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Original article

Synthesis and anticancer activity of a novel class of flavonoids: 2,4-Diarylchromane[4,3-*d*]-$\Delta^{1,9b}$-1,2,3-thiadiazolines

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Abstract

A series of 2,4-diarylchromane[4,3-*d*]-$\Delta^{1,9b}$-1,2,3-thiadiazolines have been synthesized by cyclization of corresponding 2-arylchroman-4-one-arylhydrazones with $SOCl_2$ then treated with alcohol. All the compounds have been tested for their antiproliferative activity in vitro against six human tumor cell lines, and the highly potent derivative **11a** exhibited in vivo inhibitory effect on tumor growth. Mechanism research indicated that it is due to **11a** that induces DNA fragmentation.

Keywords: 2,4-Diarylchromane[4,3-*d*]-$\Delta^{1,9b}$-1,2,3-thiadiazolines; Synthesis; Antitumor activity

1. Introduction

Flavonoids are very well known family possessing a broad range of pharmacological properties, including antihepatotoxic, antitumor, anti-inflammatory and antiviral properties [1]. In antitumor area, flavonoids can inhibit the metabolism of the benze[α]pyrene by hamster embryo cells in tissue culture [2] and markedly augment the cytotoxicity of TNF (tumor necrosis factor-α) [3]. Flavonoids are also found to have the ability for inhibiting tyrosinase and aromatase [4,5], estradiol-induced DNA synthesis [6] and inducing apoptosis [7]. Furthermore, most of the flavonoids displayed enhanced bioactivity after structure modification [8,9]. On the other hand, many compounds containing 1,2,3-thiadiazole and 1,2,4-thiadiazoline moieties were reported for their antitumor activity in recent years [10]. But there were few literatures reported that the bioactivity of 1,2,3-thiadiazoline derivatives. This prompted us to design and synthesis a series of new flavonoids with 1,2,3-thiadiazoline ring and studied their bioactivity. In this paper, we describe the synthesis of the 2,4-diarylchromane[4,3-*d*]-$\Delta^{1,9b}$-1,2,3-thiadiazolines and their antiproliferative activity in vitro against various human cancer cell lines, and we also reported the antitumor activity of **11a** in ICR mice bearing sarcoma 180 cells.

2. Results and discussions

2.1. Chemistry

2.1.1. Synthesis of derivatives

The method utilized for the synthesis of 2,4-diarylchromane[4,3-*d*]-$\Delta^{1,9b}$-1,2,3-thiadiazolines **10**–**11** is outlined in Scheme 1. The arylketones **1** and arylaldehydes **2** were commercially available and the chalones **3** and flavonones **4** were easily prepared by following the previously reported methods in high yield [11]. The obtained flavonones **4** were reacted with arylhydrazines **5** in ethanol at reflux conditions to give flavanone-4-arylhydrazones **6** or **7** in 83–96% yields. We tried to prepare target compounds by following Mohamed's method [12]. Compounds **6** or **7** were treated with thionyl chloride at 20 °C for 10 h, however, no products were obtained. Maybe the materials

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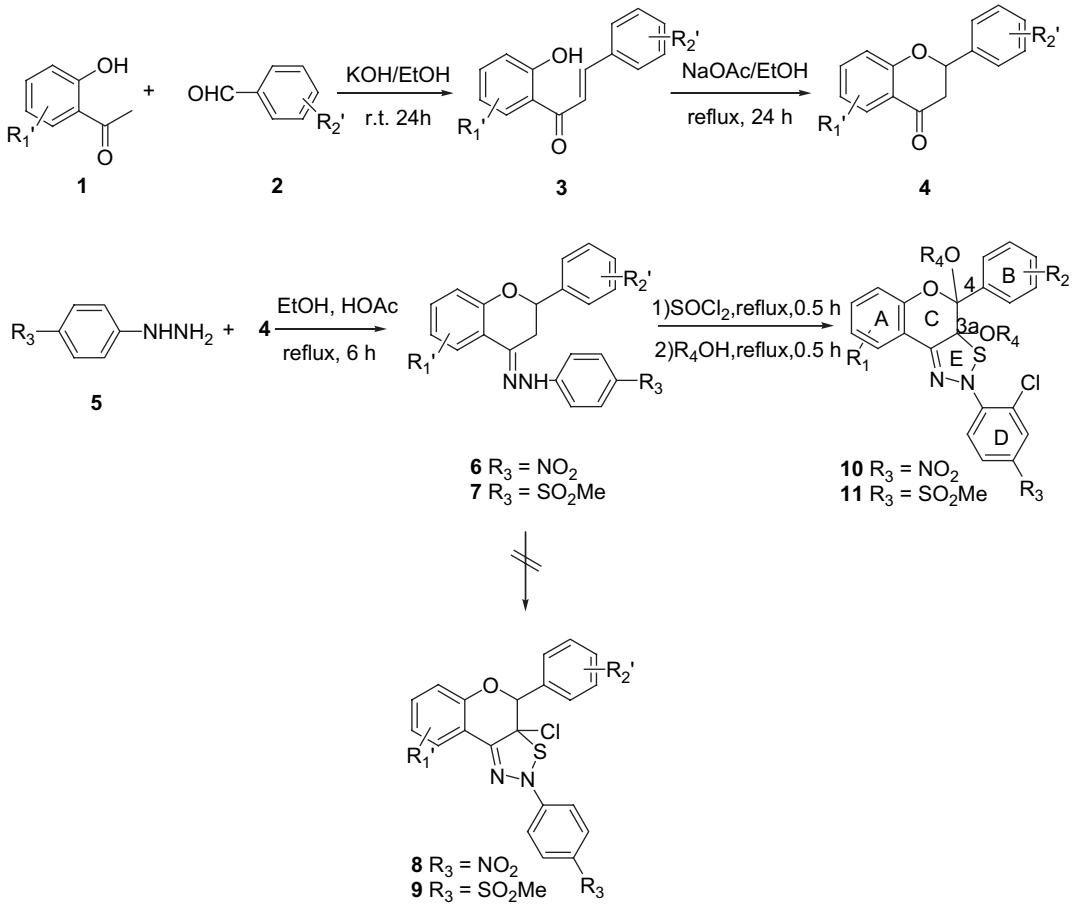


Scheme 1.

were polymerized and the byproducts were polymers. Different reaction conditions were tried; at last, we found that the compounds **10** or **11** were obtained in 37–76% yield via condensation of the corresponding hydrazones **6** or **7** in excess $SOCl_2$ under N_2 for 0.5 h, followed by refluxing in alcohol for 0.5 h. It is unexpected that all the products **10** and **11** were substituted with alkoxyl at 3a- and 4-positions, and chlorine at 2″-position. Besides that, as is listed in Table 1, some of the products were substituted with chlorine at 6-position and 2′-position: (1) when the materials with 5,7-dimethoxyl at A-ring (**6c–f** and **7d**), the products (**10c–f**, **10k** and **11d**) have a chlorine at the 6-position; (2) when the materials with 3′,4′-dioxmethylene (**6h**) or 3′,4′-dimethoxyl (**6i**) at B-ring, the products (**10h** and **10i**) were substituted with chlorine at 2′-position (Table 1).

The other desired compounds **12** were obtained by reduction of **10** with Raney-Ni and H_2 followed by acetylation with acetic anhydride in the presence of pyridine (Scheme 2). All the new compounds synthesized were characterized by ^{1}H NMR, ESI-MS and IR spectroscopy, and the structure of **10c** was confirmed by X-ray analysis [13].

2.2. Biological activities

All the synthesized 2,4-diarylchromane[4,3-*d*]-$\Delta^{1,9b}$-1,2,3-thiadiazolines were tested for antiproliferative activities in vitro against several tumor cell lines including Bel-72, ECA-109, PC-3, MCF-7, HL-60, and A-549 cells. 2-Phenylchroman-4-one, a kind of flavonone, was tested together as a positive control in the assays. The results are summarized in Table 2.

As is demonstrated in Table 2, compounds **11a** and **11e** exhibited the most potent antiproliferative activity against all the tested cell lines. Some general conclusions that can be drawn from the results, based more on the consistency of effect than on statistical significance, would be as follows: (1) the compounds without substituent at A-ring (**10a–b**, **11a**, **11c** and **12a–b**) showed similar antiproliferative activity, and all these compounds displayed more effective activity than 2-phenylchroman-4-one. (2) The effect of compounds **10a–j**, **11b** and **11e**, which have methyl at 8 position, is not uniform. Compounds **10g–j** were almost inactive, however, **11b** and **11e** showed high activity. In this series, methylsulfonyl group at D-ring was important. (3) The compounds **10c–f** and **10k** with tri-substituent at A-ring showed low activity. (4) The compounds with *para* substituent at B-ring were more effective than those with *ortho* substituent or tri-substituent. *p*-Methyl group substituted at B-ring might be the most beneficial (cf. **10e**, **10f** and **10c**). The compounds with tri-substituted at B-ring (**10h** and **10i**) or *ortho* substituent at B-ring (**10d**) almost lost their antiproliferative activity. (5)

Table 1
The structure of hydrazones and products

Compound	Substituent		Compound	Substituent		
	R1′	R2′		R1	R2	R4
6a	H	H	**10a**	H	4′-Cl	Et
6b	H	4′-MeO	**10b**	H	4′-MeO	Et
6c	5,7-diMeO	H	**10c**	6-Cl-7,9-diMeO	4′-Cl	Et
6d	5,7-diMeO	2′-Cl	**10d**	6-Cl-7,9-diMeO	2′-Cl	Et
6e	5,7-diMeO	4′-Me	**10e**	6-Cl-7,9-diMeO	4′-Me	Et
6f	5,7-diMeO	4′-MeO	**10f**	6-Cl-7,9-diMeO	4′-MeO	Et
6g	6-Me	H	**10g**	8-Me	4′-Cl	Et
6h	6-Me	3′,4′-OCH_2O–	**10h**	8-Me	2′-Cl-4′,5′-OCH_2O-	Et
6i	6-Me	3′,4′-diMeO	**10i**	8-Me	2′-Cl-4′,5′-diMeO	Et
6j	6-Me	4′-MeO	**10j**	8-Me	4′-MeO	Et
6c	5,7-diMeO	H	**10k**	6-Cl-7,9-diMeO	4′-Cl	Me
7a	H	H	**11a**	H	4′-Cl	Et
7b	6-Me	4′-MeO	**11b**	8-Me	4′-MeO	Et
7c	H	4′-MeO	**11c**	H	4′-MeO	Et
7d	5,7-diMeO	H	**11d**	6-Cl-7,9-diMeO	4′-Cl	Et
7e	6-Me	H	**11e**	8-Me	4′-Cl	Et
			12a	H	4′-Cl	Et
			12b	8-Me	4′-Cl	Et

The compound with dimethoxyl at 3a- and 4-positions (**10c**) might be more active than that with diethoxyl (**10k**).

Because compound **11a** have highly antiproliferative activity in vitro, we tested its antiproliferative activities on S180 tumor bearing mice, Cyclophosphamide (CTX), a well-known anticancer agent, was tested together as positive control (Table 3).

Since apoptosis is an important response to most chemotherapeutic agents [14,15], we evaluated the role of these compounds in inducing apoptosis. To confirm whether cell death was caused by apoptotic processes, we assessed the DNA fragmentation in the cells exposed to **11a** (0.5 μg/mL, 1.0 μg/mL, 2.0 μg/mL) for 24 h. DNA ladder on an agarose gel was observed in **11a** (Fig. 1). By flow cytometry, the percentages of apoptotic cells were 3.9, 25.3 and 68.3 after treatment with **11a** (0.5, 1.0, 2.0 μg/mL, respectively) for 48 hours (Fig. 2). The induction of DNA ladder and the increased sub-G0/G1 contents indicate that **11a** is able to induce apoptosis in HL-60 cells.

3. Conclusion

In conclusion, we synthesized a series of new flavonoids, 2,4-diarylchromane[4,3-*d*]-$\Delta^{1,9b}$-1,2,3-thiadiazolines, and studied their ability of antiproliferative activity. Most of these compounds had antiproliferative activity in vitro and compound **11a** showed in vitro as well as in vivo the inhibitory effect on tumor growth. Further mechanism research indicated that compound **11a** has the abilities to induce apoptosis in HL-60 cells. It provided an available approach to develop a novel class of flavonoids with potential as anticancer drugs.

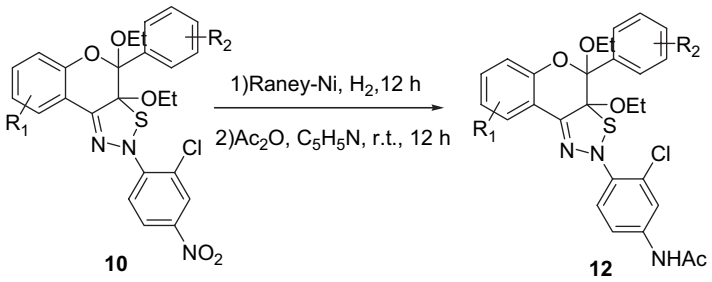
4. Experimental protocols

Melting points were not corrected and were recorded on a Buchi apparatus. IR spectra, KBr pellets, 400–4000 cm^{-1}, were recorded on a Bruker VECTOR 22 FTIR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 400 instrument at 400 and 500 MHz (chemical shifts are expressed as δ values relative to TMS as internal standard). Mass spectra (MS), ESI (positive) were recorded on an Esquire-LC-00075 spectrometer.

4.1. Synthesis

*4.1.1. General procedure for the preparation of flavanone-4-arylhydrazones **6** and **7***

An equimolar of flavonones and arylhydrozine (5 mmol) in ethanol (50 mL) and acetic acid (0.5 mL) was heated under



Scheme 2.

Table 2
Antiproliferative activities of 2,4-diarylchromane[4,3-*d*]-$\Delta^{1,9b}$-1,2,3-thiadiazolines

Compound	Cell line (IC_{50}, μg/ml)[a]					
	Bel-7402	ECA-109	PC-3	MCF-7	HL-60	A-549
10a	11.8 (±0.7)	6.9 (±0.7)	4.1 (±0.4)	15.0 (±0.6)	2.2 (±2.6)	3.8 (±4.4)
10b	8.2 (±0.3)	9.6 (±0.6)	5.7 (±0.9)	23.3 (±1.8)	3.3 (±3.4)	5.1 (±6.4)
10c	>50	>50	15.8 (±3.3)	>25	6.7 (±0.1)	>50
10d	>50	>50	>50	>25	>25	>50
10e	>50	7.3 (±1.0)	7.3 (±1.0)	>25	16.8 (±0.4)	>50
10f	>50	>50	19.2 (±13.1)	>25	23.2 (±0.04)	17.7 (±2.0)
10g	18.6 (±5.9)	14.8 (±0.9)	>50	>25	>25	7.8 (±9.8)
10h	>50	>50	>50	>25	>25	>50
10i	>50	>50	>50	>25	>25	>50
10j	21.8 (±1.3)	>50	>50	>25	>25	>50
10k	>50	5.7 (±5.1)	2.9 (±3.5)	5.5 (±0.1)	3.2 (±0.4)	5.4 (±0.5)
11a	10.3 (±0.3)	5.9 (±4.0)	3.1 (±2.5)	4.1 (±0.2)	2.8 (±0.4)	4.9 (±1.4)
11b	40.4 (±13.9)	1.5 (±0.7)	0.6 (±0.3)	>25	0.6 (±0.4)	1.1 (±0.6)
11c	23.5 (±17.5)	5.9 (±0.7)	3.5 (±0.2)	>25	1.8 (±2.3)	3.3 (±3.6)
11d	24.8 (±0.4)	20.1 (±8.9)	>50	12.6 (±2.5)	5.6 (±0.3)	14.5 (±7.9)
11e	7.3 (±0.9)	11.5 (±9.1)	6.0 (±6.0)	2.5 (±1.2)	6.0 (±0.03)	10.3 (±1.7)
12a	21.3 (±0.3)	9.6 (±0.7)	4.1 (±0.4)	23.3 (±1.8)	2.2 (±2.9)	4.2 (±4.4)
12b	10.5 (±0.6)	4.1 (±1.0)	2.4 (±0.2)	19.8 (±1.2)	1.3 (±1.5)	2.5 (±2.2)
2-Phenylchroman-4-one	>50	27.7 (±0.7)	16.6 (±3.4)	>25	3.4 (±10.0)	14.2 (±19.0)

[a] Values are means of three experiments, standard deviation is given in parentheses.

reflux for 6 h. After cooling to r.t., the product **6** or **7** that precipitated was separated by suction and washed with ethanol.

*4.1.1.1. 2-Phenylchroman-4-one-(4-nitrophenyl)hydrozone **6a**.* Yield: 87%, m.p. 249–250 °C. ESI-MS: $m/z = 360$ [M + 1]. IR (KBr): 3310, 1601 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.77–2.84 (m, 1H, 3-H), 3.35–3.40 (m, 1H, 3-H), 5.25 (dd, 1H, $J = 12.0$ Hz, 3.2 Hz, 2-H), 6.96 (d, 1H, $J = 8.0$ Hz, 8-H), 7.03 (t, 1H, $J = 8.0$ Hz, 7-H), 7.27 (t, 1H, $J = 8.0$ Hz, 6-H), 7.35 (m, 2H, 2′-H and 6′-H), 7.38 (d, 1H, $J = 7.2$ Hz, 4′-H), 7.44 (t, 2H, $J = 7.2$ Hz, 3′-H and 5′-H), 7.56 (d, 2H, $J = 8.0$ Hz, 2″-H and 6″-H), 8.07 (d, 1H, $J = 8.0$ Hz, 5-H), 8.11 (d, 2H, $J = 8.0$ Hz, 3″-H and 5″-H), 10.31 (s, 1H, NH).

*4.1.1.2. (4′-Methoxylphenyl)chroman-4-one-(4-nitrophenyl) hydrozone **6b**.* Yield: 93%, m.p. 237–239 °C. ESI-MS: $m/z =$ 390 [M + 1]. IR: 3299, 1597 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.80–2.87 (m, 1H, 3-H), 3.29–3.34 (m, 1H, 3-H), 3.75 (s, 3H, OCH_3), 5.18 (dd, 1H, $J = 12.0$ Hz, 2.8 Hz, 2-H), 6.93 (d, 1H, $J = 8.0$ Hz, 8-H), 6.98 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.27 (t, 1H, $J = 8.0$ Hz, 7-H), 7.32 (t, 1H, $J = 8.0$ Hz, 6-H), 7.47 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.56 (d, 2H, $J = 7.6$ Hz, 2″-H and 6″-H), 8.06 (d, 1H, $J = 7.6$ Hz, 5-H), 8.11 (d, 2H, $J = 7.6$ Hz, 3″-H and 5″-H), 10.31 (s, 1H, NH).

*4.1.1.3. 5,7-Dimethoxyl-2-phenylchroman-4-one-(4-nitrophenyl)hydrozone **6c**.* Yield: 89%, m.p. 214–216 °C. ESI-MS: $m/z = 420$ [M + 1]. IR (KBr): 3321, 1589 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.73–2.80 (m, 1H, 3-H), 3.30–3.35 (m, 1H, 3-H), 3.77 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.18 (dd, 1H, $J = 12.0$ Hz, 3.2 Hz, 2-H), 6.23 (d, 1H, $J = 2.4$ Hz, 8-H), 6.30 (d, 1H, $J = 2.4$ Hz, 6-H), 7.29 (d, 2H, $J = 2.4$ Hz, 2′-H and 6′-H), 7.39 (t, 1H, $J = 7.2$ Hz, 4′-H), 7.46 (t, 2H, $J = 7.2$ Hz, 3′-H and 5′-H), 7.56 (d, 2H, $J = 8.0$ Hz, 2″-H and 6″-H), 8.13 (d, 2H, $J = 8.0$ Hz, 3″-H and 5″-H), 10.12 (s, 1H, NH).

*4.1.1.4. 5,7-Dimethoxyl-2-(4-chlorophenyl)chroman-4-one-(4-nitrophenyl)hydrozone **6d**.* Yield: 85%, m.p. 185–187 °C. ESI-MS: $m/z = 454$ [M + 1]. IR (KBr): 3235, 1593 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.67–2.74 (m, 1H, 3-H), 3.31–3.36

Table 3
Effects of **11a** on S180 tumor bearing mice

Groups	No. of animals	Tumor weight (g)	Inhibition rate (%)
Control	20	1.21 (±0.33)	–
11a, 80 mg/kg	10	0.63 (±0.23)	48.2*
CTX, 50 mg/kg	10	0.66 (±0.20)	45.4*

*$P < 0.01$ versus control.

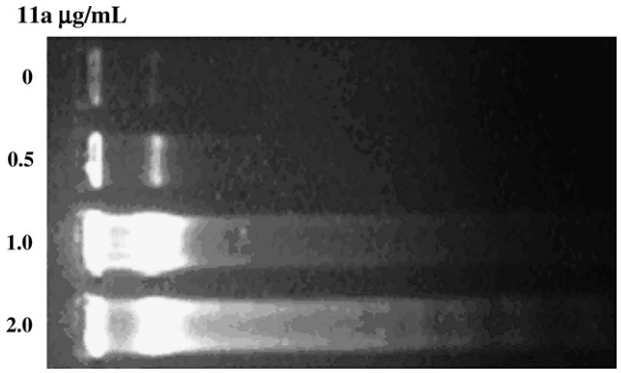


Fig. 1. DNA fragmentation induced by **11a** in HL-60 cells.

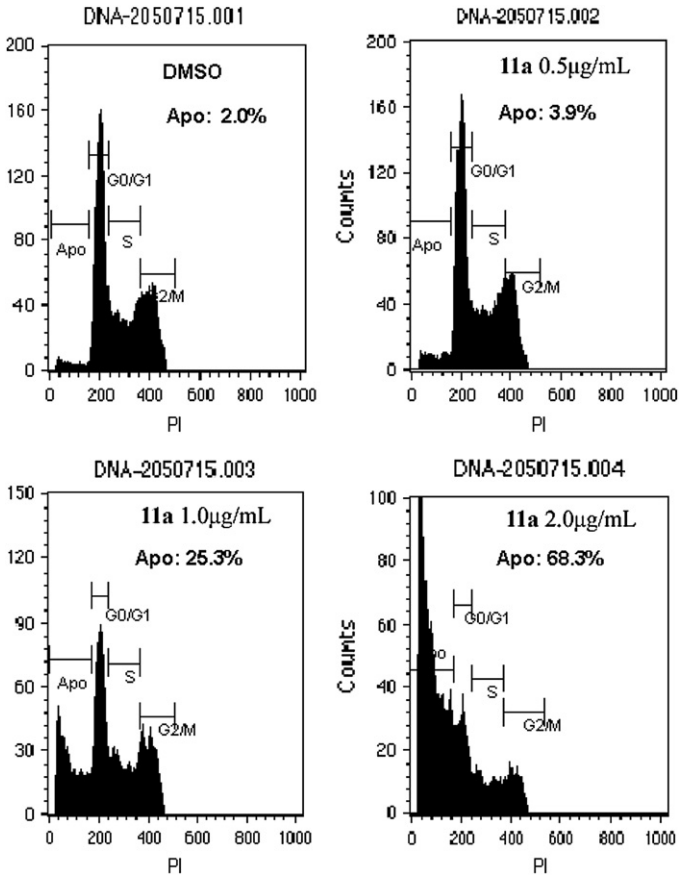


Fig. 2. Induction of apoptosis by **11a** in HL-60 cells.

(m, 1H, 3-H), 3.75 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.37 (dd, 1H, $J = 12.0$ Hz, 2.4 Hz, 2-H), 6.22 (d, 1H, $J = 2.4$ Hz, 8-H), 6.31 (d, 1H, $J = 2.4$ Hz, 6-H), 7.27 (brs, 2H, 4′-H and 6′-H), 7.40–7.54 (m, 3H, 5′-H, 2″-H and 6″-H), 7.74 (d, 1H, $J = 8.0$ Hz, 3′-H), 8.10 (d, 2H, $J = 8.8$ Hz, 3″-H and 5″-H), 10.10 (s, 1H, NH).

4.1.1.5. 5,7-Dimethoxyl-2-(4-methylphenyl)chroman-4-one-(4-nitrophenyl)hydrozone ***6e***. Yield: 90%, m.p. 208–210 °C. ESI-MS: $m/z = 434$ [M + 1]. IR (KBr): 3322, 1602 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 2.69–2.76 (m, 1H, 3-H), 3.23–3.28 (m, 1H, 3-H), 3.74 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 5.10 (dd, 1H, $J = 12.0$ Hz, 3.2 Hz, 2-H), 6.19 (d, $J = 2.0$ Hz, 8-H), 6.26 (d, $J = 2.0$ Hz, 6-H), 7.22–7.35 (m, 4H, 2′-H, 3′-H, 5′-H and 6′-H), 7.41 (d, 2H, $J = 8.8$ Hz, 2″-H and 6″-H), 8.10 (d, 2H, $J = 8.8$ Hz, 3″-H and 5″-H), 10.11 (s, 1H, NH).

4.1.1.6. 5,7-Dimethoxyl-2-(4-methoxylphenyl)chroman-4-one-(4-nitrophenyl)hydrozone ***6f***. Yield: 90%, m.p. 218–219 °C. ESI-MS: $m/z = 450$ [M + 1]. IR (KBr): 3314, 1602 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.72–2.79 (m, 1H, 3-H), 3.21–3.26 (m, 1H, 3-H), 3.73 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 5.08 (dd, 1H, $J = 12.0$ Hz, 2.8 Hz, 2-H), 6.18 (d, 1H, $J = 2.4$ Hz, 8-H), 6.24 (d, 1H, $J = 2.4$ Hz, 6-H), 6.97 (d, 2H, $J = 8.4$ Hz, 3′-H and 5′-H), 7.27 (brs, 2H, 2′-H and 6′-H), 7.45 (d, 2H, $J = 8.8$ Hz, 2″-H and 6″-H), 8.10 (d, 2H, $J = 8.8$ Hz, 3″-H and 5″-H), 10.12 (s, 1H, NH).

4.1.1.7. 6-Methyl-4-2-phenylchroman-4-one-(4-nitrophenyl)-hydrozone ***6g***. Yield: 85%, m.p. > 250 °C. ESI-MS: $m/z = 374$ [M + 1]. IR (KBr): 3307, 1591 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 2.76–2.83 (m, 1H, 3-H), 3.35–3.40 (m, 1H, 3-H), 5.22 (dd, $J = 12.0$ Hz, 2.4 Hz, 1H, 2-H), 6.88 (d, 1H, $J = 8.0$ Hz, 8-H), 7.11 (dd, 1H, $J = 8.0$, 1.2 Hz, 7-H), 7.35 (d, 2H, $J = 7.2$ Hz, 2′-H and 6′-H), 7.40 (t, 1H, $J = 7.2$ Hz, 4′-H), 7.45 (t, 2H, $J = 7.2$ Hz, 3′-H and 5′-H), 7.57 (d, 2H, $J = 7.6$ Hz, 2″-H and 6″-H), 7.89 (d, 1H, $J = 1.2$ Hz, 5-H), 8.14 (d, 2H, $J = 7.6$ Hz, 3″-H and 5″-H), 10.29 (s, 1H, NH).

4.1.1.8. 2-(Benzo[d][1,3]dioxol-5-yl)-6-methylchroman-4-one-(4-nitrophenyl)hydrozone ***6h***. Yield: 93%, m.p. > 250 °C. ESI-MS: $m/z = 418$ [M + 1]. IR (KBr): 3219, 1602 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.74–2.81 (m, 1H, 3-H), 3.27–3.32 (m, 1H, 3-H), 5.10 (dd, 1H, $J = 12.0$ Hz, 2.8 Hz, 2-H), 6.02 (s, 2H, CH_2), 6.83 (d, 1H, $J = 8.0$ Hz, 8-H), 6.94 (d, 1H, $J = 8.0$ Hz, 7-H), 7.01 (d, 1H, $J = 8.0$ Hz, 5′-H), 7.08 (d, 1H, $J = 8.0$ Hz, 6′-H), 7.14 (s, 1H, 2′-H), 7.33 (d, 2H, $J = 8.4$ Hz, 2″-H and 6″-H), 7.84 (s, 1H, 5-H), 8.12 (d, 2H, $J = 8.4$ Hz, 3″-H and 5″-H), 10.26 (s, 1H, NH).

4.1.1.9. 2-(3,4-Dimethoxyphenyl)-6-methylchroman-4-one-(4-nitrophenyl)hydrozone ***6i***. Yield: 90%, m.p. > 250 °C. ESI-MS: $m/z = 434$ [M + 1]. IR (KBr): 3317, 1595 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.82–2.89 (m, 1H, 3-H), 3.27–3.33 (m, 1H, 3-H), 3.74 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 5.10 (dd, 1H, $J = 10.0$ Hz, 2.4 Hz, 2-H), 6.84 (d, 1H, $J = 8.4$ Hz, 8-H), 6.97 (d, 1H, $J = 8.4$ Hz, 7-H), 7.04–7.09 (m, 2H, 5′-H and 6′-H), 7.16 (s, 1H, 2′-H), 7.33 (d, 2H, $J = 8.8$ Hz, 2″-H and 6″-H), 7.85 (s, 1H, 5-H), 8.11 (d, 2H, $J = 8.8$ Hz, 3″-H and 5″-H), 10.27 (s, 1H, NH).

4.1.1.10. 2-(4-Methoxyphenyl)-6-methylchroman-4-one-(4-nitrophenyl)hydrozone ***6j***. Yield: 89%, m.p. > 250 °C. ESI-MS: $m/z = 404$ [M + 1]. IR (KBr): 3315, 1604 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 2.78–2.85 (m, 1H, 3-H), 3.29–3.34 (m, 1H, 3-H), 3.78 (s, 3H, OCH_3), 5.15 (dd, 1H, $J = 12.0$ Hz, 2.4 Hz, 2-H), 6.85 (d, 1H, $J = 8.4$ Hz, 8-H), 7.00 (d, 2H, $J = 8.0$ Hz, 3′-H and 5′-H), 7.10 (dd, 1H, $J = 8.4$ Hz, 1.6 Hz, 7-H), 7.35 (d, 2H, $J = 8.0$ Hz, 2′-H and 6′-H), 7.49 (d, 2H, $J = 8.8$ Hz, 2″-H and 6″-H), 7.88 (d, 1H, $J = 1.6$ Hz, 5-H), 8.14 (d, 2H, $J = 8.8$ Hz, 3″-H and 5″-H), 10.30 (s, 1H, NH).

4.1.1.11. 2-Phenylchroman-4-one-[4-(methylsufonyl)phenyl]-hydrozone ***7a***. Yield: 92%, m.p. > 250 °C. ESI-MS: $m/z = 393$ [M + 1]. IR (KBr): 3319, 1597 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.73–2.80 (m, 1H, 3-H), 3.08 (s, 3H, SO_2CH_3), 3.29–3.34 (m, 1H, 3-H), 5.23 (dd, 1H, $J = 12.0$ Hz, 2.8 Hz, 2-H), 6.95 (d, 1H, $J = 8.0$ Hz, 8-H), 7.02 (t, 1H, $J = 8.0$ Hz,

7-H), 7.25 (t, 1H, $J = 8.0$ Hz, 6-H), 7.34–7.45 (m, 5H, 2′-H, 3′-H, 4′-H, 5′-H and 6′-H), 7.56 (d, 2H, $J = 8.8$ Hz, 2″-H and 6″-H), 7.70 (d, 2H, $J = 8.8$ Hz, 3″-H and 5″-H), 8.05 (dd, 1H, $J = 8.0$ Hz, 2.0 Hz, 5-H), 9.99 (s, 1H, NH).

4.1.1.12. 2-(4-Methoxyphenyl)-6-methylchroman-4-one-[4-(methylsufonyl)phenyl]hydrozone ***7b***. Yield: 93%, m.p. > 250 °C. ESI-MS: $m/z = 437$ [M + 1]. IR (KBr): 3307, 1600 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 2.75–2.82 (m, 1H, 3-H), 3.11 (s, 3H, SO_2CH_3), 3.27–3.32 (m, 1H, 3-H), 3.78 (s, 3H, OCH_3), 5.13 (dd, 1H, $J = 12.0$ Hz, 2.4 Hz, 2-H), 6.84 (d, 1H, $J = 8.4$ Hz, 8-H), 7.00 (d, 2H, $J = 8.0$ Hz, 2′-H and 6′-H), 7.07 (dd, 1H, $J = 8.4$ Hz, 1.6 Hz, 7-H), 7.38 (d, 2H, $J = 8.4$ Hz, 2″-H and 6″-H), 7.49 (d, 2H, $J = 8.0$ Hz, 3′-H and 5′-H), 7.73 (d, 2H, $J = 8.4$ Hz, 3″-H and 5″-H), 7.86 (d, 1H, $J = 1.6$ Hz, 5-H), 9.97 (s, 1H, NH).

4.1.1.13. 2-(4-Methoxyphenyl)chroman-4-one-[4-(methylsufonyl)phenyl]hydrozone ***7c***. Yield: 93%, m.p. > 250 °C. ESI-MS: $m/z = 423$ [M + 1]. IR (KBr): 3307, 1600 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.76–2.83 (m, 1H, 3-H), 3.08 (s, 3H, SO_2CH_3), 3.28–3.33 (m, 1H, 3-H), 3.75 (s, 3H, OCH_3), 5.16 (dd, 1H, $J = 12.0$ Hz, 3.2 Hz, 2-H), 6.92 (d, 1H, $J = 8.0$ Hz, 8-H), 6.98 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.00 (t, 1H, $J = 8.0$ Hz, 7-H), 7.24 (t, 1H, $J = 8.0$ Hz, 6-H), 7.35 (d, 2H, $J = 8.8$ Hz, 2″-H and 6″-H), 7.47 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.70 (d, 2H, $J = 8.4$ Hz, 3″-H and 5″-H), 8.05 (d, 1H, $J = 8.0$ Hz, 5-H), 9.98 (s, 1H, NH).

4.1.1.14. 5,7-Dimethoxy-2-phenylchroman-4-one-[4-(methylsufonyl)phenyl]hydrozone ***7d***. Yield: 85%, m.p. 225–227 °C. ESI-MS: $m/z = 453$ [M + 1]. IR (KBr): 3317, 1625 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.74–2.81 (m, 1H, 3-H), 3.04 (s, 3H, SO_2CH_3), 3.25–3.30 (m, 1H, 3-H), 3.71 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 5.49 (dd, 1H, $J = 10.0$ Hz, 2.8 Hz, 2-H), 6.13 (d, 1H, $J = 2.0$ Hz, 6-H), 6.20 (d, 1H, $J = 2.0$ Hz, 8-H), 6.93 (d, 2H, $J = 8.8$ Hz, 2″-H and 6″-H), 7.23–7.39 (m, 5H, 2′-H, 3′-H, 4′-H, 5′-H and 6′-H), 7.65 (d, 2H, $J = 8.8$ Hz, 3″-H and 5″-H), 11.42 (s, 1H, NH).

4.1.1.15. 6-Methyl-2-phenylchroman-4-one-[4-(methylsufonyl)phenyl]hydrozone ***7e***. Yield: 86%, m.p. > 250 °C. ESI-MS: $m/z = 407$ [M + 1]. IR (KBr): 3323, 1599 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.70–2.77 (m, 1H, 3-H), 3.08 (s, 3H, SO_2CH_3), 3.31–3.36 (m, 1H, 3-H), 5.18 (dd, $J = 12.0$ Hz, 2.8 Hz, 2-H), 6.84 (d, 1H, $J = 8.4$ Hz, 8-H), 6.93 (d, 2H, $J = 8.8$ Hz, 2″-H and 6″-H), 7.06 (d, 1H, $J = 8.4$ Hz, 7-H), 7.36 (t, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.43 (t, 1H, $J = 8.4$ Hz, 4′-H), 7.54 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.70 (d, 2H, $J = 8.8$ Hz, 3″-H and 5″-H), 7.84 (s, 1H, 5-H), 9.94 (s, 1H, NH).

4.1.2. General procedure for the preparation of 2,4-diarylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazolines ***10*** *and* ***11***

A mixture of the 2-arylchroman-4-one-arylhydrazone (5 mmol) and $SOCl_2$ (4 mL) was refluxed for 30 min under nitrogen atmosphere. The excess $SOCl_2$ was evaporated under reduced pressure. Then alcohol (4 mL) was added into the residue and refluxed for 30 min. The reaction mixture was diluted with water (10 mL) and extracted thoroughly with CH_2Cl_2 (5 mL × 3). Then the CH_2Cl_2 layer was washed successively with brine (5 mL × 2), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Usual workup afforded the crude product which was further purified by chromatogram.

4.1.2.1. (3aRS,4RS)2-(2-Chloro-4-nitrophenyl)-3a,4-diethoxyl-4-(4-chlorophenyl)chromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10a***. Yield: 58%, m.p. 192–194 °C. ESI-MS: $m/z = 545$ [M + 1]. IR (KBr): 2982, 1609 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.80 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 0.88 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.86–2.90 (m, 1H, OCH_2CH_3), 3.08–3.12 (m, 1H, OCH_2CH_3), 3.26–3.30 (m, 1H, OCH_2CH_3), 3.37–3.41 (m, 1H, OCH_2CH_3), 7.24–7.31 (m, 2H, 7-H and 8-H), 7.57 (d, 3H, $J = 8.8$ Hz, 6-H, 2′-H and 6′-H), 7.65 (d, 2H, $J = 8.8$ Hz, 3′-H and H-5′), 7.74 (d, 1H, $J = 9.2$ Hz, 6″-H), 8.09 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz, 9-H), 8.21 (dd, 1H, $J = 9.2$ Hz, 2.8 Hz, 5″-H), 8.42 (d, 1H, $J = 2.8$ Hz, 3″-H).

4.1.2.2. (3aRS,4RS)2-(2-Chloro-4-nitrophenyl)-3a,4-diethoxyl-4-(methoxylphenyl)chromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10b***. Yield: 50%, m.p. 170–172 °C. ESI-MS: $m/z = 541$ [M + 1]. IR (KBr): 2921, 1609 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.80 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 0.88 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.86–2.90 (m, 1H, OCH_2CH_3), 3.08–3.12 (m, 1H, OCH_2CH_3), 3.26–3.30 (m, 1H, OCH_2CH_3), 3.37–3.41 (m, 1H, OCH_2CH_3), 3.96 (s, 3H, OCH_3), 6.93 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.24–7.31 (m, 2H, 7-H and 8-H), 7.57 (d, 1H, $J = 8.8$ Hz, 6-H), 7.61 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.71 (d, 1H, $J = 9.2$ Hz, 6″-H), 8.09 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz, 9-H), 8.19 (dd, 1H, $J = 9.2$ Hz, 2.8 Hz, 5″-H), 8.29 (d, 1H, $J = 2.8$ Hz, 3″-H).

4.1.2.3. (3aRS,4RS)6-Chloro-2-(2-chloro-4-nitrophenyl)-4-(4-chlorophenyl)-3a,4-diethoxyl-7,9-dimethoxylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10c***. Yield: 53%, m.p. 219–221 °C. ESI-MS: $m/z = 639$ [M + 1]. IR (KBr): 2924, 1601 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (t, 3H, $J = 7.4$ Hz, OCH_2CH_3), 0.97 (t, 3H, $J = 7.4$ Hz, OCH_2CH_3), 2.84–2.91 (m, 1H, OCH_2CH_3), 3.15–3.22 (m, 1H, OCH_2CH_3), 3.33–3.40 (m, 1H, OCH_2CH_3), 3.44–3.51 (m, 1H, OCH_2CH_3), 4.03 (s, 3H, OCH_3), 4.06 (s, 3H, OCH_3), 6.39 (s, 1H, H-8), 7.41 (d, 2H, $J = 8.0$ Hz, 3′-H and 5′-H), 7.68 (d, 3H, $J = 8.0$ Hz, 2′-H, 6′-H and H-6″), 8.10 (dd, 1H, $J = 8.0$ Hz, 2.4 Hz, 5″-H), 8.30 (d, 1H, $J = 2.4$ Hz, 3″-H). ^{13}C NMR ($CDCl_3$) δ: 14.7 (q, OCH_2CH_3), 15.2 (q, OCH_2CH_3), 56.6 (q, OCH_3), 56.7 (q, OCH_3), 59.9 (t, OCH_2CH_3), 60.2 (t, OCH_2CH_3), 90.5 (d, 8-C), 99.0 (s, 6-C), 102.8 (s, 3a-C), 103.6 (s, 9a-C), 108.0 (s, 4-C), 122.6 (d, 3′-C and 5′-C), 123.0 (d, 5″-C), 123.7 (s, 4-C), 126.6 (d, 3″-C), 127.2 (s, 2″-C), 128.4 (s, 1′-C), 128.6 (d, 6″-C), 128.8 (d, 2′-C and 6′-C), 135.7 (s, 4′-C), 135.9 (s,

4″-C), 143.7 (s, 1″-C), 144.5 (s, 5a-C), 149.8 (s, 9-C), 151.0 (s, 9b-C), 158.2 (s, 7-C).

4.1.2.4. (3aRS,4RS)6-Chloro-2-(2-chloro-4-nitrophenyl)-4-(2-chlorophenyl)-3a,4-diethoxyl-7,9-dimethoxylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10d***. Yield: 45%, m.p. 222–224 °C. ESI-MS: $m/z = 639$ [M + 1]. IR (KBr): 2972, 1603 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.91 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 1.01 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.87–2.95 (m, 1H, OCH_2CH_3), 3.22–3.29 (m, 1H, OCH_2CH_3), 3.45–3.61 (m, 2H, OCH_2CH_3), 4.03 (s, 3H, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.39 (s, 1H, 6-H), 7.30–7.35 (m, 2H, 4′-H and 5′-H), 7.42–7.44 (m, 1H, 6′-H), 7.71 (d, 1H, $J = 9.2$ Hz, 5″-H), 7.87–7.69 (m, 1H, 3′-H), 8.10 (d, 1H, $J = 9.2$, 6″-H), 8.29 (s, 1H, 3″-H).

4.1.2.5. (3aRS,4RS)6-Chloro-2-(2-chloro-4-nitrophenyl)-4-(4-methylphenyl)-3a,4-diethoxyl-7,9-dimethoxylchromane [4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10e***. Yield: 58%, m.p. 134–136 °C. ESI-MS: $m/z = 619$ [M + 1]. IR (KBr): 2927, 1598 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.82 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 0.90 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.43 (s, 3H, CH_3), 2.86–2.90 (m, 1H, OCH_2CH_3), 3.08–3.12 (m, 1H, OCH_2CH_3), 3.26–3.30 (m, 1H, OCH_2CH_3), 3.37–3.41 (m, 1H, OCH_2CH_3), 6.41 (s, 1H, 8-H), 7.03 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.22 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.75 (d, 1H, $J = 9.2$ Hz, 6″-H), 8.19 (dd, 1H, $J = 9.2$ Hz, 2.8 Hz, 5″-H), 8.39 (d, 1H, $J = 2.8$ Hz, 3″-H).

4.1.2.6. (3aRS,4RS)6-Chloro-2-(2-chloro-4-nitrophenyl)-4-(4-methoxylphenyl)-3a,4-diethoxyl-7,9-dimethoxylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10f***. Yield: 40%, m.p. 171–173 °C. ESI-MS: $m/z = 635$ [M + 1]. IR (KBr): 2927, 1583 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 1.00 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.84–2.92 (m, 1H, OCH_2CH_3), 3.19–3.26 (m, 1H, OCH_2CH_3), 3.34–3.42 (m, 1H, OCH_2CH_3), 3.48–3.56 (m, 1H, OCH_2CH_3), 3.85 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.61 (s, 1H, 8-H), 6.94 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.60 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.69 (d, 1H, $J = 9.2$ Hz, 6″-H), 8.08 (dd, 1H, $J = 9.2$ Hz, 1.6 Hz, 5″-H), 8.30 (d, 1H, $J = 1.6$ Hz, 3″-H).

4.1.2.7. (3aRS,4RS)2-(2-Chloro-4-nitrophenyl)-4-(4-chlorophenyl)-3a,4-diethoxyl-8-methylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10g***. Yield: 76%, m.p. 172–174 °C. ESI-MS: $m/z = 559$ [M + 1]. IR (KBr): 2977, 1582 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.80 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 0.89 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.38 (s, 3H, CH_3), 2.86–2.90 (m, 1H, OCH_2CH_3), 3.07–3.11 (m, 1H, OCH_2CH_3), 3.24–3.28 (m, 1H, OCH_2CH_3), 3.35–3.39 (m, 1H, OCH_2CH_3), 7.18 (d, 1H, $J = 8.4$ Hz, 6-H), 7.38 (dd, 1H, $J = 8.4$ Hz, 1.6 Hz, 7-H), 7.56 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.64 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.74 (d, 1H, $J = 9.2$ Hz, 6″-H), 7.89 (d, 1H, $J = 1.6$ Hz, 9-H), 8.21 (dd, 1H, $J = 9.2$ Hz, 2.8 Hz, 5″-H), 8.42 (d, 1H, $J = 2.8$ Hz, 3″-H).

4.1.2.8. (3aRS,4RS)4-(6-Chlorobenzo[d][1,3]dioxol-5-yl)-2-(4-nitrophenyl)-3a,4-diethoxyl-8-methylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10h***. Yield: 53%, m.p. 103–106 °C. ESI-MS: $m/z = 603$ [M + 1]. IR (KBr): 2927, 1587 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.85 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 0.92 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 2.85–2.93 (m, 1H, OCH_2CH_3), 3.11–3.19 (m, 1H, OCH_2CH_3), 3.36–3.50 (m, 2H, OCH_2CH_3), 6.14 (d, 1H, $J = 1.2$ Hz, OCH_2O), 6.17 (d, 1H, $J = 1.2$ Hz, OCH_2O), 7.10 (s, 1H, 3′-H), 7.13 (d, 1H, $J = 8.0$ Hz, 6-H), 7.22 (s, 1H, 6′-H), 7.37 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz, 7-H), 7.75 (d, 1H, $J = 9.2$ Hz, 6″-H), 7.87 (d, 1H, $J = 1.6$ Hz, 9-H), 9.19 (dd, 1H, $J = 9.2$ Hz, 2.4 Hz, 5″-H), 8.41 (d, 1H, $J = 2.4$ Hz, 3″-H).

4.1.2.9. (3aRS,4RS)4-(2-Chloro-4,5-dimethoxyphenyl)-2-(2-chloro-4-nitrophenyl)-3a,4-diethoxyl-8-methylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10i***. Yield: 37%, m.p. 105–107 °C. ESI-MS: $m/z = 619$ [M + 1]. IR (KBr): 2977, 1582 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.81 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 0.89 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.37 (s, 3H, CH_3), 2.80–2.87 (m, 1H, OCH_2CH_3), 3.05–3.12 (m, 1H, OCH_2CH_3), 3.31–3.37 (m, 1H, OCH_2CH_3), 3.41–3.48 (m, 1H, OCH_2CH_3), 3.73 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.97 (s, 1H, 3′-H), 7.10 (d, 1H, $J = 8.4$, 6-H), 7.21 (s, 1H, 6′-H), 7.32 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz, 7-H), 7.71 (d, 1H, $J = 9.2$ Hz, 6″-H), 7.31 (dd, 1H, $J = 8.4$ Hz, 1.6 Hz, 7-H), 7.83 (d, 1H, $J = 1.6$ Hz, 9-H), 8.15 (dd, 1H, $J = 9.2$ Hz, 2.4 Hz, 5″-H), 8.28 (d, 1H, $J = 2.4$ Hz, 3″-H).

4.1.2.10. (3aRS,4RS)2-(2-Chloro-4-nitrophenyl)-3a,4-diethoxyl-4-(4-methoxylphenyl)-8-methylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10j***. Yield: 53%, m.p. 221–224 °C. ESI-MS: $m/z = 555$ [M + 1]. IR (KBr): 2927, 1589 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.89 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 0.99 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.85–2.93 (m, 1H, OCH_2CH_3), 3.21–3.27 (m, 1H, OCH_2CH_3), 3.43–3.60 (m, 2H, OCH_2CH_3), 3.92 (s, 3H, OCH_3), 6.93 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.18 (d, 1H, $J = 8.4$ Hz, 6-H), 7.38 (dd, 1H, $J = 8.4$ Hz, 1.6 Hz, 7-H), 7.61 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.73 (d, 1H, $J = 9.2$ Hz, 5″-H), 7.89 (d, 1H, $J = 1.6$ Hz, 9-H), 8.10 (d, 1H, $J = 9.2$ Hz, 6″-H), 8.29 (s, 1H, 3″-H).

4.1.2.11. (3aRS,4RS)2-[2-Chloro-4-nitrophenyl]-4-phenyl-3a,4,7,9-tetramethoxylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***10k***. Yield: 52%, m.p. 107–108 °C. ESI-MS: $m/z = 611$ [M + 1]. IR (KBr): 2980, 1597 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.90 (s, 3H, OCH_3), 3.13 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 4.06 (s, 3H, OCH_3), 6.41 (s, 1H, 8-H), 7.43 (d, 2H, $J = 8.4$ Hz, 3′-H and 5′-H), 7.57–7.72 (m, 3H, 2′-H, 6′-H and 6″-H), 8.10 (d, 1H, $J = 9.6$ Hz, 5″-H), 8.30 (s, 1H, 3″-H).

4.1.2.12. (3aRS,4RS)2-[2-Chloro-4-(methylsulfonyl)-phenyl]-4-(4-chlorophenyl)-3a,4-diethoxylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***11a***. Yield: 78%, m.p. 112–113 °C. ESI-MS: $m/z = 578$ [M + 1]. IR (KBr): 2954, 1608 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.90 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 0.95 (t, 3H, $J = 7.2$ Hz, OCH_2CH_3), 2.91–2.95 (m, 1H, OCH_2CH_3), 3.08 (s, 3H, SO_2CH_3), 3.27–3.46 (m, 3H,

OCH_2CH$_3$), 7.14–7.18 (m, 2H, 6-H and 7-H), 7.39 (d, 2H, $J = 8.4$ Hz, 3′-H and 5′-H), 7.48 (m, 1H, 8-H), 7.63–7.66 (m, 3H, 2′-H, 6′-H and 6″-H), 7.79 (dd, 1H, $J = 8.8$ Hz, 2.0 Hz, 5″-H), 7.98 (d, 1H, $J = 2.0$ Hz, 3″-H), 8.05 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz, 9-H).

4.1.2.13. (3aRS,4RS)2-[2-Chloro-4-(methylsulfonyl)phenyl]-4-(4-methoxylphenyl)-3a,4-diethoxyl-8-methylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***11b***. Yield: 69%, m.p. 131–133 °C. ESI-MS: $m/z = 588$ [M + 1]. IR (KBr): 2936, 1608 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ: 0.90 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 0.95 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 2.39 (s, 3H, CH$_3$), 3.07 (s, 3H, SO$_2$CH$_3$), 2.89–2.96 (m, 1H, OCH_2CH$_3$), 3.22–3.45 (m, 3H, OCH_2CH$_3$), 3.87 (s, 3H, OCH$_3$), 6.95 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.06 (d, 1H, $J = 8.4$ Hz, 6-H), 7.25 (dd, 1H, $J = 8.4$ Hz, 2.0 Hz, 7-H), 7.60 (d, 2H, $J = 8.8$ Hz, 2′-H, 6′-H), 7.66 (d, 1H, $J = 8.4$ Hz, 6″-H), 7.78 (dd, 1H, $J = 8.4$ Hz, 2.4 Hz, 5″-H), 7.85 (d, 1H, $J = 2.0$ Hz, 9-H), 7.97 (d, 1H, $J = 2.4$ Hz, 3″-H).

4.1.2.14. (3aRS,4RS)2-[2-Chloro-4-(methylsulfonyl)phenyl]-4-(4-methoxylphenyl)-3a,4-diethoxylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***11c***. Yield: 66%, m.p. 195–197 °C. ESI-MS: $m/z = 574$ [M + 1]. IR (KBr): 2936, 1608 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ: 0.88 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 0.93 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 2.89–2.96 (m, 1H, OCH_2CH$_3$), 3.07 (s, 3H, SO$_2$CH$_3$), 3.24–3.45 (m, 3H, OCH_2CH$_3$), 3.86 (s, 3H), 6.92 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.14–7.18 (m, 2H, 6-H and 7-H), 7.60 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.66 (d, 1H, $J = 8.4$ Hz, 6″-H), 7.78 (dd, 1H, $J = 8.4$ Hz, 2.4 Hz, 5″-H), 7.97 (d, 1H, $J = 2.4$ Hz, 3″-H), 8.05 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz, 9-H).

4.1.2.15. (3aRS,4RS)6-Chloro-2-[2-chloro-4-(methylsulfonyl)-phenyl]-4-(4-chlorophenyl)-3a,4-diethoxyl-7,9-dimethoxylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***11d***. Yield: 55%, m.p. 112–113 °C. ESI-MS: $m/z = 672$ [M + 1]. IR (KBr): 2936, 1608 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ: 0.87 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 0.96 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 2.84–2.90 (m, 1H, OCH_2CH$_3$), 3.07 (s, 3H, SO$_2$CH$_3$), 3.18–3.25 (m, 1H, OCH_2CH$_3$), 3.32–3.40 (m, 1H, OCH_2CH$_3$), 3.43–3.51 (m, 1H, OCH_2CH$_3$), 4.03 (s, 3H, OCH$_3$), 4.04 (s, 3H, OCH$_3$), 6.39 (s, 1H, 8-H), 7.40 (d, 2H, $J = 8.4$ Hz, 2′-H and 6′-H), 7.6–7.69 (m, 3H, 3′-H, 5′-H and 6″-H), 7.77 (dd, 1H, $J = 8.4$ Hz, 2.4 Hz, 5″-H), 7.86 (d, 1H, $J = 2.4$ Hz, 3″-H).

4.1.2.16. (3aRS,4RS)2-[2-Chloro-4-(methylsulfonyl)phenyl]-4-(4-chlorophenyl)-3a,4-diethoxyl-8-methylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***11e***. Yield: 72%, m.p. 130–132 °C. ESI-MS: $m/z = 592$ [M + 1]. IR (KBr): 2979, 1609 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ: 0.90 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 0.95 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 2.40 (s, 3H, CH$_3$), 2.89–2.96 (m, 1H, OCH_2CH$_3$), 3.06 (s, 3H, SO$_2$CH$_3$), 3.25–3.46 (m, 3H, OCH_2CH$_3$), 7.06 (d, 1H, $J = 8.4$ Hz, 6-H), 7.25 (dd, 1H, $J = 8.4$ Hz, 2.0 Hz, 7-H), 7.38 (d, 2H, $J = 8.8$ Hz, 3′-H and 5′-H), 7.63 (d, 2H, $J = 8.8$ Hz, 2′-H and 6′-H), 7.66 (d, 1H, $J = 8.4$ Hz, 6″-H), 7.78 (dd, 1H, $J = 8.4$ Hz, 2.4 Hz, 5″-H), 7.84 (d, 1H, $J = 2.0$ Hz, 9-H), 7.98 (d, 1H, $J = 2.4$ Hz, H-3″).

4.1.3. General procedure for the preparation of 4-aryl-2-[2-chloro-4-(acetamide)phenyl]-3a,4-diethoxychromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazolines ***12***

A mixture of **10** (2 mmol) and Raney-Ni (1.0 g) in EtOH (6 mL) was stirred at r.t. under H_2 for 12 h. The mixture was filtered, and the filtrate concentrated. Ac_2O (0.6 mL) and pyridine (0.06 mL) were added into the residue and stirred for 12 h. Then the mixture was diluted with water and extracted thoroughly with CH_2Cl_2 and then the CH_2Cl_2 layer washed successively with aqueous sodium bicarbonate and brine, dried over anhydrous Na_2SO_4. After removal of the solvent, and the crude product was further purified by silica gel column chromatography.

4.1.3.1. (3aRS,4RS)2-[2-Chloro-4-(acetamide)phenyl]-4-(4-chlorophenyl)-3a,4-diethoxylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***12a***. Yield: 90%, m.p. 150–152 °C. ESI-MS: $m/z = 558$ [M + 1]. IR (KBr): 3413, 2980, 1703, 1681, 1597 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ: 0.82 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 0.90 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 2.86–2.90 (m, 1H, OCH_2CH$_3$), 3.08–3.12 (m, 1H, OCH_2CH$_3$), 3.26–3.30 (m, 1H, OCH_2CH$_3$), 3.37–3.41 (m, 1H, OCH_2CH$_3$), 7.24–7.31 (m, 2H, 6-H and 7-H), 7.35–7.42 (brs, 4H, 3′-H, 5′-H, 5″-H and 6″-H), 7.59 (m, 2H, 8-H and 3″-H), 7.62 (d, 2H, $J = 8.0$ Hz, 2′-H and 6′-H), 8.07 (dd, 1H, $J = 8.0$ Hz, 1.6 Hz, 9-H).

4.1.3.2. (3aRS,4RS)2-[2-Chloro-4-(acetamide)phenyl]-4-(4-chlorophenyl)-3a,4-diethoxyl-8-methylchromane[4,3-d]-$\Delta^{1,9b}$-1,2,3-thiadiazoline ***12b***. Yield: 85%, m.p. 187–189 °C. ESI-MS: $m/z = 572$ [M + 1]. IR (KBr): 3417, 2979, 1698, 1595 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ: 0.89 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 0.97 (t, 3H, $J = 7.2$ Hz, OCH$_2$CH_3), 2.18 (s, 3H, COCH$_3$), 2.38 (s, 3H, CH$_3$), 2.92–2.99 (m, 1H, OCH_2CH$_3$), 3.29–4.44 (m, 3H, OCH_2CH$_3$), 7.04 (d, 1H, $J = 8.4$ Hz, 6-H), 7.19–7.22 (m, 1H, 7-H), 7.31–7.37 (m, 4H, 3′-H, 5′-H, 5″-H and 6″-H), 7.62 (d, 2H, $J = 8.0$ Hz, 2′-H and 6′-H), 7.69 (s, 1H, 3″-H), 7.82 (d, 1H, $J = 2.0$ Hz, 9-H).

4.2. Biology

The tumor cell lines (Bel-72, ECA-109, PC-3, MCF-7, HL-60, and A-549, S180) were obtained from Shanghai Institute of Pharmaceutical Industry.

4.2.1. Cytotoxic assay

The cytotoxic activity in vitro was measured using the MTT assay [16]. The MTT solution (10.0 μl/well) in RPMI-1640 (Sigma, St. Louis, MO) was added after cells were treated with the drug for 72 h, and cells were incubated for further 4 h at 37 °C. The purple formazan crystals were dissolved in 100.0 μl DMSO. After 5 min, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT) at 570 nm. Assays were performed in triplicate

on three independent experiments. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software ''Dose-Effect Analysis with Microcomputers''. The tumor cell lines panel consisted of Bel-7402, ECA-109, PC-3, MCF-7, HL-60 and A-549. In all of these experiments, three replicate wells were used to determine each point.

4.2.2. Antitumor activity in ICR mice bearing sarcoma 180 cells

Tumor cells of S180 sarcoma were inoculated to mice. After seven days, tumors were taken out and cells were harvested. Viable tumor cells (2.5×10^6 cells/mouse) were inoculated to the armpit of mice by subcutaneous injection. The compound **11a** and CTX were injected intraperitoneally (i.p.) to different groups of mice (negative control group containing 20 female mice, other groups containing 10 female mice) 24 h after the inoculation once a day for consecutive seven days. Cytophosphane (CTX) of 50.0 mg kg^{-1} was used as a positive control and physiological saline as negative control. Tumors were dissected and weighed, and inhibition rates were calculated at day 8. The inhibition rate was calculated as follows: $C - T/C \times 100$, T: average tumor weight of treated group; C: average tumor weight of negative control group.

4.2.3. DNA fragmentation

HL-60 cells at a density of 2×10^5 cells/mL were treated with various concentrations of **11a** (0.5 μg/mL, 1.0 μg/mL, 2.0 μg/mL) for 24 h and then collected by centrifugation. The cell pellets were lysed in 200.0 μl lysis buffer (10.0 mM EDTA; 50.0 mM Tris–HCl, pH 8.0; 0.5% sodium lauryl sulfate; 100.0 mg/ml proteinase K) at 37 °C for 12 h, then incubated with RNase (50.0 mg/ml) at 37 °C for an additional 1 h. After incubation, DNA in the lysate was extracted with equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), then with chloroform. DNA was precipitated with two volumes of ethanol in the presence of 0.3 M sodium acetate. After centrifugation at 12,000*g* for 15 min, the DNA pellets were washed with 70% ethanol, air-dried, and resuspended in 20.0 μl TE (10.0 mM Tris–HCl and 1.0 mM EDTA, pH 8.0). DNA was separated on 1.5% agarose gels containing 0.5 mg/mL ethidium bromide and photographed by Bio-Rad GD2000 (Bio-Rad, Hercules, CA) [17].

4.2.4. Flow cytometric analysis of apoptosis

For flow cytometry analysis of DNA content, HL-60 cells in exponential growth were treated with graded concentrations of **11a** (0.8–3.2 μM) for 48 h. Cells were washed twice with PBS and fixed in 70% ethanol at −20 °C. The cell pellet was resuspended in 100.0 μl of PBS containing 50.0 mg/ml RNase (Amersco, Solon, OH) and then incubated at 37 °C for 1 h. After incubation, the cells were stained with 200.0 mg/ml propidium iodide (PI, Sigma, St. Louis, MO) at 4 °C for 30 min. The fluorescence of 2×10^4 cell was measured with FACSCalibur (Becton Dickinson, Lincoln Park, NJ) [18].

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