluting with chloroform and crystallization from diethyl ether gave compound 32 (50 mg, 66%). M.p. 82–83 °C (ether). IR (KBr, ν, cm⁻¹): 3435, 3059, 2979, 2935, 2877, 2185, 1732, 1685, 1625, 1577, 1471, 1433, 1388, 1321, 1286, 1249, 1211, 1197, 1137, 1116, 1047, 869, 819, 721, 694. 1H-NMR (400 MHz, CDCl3, δH): 1.25 (d, J = 7 Hz, 3H, CH3), 1.29 (d,3H, J = 7 Hz, CH3), 2.37 (s, 1H, =CH), 3.19 [m, 1H, CH(CH3)2], 6.32 (d, J = 9.6 Hz, 1H, H-6), 7.33 (s, 1H, H-9), 7.50 (s, 1H, H-4), 7.71 (d, J = 9.6 Hz, 1H, H-5). 13C-NMR (100 MHz, CDCl3, δC): 20.2 (CH3), 20.4 (CH3), 25.8 (CH), 80.5 (C-1a), 87.6 (C-1b), 96.1 (C-3), 100.4 (C-9), 107.3 (C-3a), 115.6 (C-6), 116.0 (C-4a), 116.4 (C-4), 116.6 (C-3), 143.5 (C-5), 152.2 (C-8a), 153.1 (C-9a), 156.9 (C-2), 160.2 (C-7). UV (EtOH) λmax (lgε): 224 (3.91), 250 (3.94), 285 (sh), 335 (3.34) nm. Anal. Calcd for C16H12O2: C, 76.18; H, 4.79; found C, 76.31; H, 5.09.
3.3. Cell Culture and Cytotoxicity Assay

The human cancer cells of the MT-4, CEM-13 (the cells of T-cellular human leucosis), and U-937 (human monocytes) were used in this study. The cells were cultured in the RPMI-1640 medium that contained 10% embryonic calf serum, L-glutamine (2 mmol/L), gentamicin (80 lg/mL), and lincomycin (30 mg/mL) in a CO2 incubator at 37 °C. The tested compounds were dissolved in DMSO and added to the cellular culture at the required concentrations. Three wells were used for each concentration. The cells which were incubated without the compounds were used as a control. Cells were placed on 96-well microliter plates and cultivated at 37 °C in 5% CO2/95% air for 72 h. The cell viability was assessed through an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide] conversion assay. 1% MTT was added to each well. Four hours later DMSO was added and mixed for 15 min. Optical density (D) of the samples was measured on a BioRad 680 spectrophotometer Microplate Reader (BioRad, Hercules, CA, USA) at the wavelength of 570 nm. The 50% cytotoxic dose (CTD50) of each compound (i.e., the compound concentration that causes the death of 50% of cells in a culture, or decreases the optical density twice as compared to the control wells) was calculated from the data obtained. Statistical processing of the results was performed using the Microsoft Excel-2007, STATISTICA 6.0, and GraphPad Prism 5.0 programs. The results are given as an average value ± a deviation from the average. Reliability of differences (p) was estimated using the Student t test. The differences with p < 0.05 were considered as reliable. The experimental results are given as the data average values obtained from three independently conducted experiments.

4. Conclusions

A series of original furocoumarin derivatives having 2-(Z)- or 3-(Z)-styryl substitution in their structures have been synthesized. The cytotoxic activity of the resulting compounds against several cancer lines have been determined in the conventional MTT assay. The cytotoxicity data of compounds 2–13 demonstrate that they exhibit anticancer activity in micromolar range. Structure-activity comparison provides evidence that a 2-(Z)-styryl substitution in the furocoumarin scaffold is preferred for cytotoxicity over the subsequent 3-(Z)-styryl substitution; the (4-methylpiperazin-1-ylmethyl) substitution in the 9-position of 3-styrylfurocoumarins increases the cytotoxic activity in MT-4 and U-937 cell lines. The biological results for the furocoumarin analogs of CA-4 1, reported herein, shown that the structural modification of furocoumarins with the introduction of (Z)-styryl moiety may prove of great importance to obtain cytotoxic anti-cancer agents.

Acknowledgments

This investigation was supported in part by the Russian Federation Basic Research (projects 12-03-92200 and 11-03-00242) and the Grants Council of the president of the Russian Federation (grants NS-3986.2012.3 and NS-2625.2014.3).

Author Contributions

The contributions of the respective authors are as follows: A. Lipeeva performed synthesis, identification, and structure elucidation of the compounds, and prepared the manuscript. M.M.
Shakirov contributed to checking and confirming the procedures of the structural identification, especially interpretation of the NMR spectra. M.A. Pokrovsky and A.G. Pokrovsky contributed to the cytotoxicity experiments. This study was performed based on the planning of E. Shults, the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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*Sample Availability:* Samples of the compounds reported in this paper are available from the authors.

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