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Synthesis and anticancer activity of 4-hydroxy naphtho coumarin derivatives and naphtho coumestans

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ABSTRACT

*We have synthesized various coumestan derivatives from 4-hydroxy-2H-benzo[h]chromen-2-one **2**. Oxidative cyclization of 4-hydroxy-2H-benzo[h]chromen-2-one with catechol and pyrogallol in presence of sodium acetate and potassium iodate gave 8, 9-dihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **3** and 8, 9, 10-trihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one **4** respectively. These coumestan derivatives and 4-hydroxy-2H-benzo[h]chromen-2-one were condensed with dimethyl sulphate and different mono and/or di alkyl halides in presence of base like anhydrous K_2CO_3 and dry acetone gave corresponding condensed or cyclized coumestan **6a-d**, **7a-d** and 4-hydroxy-2H-benzo[h]chromen-2-one **5a-b** derivatives.*

Keywords: naphthocoumarin, naphthocoumestan, anticancer activity

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INTRODUCTION

Coumestan ring system is present in number of natural products like coumestrol, psoralidine, pterocarsin [1], lucernol [2] and wedelolactone [3]. Coumestans represent an important class of natural oxygenated aromatic products responsible for medicinal effects. Eclipta alba [4] and wedelia calendulacea [5] are the plant sources of nor-wedelolactone and wedelolactone. Both of them show medicinal effects such as antihepatotoxic, antihypertensive, antitumor, antiphospholipase A_2 and antidote activities against snake venome [6-9].

Coumestans belongs to the flavonoids category of phytoestrogens, which have diverse pharmacological properties such as antihemorrhagic, antiproteolytic, antihepatotoxic [10, 6], antiphospholipase and antimyotoxic activity [11]. In traditional Chinese medicine, coumestans are used in the treatment of septic shock and in Indian Ayurvedic medicine as a treatment for liver diseases [12], skin disorders and viral infections. Coumestans have also been shown to reduce cancer risk [13] due to their structural similarity to phytoestrogens. A series of coumestan derivatives were recently reported as HCVNS5B polymerase inhibitors [14] and they also found to inhibit binding to the $GABA_A$ receptors from the rat brain [11]. Wedelolactone has been shown to inhibit the NF-κB mediated gene transcription in cells by blocking the phosphorylation and degradation [15] selective 5-lipoxygenase-inhibitor [15] and LPS- induced caspase-11 expression inhibitor [15]. Coumestrol has been reported to have strong estrogenic activity [16]. Coumestan derivatives have been reported to show inhibitory effect of lipid peroxidation [17] and Na^+, K^+ ATPase activity [18, 19].

MATERIALS AND METHODS

Chemistry

Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR

spectra were recorded as KBr discs on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded from a Bruker Advance 300 spectrometer (300 MHz) and Advance 400 spectrometer (400 MHz). TLC was performed on silica gel F254 plates (Merck). CHN elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA.

Synthesis of 4-hydroxy-2H-benzo[h]chromen-2-one (2)

A solution of 2-acetyl 1-naphthol **1** (0.00107 mol) in diethyl carbonate (30 ml) was added slowly to pulverized sodium (0.01739 mol) under anhydrous conditions. Highly exothermic reaction was observed. It was then allowed to cool to room temperature. Ethanol (50 ml) was added to decompose the unreacted sodium. The reaction mass was then poured into water (250 ml) and the aqueous layer washed twice with petroleum ether (50 ml). Concentrated hydrochloric acid was added slowly to the aqueous layer until pH 2 and the solid obtained was collected by filtration. The crude product crystallized from ethanol to give 4-hydroxy-2H-benzo[h]chromen-2-one **2** as light-yellow solid. Yield 96%; mp 283-285°C (Lit. 284°C [4]); IR (KBr, cm^{-1}): 3423, 2926, 1604, 1561; ^{1}H NMR (DMSO, d_6, δ ppm): 5.83 (s, 1H, C-3 proton), 7.60-7.76 (m, 3H, ArH), 7.86-7.94 (m, 2H, ArH), 8.48-8.53 (m, 1H, ArH), 11.94 (s, 1H, OH); ^{13}C NMR (DMSO, d_6, δ ppm): 91.08, 111.62, 119.43, 122.15, 122.66, 124.06, 127.80, 128.57, 129.25, 135.27, 151.12, 162.31, 167.16; Elemental Analysis for $C_{13}H_8O_3$; Calculated, %: 73.58; H, 3.80; Found, %: C 73.75; H 3.68.

General procedure for synthesis of 3 and 4

In a solution of **2** (3 gm, 1.415 mmol) in 1:1 water: acetone (50 ml), sodium acetate (4 gm, 4.878 mmol) and catechol or pyrogallol (1.964 mmol) were added. Reaction mixture was stirred at room temperature for 10 minutes. Mixture of KIO_3 (9 gm, 4.2056 mmol) and sodium acetate (4 gm, 4.878 mmol) in hot water (50 ml) was added slowly in the reaction flask in period of 20 minutes and stirred at room temperature for 30 minutes. Solid product separated was filtered and washed with hot water. Crude product washed with hot ethanol (3×50 ml) and hot petroleum ether (3×50 ml) and dried.

Spectral Data:

8, 9-dihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (3)

Yield: 89%; mp: >300°C; IR (KBr, cm^{-1}): 3416, 1702, 1609; Mol. Formula: $C_{19}H_{10}O_5$.

8, 9, 10-trihydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (4)

Yield: 84%; mp: >300°C; IR (KBr, cm^{-1}): 3843, 3008, 1734, 1583; Mol. Formula: $C_{19}H_{10}O_6$.

General Procedure for synthesis of 5a-b, 6a-b and 7a-b

2, 3 and **4 (**1 gm, 1 eq.) dissolved in 20 ml dry acetone. Freshly fused K_2CO_3 (3.5 eq.) and dimethyl sulphate or allyl bromide was added (1.1 eq.) in a reaction flask and it was refluxed for 10 hours. Reaction mass was poured in ice-water, solid crude product obtained was filtered and washed with water. Crude product was washed with hot ethanol (3×50 ml) and hot petroleum ether (3×50 ml) and dried. Compound **5a-b** was purified by column chromatography using 5% Ethyl acetate/ petroleum ether.

Spectral Data:

4-methoxy-2H-benzo[h]chromen-2-one (5a)

Yield: 10%; mp: 154-156°C; IR (KBr, cm^{-1}): 3078, 2916, 2846, 1734; ^{1}H NMR ($CDCl_3$, d_6, δ ppm): 4.07 (3H, s, -OCH_3), 5.81 (1H, s, C-3 proton), 7.64-7.72 (3H, m, ArH), 7.82-7.91 (2H, m, ArH), 8.52-8.6 (1H, m, ArH); ^{13}C NMR ($CDCl_3$, d_6, δ ppm): 56.5, 89.6, 110.9, 118.5, 122.7, 122.9, 123.9, 127.1, 127.8, 128.8, 135.2, 150.7, 163.1, 167.4; Elemental Analysis for $C_{14}H_{10}O_3$: Calculated, %: C 74.33; H 4.46, Found, %: C 74.46; H 4.18.

4-allyloxy-2H-benzo[h]chromen-2-one (5b)

Yield: 9%; mp: 200-205°C; IR (KBr, cm^{-1}): 3092, 3022, 1726; ^{1}H NMR ($CDCl_3$, d_6, δ ppm): 4.73 (2H, dd, -OCH_2), 5.45-5.48 (1H, dd, vinyl proton), 5.53- 5.58 (1H, dd, vinyl proton), 5.79 (1H, s, C-3 proton), 6.10- 6.17 (1H, m, vinyl proton), 7.64-7.70 (3H, m, ArH), 7.83-7.89 (2H, m, ArH), 8.55-8.57 (1H, m, ArH); ^{13}C NMR ($CDCl_3$, d_6, δ ppm): 69.9, 90.5, 110.9, 118.6, 119.7, 122.7, 122.9, 123.9, 127.1, 127.8, 128.8, 130.7, 135.2, 150.8, 163.0, 166.2; Elemental Analysis for $C_{16}H_{12}O_3$: Calculated, %: C 76.18; H 4.79, Found, %: C 76.43; H 4.53.

8, 9-dimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (6a)

Yield: 22%; mp: 259-261°C; IR (KBr, cm^{-1}): 1731, 1296, 1079, 998; ^{1}H NMR (DMSO, d_6, δ ppm): 3.92-3.93 (6H, d, CH_3), 7.31 (1H, s, ArH), 7.44 (1H, s, ArH), 7.63-7.66 (2H, m, ArH), 7.77-7.82 (1H, m, ArH), 7.91-7.94 (2H, m, ArH), 8.49-8.51 (1H, d, ArH); Elemental Analysis for $C_{21}H_{14}O_5$ %: Calculated, %: C 72.83; H 4.07, Found, %: C 72.57; H 3.82.

8, 9-bis(allyloxy)-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (6b)
Yield: 44%; mp: >300°C; IR (KBr, cm^{-1}): 3020, 2849, 1726; ^1H NMR (DMSO, d_6, δ ppm): 4.64- 4.65 (4H, d, -OCH_2 protons), 5.25- 5.30 (2H, dd, vinyl protons), 5.42- 5.47 (2H, dd, vinyl protons), 6.03- 6.11 (2H, m, vinyl protons), 7.30 (1H, s, ArH), 7.45 (1H, s, ArH), 7.59-7.66 (2H, m, ArH), 7.78-7.81 (1H, d, ArH), 7.90-7.92 (2H, m, ArH), 8.48-8.50 (1H, d, ArH); Elemental Analysis for $C_{25}H_{18}O_5$: Calculated, %: C 75.37; H 4.55, Found, %: C 75.76; H 4.72.

8, 9, 10-trimethoxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (7a)
Yield: 41%; mp: >300°C; IR (KBr, cm^{-1}): 3210, 3075, 2946, 2924, 2851, 1733, 1684; Mol. Formula: $C_{22}H_{16}O_6$.

8, 9, 10-tris(allyloxy)-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (7b)
Yield: 32%; mp: >300°C; IR (KBr, cm^{-1}): 2918, 2851, 1675; Mol. Formula: $C_{28}H_{22}O_6$.

General Procedure for synthesis of 6c-d and 7c-d
Compound **3** or **4 (**1 gm, 1 eq.) dissolved in 20 ml dry acetone in a round bottom flask. Freshly fused K_2CO_3 (3.5 eq.) and di substituted bromo alkane were added (0.6 eq.) in a reaction flask and it was refluxed for 10 hours. Reaction mass poured in ice-water, solid crude product obtained. It was filtered and washed with water. Crude product was refluxed with hot ethanol (3×50 ml) and hot petroleum ether (3×50 ml), filtered and dried.

Spectral Data:
8, 9-ethylenedioxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (6c)
Yield: 54%; mp: >300°C; IR (KBr, cm^{-1}): 1717, 1359, 1255, 1180, 1030, 763; Mol. Formula: $C_{21}H_{12}O_5$.

8, 9-methylenedioxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (6d)
Yield: 27%; mp: >300°C; IR (KBr, cm^{-1}): 1731, 1362, 1278, 1085, 1001, 769; Mol. Formula: $C_{20}H_{10}O_5$.

8, 9-ethylenedioxy, 10-hydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (7c)
Yield: 37%; mp: >300°C; IR (KBr, cm^{-1}): 3624, 1720, 1552, 1530, 1241, 1090, 1050, 769; Mol. Formula: $C_{21}H_{12}O_6$.

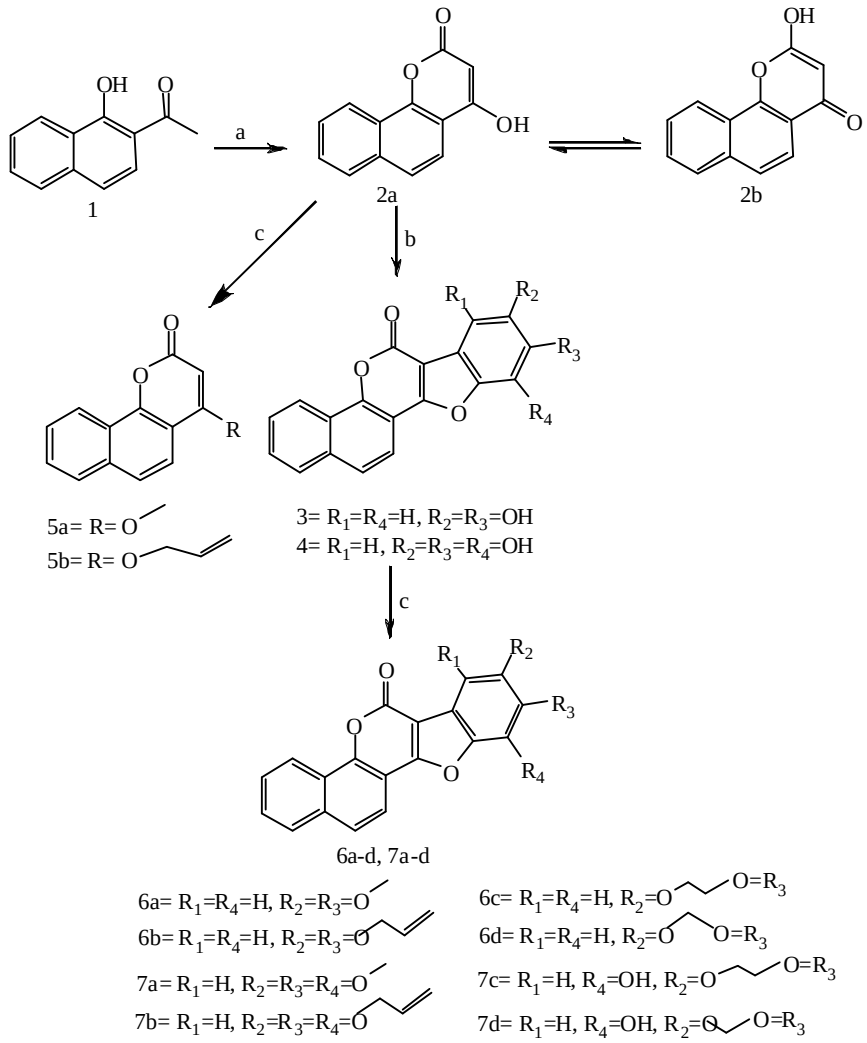
8, 9-methylenedioxy, 10-hydroxy-6H-benzo[h]benzofuro[3,2-c]chromen-6-one (7d)
Yield: 53%; mp: >300°C; IR (KBr, cm^{-1}): 3473, 1731, 1376, 1247, 1120, 970, 920, 830, 763; Mol. Formula: $C_{20}H_{10}O_6$.

Anticancer activity: Procedure to assess the effect of the coumestan derivatives on melanoma cell survival using the MTS Method
96 well plates were plated with 100µl Media (DMEM + 10% Fetal bovine serum and L-Glutamine) containing 5000 cells/well. Stock solution of 20 mM was prepared for compounds to get a series of concentration ranging from 50 µM to 0.625 µM. 100 µl of these compounds were added to the 96 well plates. These 96 well plates were incubated at 37°C in humidified incubator under 5% CO_2 atmosphere for 24, 48 and 72 hours.

In vitro inhibitory efficacy of cancer cell lines representing different cancer types following treatment with compounds was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (Promega, Madison, WI). In brief, 5×10^3 cells per well in 100 µL of DMEM containing 10% FBS were grown in a 96-well plate for 24 h and treated with either control DMSO vehicle or increasing concentrations (0.625-50 µM) of these compounds for 24, 48 and 72 h. The proportion of viable cells compared to control DMSO treated cells were determined using MTS assay and IC50 values calculated using GraphPad Prism, version 4.01 (GraphPad software, San Diego, CA). The IC_{50} value for each compound was determined by at least three independent experiments and represented with a standard error. IC_{50} values in µM concentration of all compounds were given in Table 1, Figure 1 and Figure 2.

RESULTS AND DISCUSSION

Chemistry
2-Acetyl 1-naphthol **1** on Hoesch reaction [20] with diethyl carbonate in presence of pulverized sodium gave 4-hydroxy-2H-benzo[h]chromen-2-one **2** [21]. Oxidative cyclization [22] of **2** with catechol and pyrogallol in presence of sodium acetate and potassium iodate gave corresponding coumestan derivatives 8,9-dihydroxy-*6H*-benzo[*h*]benzofuro[3,2-c]chromen-6-one **3** and 8,9,10-trihydroxy-*6H*-benzo[*h*]benzofuro[3,2-c]chromen-6-one **4**. Reaction of 4-hydroxy-2H-benzo[h]chromen-2-one **2** and coumestan derivatives **3** and **4** with dimethyl sulphate and various mono or dihaloalkanes in presence of base like anhydrous K_2CO_3 [22] gave corresponding alkyl derivatives of coumestans **5a-b**, **6a-d** and **7a-d** as shown in Scheme 1.



Scheme 1: Reagents and conditions: (a) pulverized sodium, diethyl carbonate, 30 min; (b) Catechol 3 and pyrogallol 4, CH_3COONa, KIO_3, Acetone, water, 30 min. (c) K_2CO_3, Dry acetone, dimethyl sulphate or mono or di substituted halide, 10 h

The IR spectrum of compound **2** showed band at 3423 cm^{-1} for hydroxyl group and band at 1604 cm^{-1} for carbonyl group which exist in **2a** and **2b** form. In ^1H NMR of **2**, singlet at δ 5.83 indicated proton at C-3, broad peak at δ 11.94 indicated hydroxy proton at C-4 and all aromatic protons were observed between δ 7.60-8.53 thus confirmed the formation of **2**.

The IR spectrum of compound **5a** showed disappearance of band at 3423 cm^{-1} and appearance of band at 1734 cm^{-1} which confirmed presence of lactone ring. In ^1H NMR of **5a** singlet at δ 4.07 for three protons indicated methoxy group, singlet at δ 5.81 indicated proton at C-3 and all aromatic protons were observed between δ 7.64-8.6 confirmed the formation of **5a**. In ^1H NMR of **5b** multiplet at δ 4.73-4.75 for two protons indicated $–OCH_2$ protons, two doublet of doublets at δ 5.45-5.48 and δ 5.53-5.58 each indicated presence of vinyl protons while all aromatic protons were observed between δ 7.64-8.57 thus confirmed formation of **5b**.

The IR spectrum of compound **3** showed band at 3416 and 1702 cm^{-1} for hydroxyl group and lactone carbonyl group respectively. The ^1H NMR of **6a** showed doublet at δ 3.92-3.93 for two methoxy groups and all aromatic protons were observed between δ 7.31-8.51 confirmed formation of **6a**. The ^1H NMR of **6b** showed doublet at δ 4.64-4.65 for two protons indicated $–OCH_2$ group, doublet of doublet at δ 5.25-5.29 for two protons, another doublet of doublet at δ 5.42-5.47 for two protons indicated two vinyl protons ($=CH_2$). One multiplet at δ 6.08 for two protons indicated one vinyl proton (=CH) each thus confirmed the presence of two allyloxy groups and all aromatic protons were observed between δ 7.30-8.50 confirmed formation of **6b**. The IR spectrum of compound **6c** showed disappearance of band at 3416 cm^{-1} for hydroxyl group and presence of band at 1717 cm^{-1} for lactone group confirmed formation of **6c**. The IR spectrum of compound **6d** showed disappearance of band at 3416 cm^{-1} for hydroxyl group and presence of band at 1731 cm^{-1} for lactone group indicated formation of **6d**.

The IR spectrum of compound **4** showed band at 3843 cm^{-1} for hydroxyl group and band at 1734 cm^{-1} for lactone carbonyl group confirmed formation of **4**. The IR spectrum of compound **7a** showed disappearance of band at 3843 cm^{-1} for hydroxyl group and presence of bands at 3210, 3075, 2946, 2924 and 2851 cm^{-1} and band at 1733 cm^{-1} for lactone group confirmed formation of **7a**. The IR spectrum of compound **7b** showed disappearance of band at 3843 cm^{-1}, while presence of bands at 2918 and 2851 cm^{-1} and band at 1675 for carbonyl group confirmed formation of **7b**. The IR spectrum of compound **7c** showed disappearance of band at 3843 $cm^{-1}$and presence of band at 1734 cm^{-1} for lactone group confirmed formation of **7c**. The IR spectrum of compound **7d** showed disappearance of band at 3843 cm^{-1} while presence of band at 1720 cm^{-1} for lactone group confirmed formation of **7d**.

Anticancer activity

All synthesized coumestan derivatives were screened against two melanoma cancer cell lines UACC-903 and A375M, one breast cancer cell line MCF-7 and fibroblast (FF2441-Precursors of normal cells) to determine IC_{50} values of synthesized compounds by MTS Assay method [23]. The results are shown in Table-1, Figure-1 and Figure-2 respectively.

Table 1: IC_{50} of compounds against melanoma cell lines UACC903 and A375M

Cell lines	UACC-903			A375M		
	24h	48h	72h	24h	48h	72h
JS-20 (3)	180.8	191.9	58.01	614.3	266.8	112.7
JS-21 (3a)	463.3	280.8	149.3	140.1	269.3	DNC
JS-22 (3b)	144.1	200	109.3	362.3	234.4	270.5
JS-23 (3c)	150.8	126.5	118.5	DNC	617.5	DNC
JS-24 (3d)	241.5	240.9	147.4	533.2	623.5	DNC
JS-25 (4)	193.5	145.7	108.2	DNC	DNC	DNC
JS-26 (4a)	98.92	107.8	44.95	218.6	237.4	DNC
JS-28 (4c)	431.6	526.3	DNC	219.0	605.4	100.9
JS-29 (4d)	462.3	DNC	DNC	DNC	386	219.3

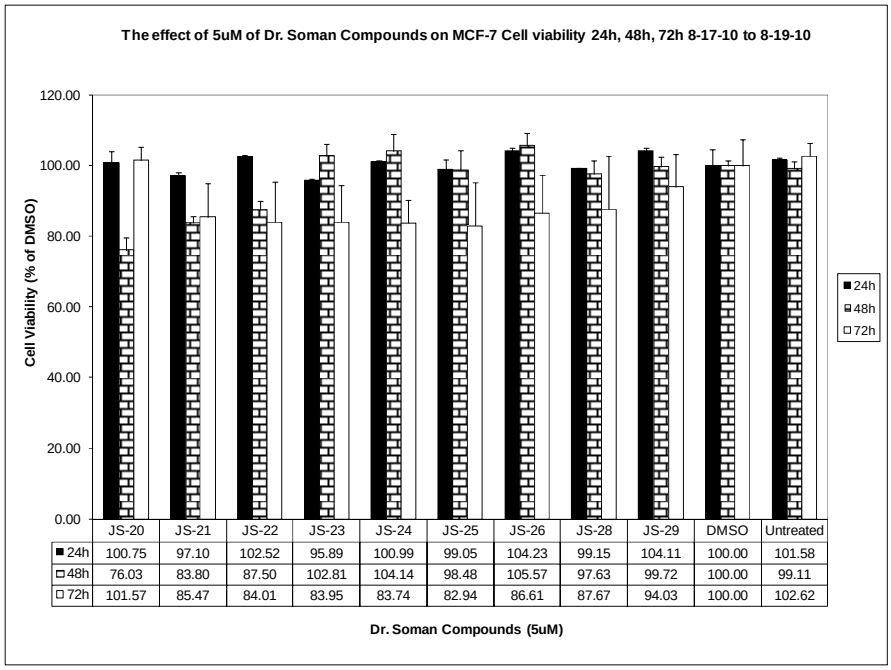
DNC: Does not calculate

Compound **3** showed no significant activity till 48 h then show 69% inhibition up to 72 h against UACC903 cell line while 57% and 58% inhibition observed after 48h and 72 h respectively against A375M cell line. Compound **7a** showed 39% and 47% inhibition after 48 h and 72 h respectively against UACC903 cell line while 192% cell growth up to 48 h against A375M cell line was observed. Compound **7b** showed 138% cell growth up to 48 h then show 45% inhibition against UACC7 cell line while 35% inhibition observed up to 48 h and then no significant change up to 72 h against A375M cell line. Compound **7c** showed 16% inhibition after 48 h then decreasing activity to 6% after 72 h against UACC703 cell line. Compound **7d** showed no significant activity up to 48 h but then show 39% inhibition up to 72 h against UACC903 cell line. Compound **4** showed 25% and 26% inhibition after 48 h and 72 h respectively against UACC903 cell line. Compound **8a** showed no significant activity up to 48 h but then show 58% inhibition after 72 h against UACC903 cell line. Compound **8c** showed 276% cell growth up to 48 h and then 83% inhibition up to 72 h against A375M cell line. Compound **8d** showed no significant activity up to 48 h then 43% inhibition observed against A375M cell line. Compound **8c** and **8d** were inactive up to 72 h against UACC903 cell line while compound **7c**, **7d**, **4** and **8a** were inactive up to 72h against A375M cell line as shown in Table 1.

All Synthesized compounds showed moderate activity against breast cancer cell line MCF-7 in 5 µM concentration as shown in Figure 1. All compounds did not show activity up to 24 h. Compound **3**, **7a** and **7b** showed 23%, 15% and 12% inhibition up to 48 h but then no significant change in activity up to 72 h for compound **3** but compound **7a** and **7b** showed 17%, and 18% inhibition respectively. While compounds **7c**, **7d**, **4**, **8a**, **8c** and **8d** showed no significant change in activity up to 48 h but then showed 19%, 19%, 19%, 16%, 16% and 8% inhibition up to 72 h respectively.

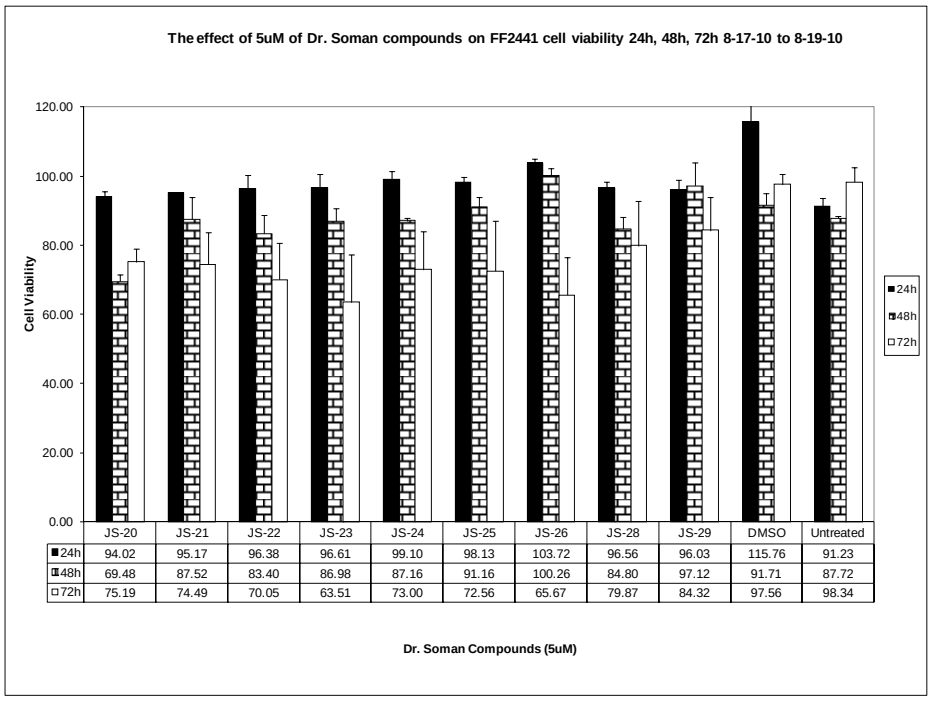
All Synthesized compounds showed moderate activity against fibroblast FF2441 in 6 µM concentration as shown in Figure 2. All compounds were not showing activity up to 24 h. Compound **3** showed 18% inhibition up to 48 h and 23% inhibition up to 72 h. Compounds **7a**, **7b**, **7c**, **7d**, **4**, **8a**, **8c** and **8d** showed no significant change in activity up to 48 h but then showed 24%, 28%, 35%, 25%, 26%, 33%, 17% and 14% inhibition up to 72 h respectively.

Figure 1: IC_{50} of compounds against Breast Cancer cell lines MCF-7



	JS-20	JS-21	JS-22	JS-23	JS-24	JS-25	JS-26	JS-28	JS-29	DMSO	Untreated
24h	100.75	97.10	102.52	95.89	100.99	99.05	104.23	99.15	104.11	100.00	101.58
48h	76.03	83.80	87.50	102.81	104.14	98.48	105.57	97.63	99.72	100.00	99.11
72h	101.57	85.47	84.01	83.95	83.74	82.94	86.61	87.67	94.03	100.00	102.62

Figure 2: IC_{50} of compounds against Fibroblast (FF2441-precursors of normal cells)



	JS-20	JS-21	JS-22	JS-23	JS-24	JS-25	JS-26	JS-28	JS-29	DMSO	Untreated
24h	94.02	95.17	96.38	96.61	99.10	98.13	103.72	96.56	96.03	115.76	91.23
48h	69.48	87.52	83.40	86.98	87.16	91.16	100.26	84.80	97.12	91.71	87.72
72h	75.19	74.49	70.05	63.51	73.00	72.56	65.67	79.87	84.32	97.56	98.34

CONCLUSION

All coumestan derivatives showed moderate activity. Methoxy and allyloxy coumestan derivatives were more active than methylenedioxy and ethylenedioxy coumestan derivatives. Methoxy derivatives showed better activity than allyloxy derivatives. Compound **3** showed better activity than other synthesized compounds against melanoma cancer cell lines, Breast cancer cell line and Fibroblast. Some compounds showed activity up to 48 h while some compounds were not so active till 48 h but then show activity up to 72 h.

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