Synthesis and Spectroscopic Characterization of Some Hydrazone and 2*H*-benzopyranone Derivatives as Antitumor Agents

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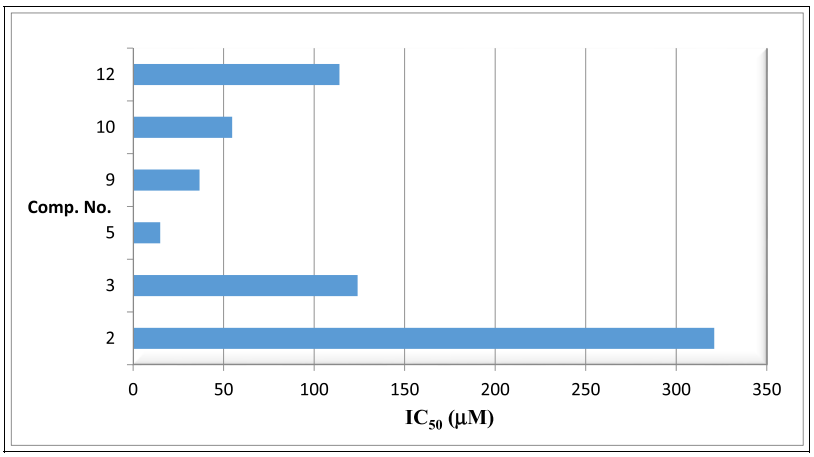

A new series of hydrazone, 2*H*-benzopyranone-3-carboxamide and 2*H*-benzopyranone-3-carbonylthiosemicarbazide derivatives were synthesized from the alkyl 7-hydroxy-2*H*-benzopyranone-3-carboxylate (**1a**,**b**) and dibromo derivatives (**6a**,**b**) as a key starting materials. The structures of the synthesized new compounds were confirmed by *IR*, ^{1}H, ^{13}C-*NMR*, *MS*, and elemental analysis. Some hydrazone derivatives and *N*-substituted 2*H*-benzopyranone-3-carboxamides were evaluated for their anticancer activity against *HepG-2* cell lines. Some of these compounds shared good cytotoxicity.

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INTRODUCTION

2*H*-Benzopyranone and its derivatives are biologically interesting compounds known for their anti-inflammatory [1], antimicrobial [2], antiviral [3], antioxidant [4], antinociceptive [5], antitumor [6], antiastharnatic [7], antidepressant [8], anti-*HIV* [9], antituberculosis [10], anti-Alzheimer [5], anti-influenza [11], antihyperlipidemic [12], being only some of them.

Antitumor activities of natural and synthetic 2*H*-benzopyranone derivatives have been extensively explored by many researchers [13–18], and it has been proven that 2*H*-benzopyranones, depending on their structures, can act on various tumor cells by different mechanisms. 2*H*-Benzopyranone and its derivatives possess cytotoxic properties [19]. 7-Hydroxy-2*H*-benzopyranone showed growth inhibition in human cancer cell lines [20], such as *A549* (lung), *ACHN* (renal), *H727* (lung), *MCF* (breast), and *HL-60* (leukemia); and, in some clinical trials, they exhibited antiproliferative activity in prostate cancer [21], malignant melanoma [22], and renal cell carcinoma [23]. Research in this area is still very active and directed toward the synthesis of compounds with enhanced pharmacological activities.

On the basis of importance of heterocyclic compounds herein, we report a protocol for the synthesis, spectroscopic characterization of 2*H*-benzopyranone derivatives by using 2,4-dihydroxy benzaldehyde and diethylmalonate, then studying the cytotoxic activities of the 2*H*-benzopyranone derivatives against various tumor cells.

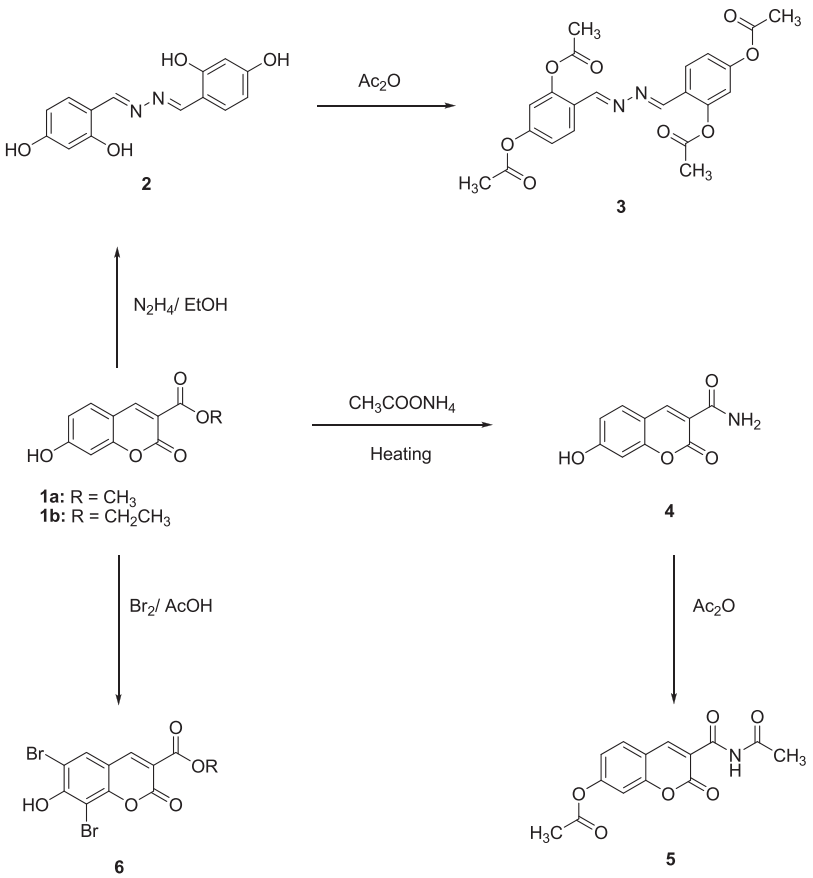
RESULTS AND DISCUSSION

Alkyl 7-hydroxy-2*H*-benzopyranone-3-carboylate (**1a**,**b**) were prepared *via* cyclocondensation of 2,4-dihydroxybenzaldehyde with dialkyl malonate in the presence of piperidine as catalyst according to the literature method [24] as a key starting material. Treatment of ester (**1a**,**b**) with hydrazine hydrate in ethanol afforded the corresponding 1,2-bis(2,4-dihydroxybenzaldehyde) hydrazone (**2**). The structure of compound **2** was supported *via* acetylation of hydrazone derivative (**2**) with acetic anhydride under reflux to give 1,2-bis(2,4-diacetoxybenzaldehyde)-hydrazone (**3**) [25,26]. Amono-lysis of alkyl 7-hydroxy-2*H*-benzopyranone-3-carboxylate (**2**) with ammonia from furnished ammonium acetated and/or formamide under fusion led to the formation of 7-hydroxy-2*H*-benzopyranone-3-carboxamide (**4**). Acetylation of

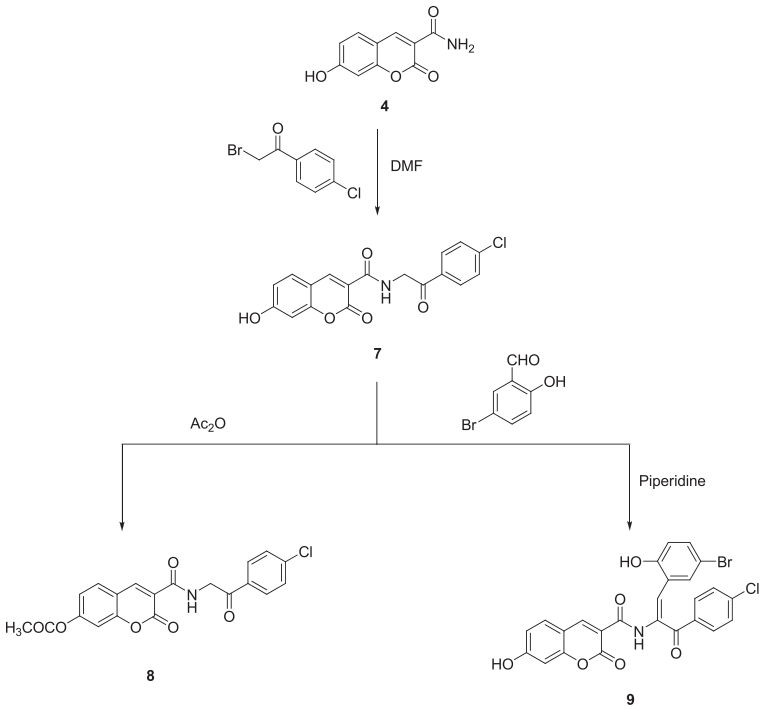
carboxamide (**4**, Scheme 1) with acetic anhydride under reflux yielded the corresponding *N*-acetyl-7-acetoxy-2*H*-benzopyranone-3-carboxamide (**5**). Bromination of alkyl 7-hydroxy-2*H*-benzopyranone-3-carboylate (**1a**,**b**) with two mole bromine in glacial acetic acid at room temperature yielded the corresponding alkyl 6,8-dibromo-7-hydroxy-2*H*-benzopyranone-3-carboxylate (**6a**,**b**). The 7-hydroxy-2*H*-benzopyranone-3-carboxamide (**4**) was reacted with *p*-chlorophenacyl bromide in refluxing dimethyl formamide to yield the corresponding *N*-(*p*-chlorobenzoyl)methyl-7-hydroxy-2*H*-benzopyranone-3-carboxamide (**7**). Compound **7** showed the absence of the absorption band corresponding to amino group (NH_2), and the appearance of new absorption band at 3210 cm^{-1} due to the NH group. Heating of compound **7** with acetic anhydride under refluxing afforded the corresponding *N*-(*p*-chlorobenzoyl)methyl-7-acetoxy-2*H*-benzopyranone-3-carboxamide (**8**). Compound **8** was confirmed from *IR* spectra which showed the new absorption band at 1776 cm^{-1} due to the carbonyl of acetoxy group ($OCOCH_3$) with disappearance of the absorption band corresponding to the hydroxyl group (OH). Also, ^{13}C-*NMR* spectra of compound **8** showed signal at δ 21.36 ppm attributed to the methyl carbon of acetoxy group ($OCOCH_3$).

Condensation of *N*-(*p*-chlorobenzoyl)methyl-7-hydroxy-2*H*-benzopyranone-3-carboxamide (**7**, Scheme 2) with 5-bromo-2-hydroxybenzaldehyde in presence of piperidine under fusion gave the corresponding *N*-(*β*-2-hydroxy-5-bromophenyl-*α*-*p*-chlorobenzoyl)vinyl-7-hydroxy-2*H*-benzopyranone-3-carboxamide (**9**). Treatment of alkyl 6,8-dibromo-7-hydroxybenzopyranone-3-carboxylate with hydrazine hydrate in refluxing ethanol yielded the corresponding 1,2-bis(2,4-dihydroxy-3,5-dibromobenzaldehyde)hydrazone (**10**). Subsequently, the dibromo derivative (**6**) was allowed to react with *N*(4-methoxyphenyl) thiosemicarbazide in dimethyl formamide under reflux led to the formation of 1-(6,8-dibromo-7-hydroxycoumarin-3-carbonyl)-4-(*p*-methoxyphenyl)thiosemicarbazide (**11**). Alkylation of thiosemicarbazide derivative (**11**) with methyl iodide in the presence of anhydrous potassium carbonate in acetone under reflux gave the corresponding 1-(6,8-dibromo-7-methoxycoumarin-3-carbonyl)-4-(*p*-methoxyphenyl)thiosemicarbazide (**12**, Scheme 3). Acetylation of methyl 6,8-dibromo-7-hydroxy-2*H*-benzopyranone-3-carboxylate with acetic anhydride under reflux led to the formation of methyl 6,8-dibromo-7-acetoxy-2*H*-benzopyranone-3-carboxylate (**13**, Scheme 3).

Scheme 1. Synthesis of hydrazone, carboxamide, and dibromo derivatives.



Scheme 2. Synthesis of 3,7-disubstituted-2*H*-benzopyranones from 7-hydroxycoumarin-3-carboxamide (**4**).



Scheme 3. Synthesis of hydrazone and tetrasubstituted-2*H*-benzopyranone derivatives.

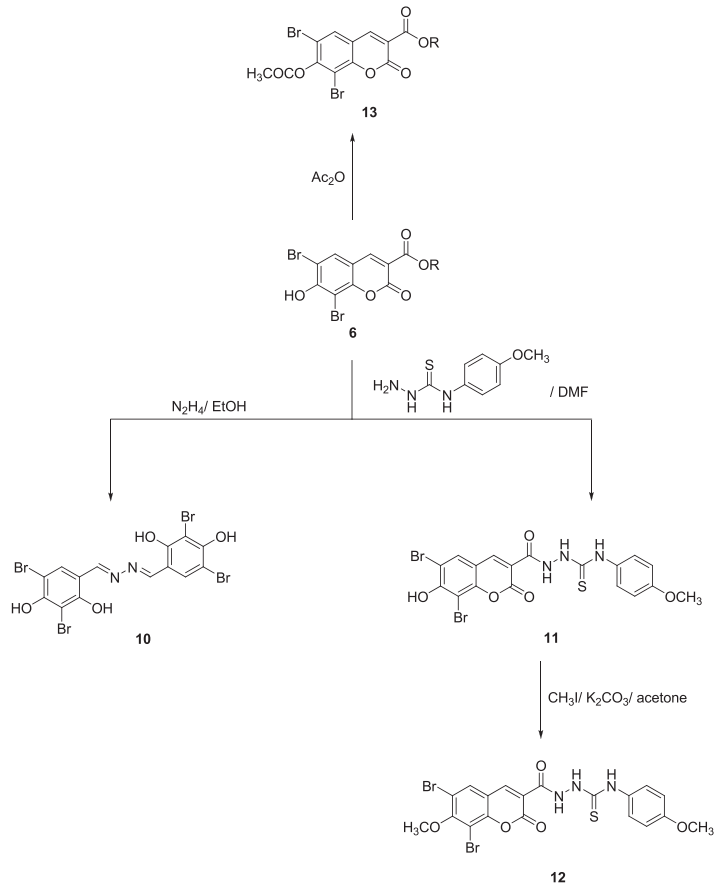


Table 1

Evaluation of cytotoxicity of salicylaldazine derivatives (**2**, **3**, and **10**).

Compound/sample conc. (μg/mL)	**2**			**3**			**10**		
	Viab. (%)	Inhib. (%)	SD (+/−)	Viab. (%)	Inhib. (%)	SD (+/−)	Viab. (%)	Inhib. (%)	SD (+/−)
500	34.67	65.33	1.45	23.15	76.85	0.31	13.68	86.32	0.34
250	56.08	43.92	2.31	38.96	61.04	0.86	26.02	73.98	0.59
125	72.31	27.69	0.92	49.72	50.28	1.43	36.14	63.86	0.42
62.50	89.42	10.58	0.36	71.56	28.44	2.72	45.39	54.61	1.75
31.25	97.63	2.37	0.59	88.40	11.60	0.64	63.78	36.22	2.34
15.60	100	0.00		5.28	4.72	0.15	80.43	19.57	0.71
7.80	100	0.00		98.17	1.83	0.32	93.04	6.96	0.19
3.90	100	0.00		100	0.00		98.76	1.24	0.0
0	100	0.00		100	0.00		100	0.00	

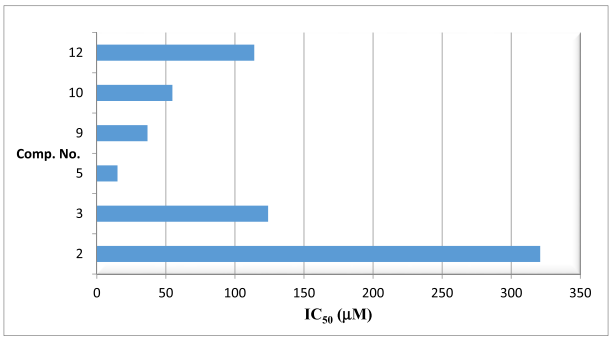


Figure 1. IC_{50} (μg) values of tested compounds **2**–**12** after 24 h continuous exposure of tumor cell. [Color figure can be viewed at wileyonlinelibrary.com]

Antitumor assay. Salicylaldazine (**2**, **3**, and **10**) and 7-substituted coumarin derivatives (**5**, **9**, and **12**) were evaluated for their human tumor cell growth inhibitory activity against *HepG-2* (Hepatocellular carcinoma cells). The measurement of cell growth and viability were determined as described in the literature [27,28]. Cytotoxicity evaluation using viability assays were performed by a regional center for Mycology and Biotechnology (RCMP), Al-Azhar University, Egypt.

The inhibitory activity of the salicylaldazine derivatives (**2**, **3**, and **10**) against human tumor cell lines *HepG-2* (Hepatocellular carcinoma cells) are given in Table 1 and Figure 1.

The inhibitory activity of 7-substituted coumarin derivatives (**5**, **9**, and **12**) against human tumor cell lines *HepG-2* (Liver cancer) are given in Table 2. The results of 50% inhibitory concentration (IC_{50}) data are summarized in Table 3.

Bromo derivatives of salicylaldazine **10** (IC_{50} = 54.7 μg/mL) was found to exhibit good cytotoxic activity than the other salicylaldazine derivatives (**2**, IC_{50} = 321 μg/mL and **3** IC_{50} = 124 μg/mL) against HepG-2 cells. It was observed that, *N*-acetyl-7-acetoxycoumarin-3-carboxamide (**5**, IC_{50} = 15 μg/mL) and *N*-substituted-7-hydroxycoumarin-3-carboxamide (**9**, IC_{50} = 36.7 μg/mL) displayed good activity on HepH-2 than the coumarin derivative (**12**, IC_{50} = 114 μg/mL).

The structure activity relationship of salicylaldazine derivatives demonstrates that substitution of the bromine atoms at the 3,5-position of the two phenyl rings in the salicylaldazine generally increased the activity profile.

Structure activity relationship study has revealed that a substituent at 3,7-positin of coumarin derivatives plays a pivotal role in incurring anticancer activity. Esteric

Table 2

Evaluation of inhibitory activity of 7-substituted coumarin derivatives (**5**, **9**, and **12**).

Compound /sample conc. (μg/mL)	**5**			**9**			**12**		
	Viab. (%)	Inhib. (%)	SD (+/−)	Viab. (%)	Inhib. (%)	SD (+/−)	Viab. (%)	Inhib. (%)	SD (+/−)
500	4.23	95.77	0.54	6.84	93.16	0.31	21.47	78.53	0.31
250	9.79	90.21	0.15	17.43	82.57	0.86	30.68	69.32	0.46
125	16.42	83.58	0.34	29.08	70.92	1.43	47.29	52.71	1.95
62.50	25.10	74.90	0.17	38.71	61.29	2.72	63.15	36.85	2.43
31.25	36.28	63.72	0.64	52.36	47.64	0.64	84.92	15.08	1.06
15.60	48.91	51.09	1.73	69.24	30.76	0.15	92.79	7.21	0.53
7.80	63.18	36.82	2.34	85.97	14.03	0.32	97.41	2.59	0.15
3.90	75.83	24.17	0.75	94.02	5.98		99.86	0.14	0.03
0	100	9.36		100	0.00		100		

Table 3

IC_{50} (μg) values of tested compounds **2**–**12** after 24 h continuous exposure of tumor cell line.

Compound no.		**2**	**3**	**5**	**9**	**10**	**12**
HepG-2 cell Lines	IC_{50}	321	124	15	36.7	54.7	114
	SD (+/−)	8.7	4.9	0.9	2.30	3.90	3.60

groups incur/increase the activity significantly better than other groups. The compound **5** containing esteric group at 7-position and *N*-acetyl carboxamide group at 3-position, which led to the increase the anticancer activity.

CONCLUSION

In conclusion, we report an efficient synthesis of novel series of salicylaldazine and 4-methylcoumarin derivatives, and the structures of these compounds were confirmed *via* IR, ^{1}H-NMR, ^{13}C-NMR, MS, and elemental analysis. The investigation of antitumor activity revealed that compounds **5** and **9** are most potent against HepG-2 cell lines (Hepatocellular carcinoma cells), IC_{50} = 15.00, 36.70 μg/mL, respectively. While compound **10** has moderate activity, compounds **2**, **3**, and **12** have weak activity against HepG-2 cell lines.

EXPERIMENTAL

All reagents and solvents were purchased from Sigma-Aldrich. All the solvents were distilled and dried before use, and melting points of crystalline compounds were measured with an electrothermal melting point apparatus and are uncorrected. *^{1}H-NMR* (400 MHz) and *^{13}C-NMR* (400 MHz) spectra were run with a Bruker 400 DRX-Avance NMR spectrometer. The compounds were dissolved in deuterated *DMSO* as solvent. The IR data were obtained with a Shimadzu 470 spectrometer (Kyoto, Japan). The molecular weight of the compounds were determined by electron ionization (EI) mass spectrometer performed using a probe Agilent MSD 5975 spectrometer operating at 70 eV. The elemental analysis was performed on a Perkin-Elmer 2400 series II CHN elemental analyzer.

Alkyl 7-hydroxy-2*H*-benzopyranone-3-carboxylate (1a,b). A mixture of equimolar quantity of 2,4-dihydroxybenzaldehyde (0.01 mol) and dialkyl malonate (0.01 mol) in the presence of piperidine was stirred at room temperature for 2 h. The reaction mixture was added ethanol (50 mL), then heating under reflux for 1 h, then cooled and poured into water. The reaction mixture was neutralized with dilute hydrochloric acid (2%). The solid product was filtered off, washed with water, dried, and recrystallized from ethanol to give **1**. Methyl 7-hydroxy-2*H*-benzopyranone-3-carboxylate (**1a**) as colorless, yielded 73%, m.p. 156°C. IR (KBr): cm^{-1} 3540 (br. OH), 1774, 1725 (C═O), 1617, 1585 (C═C), 1130, 1024 (C─O). ^{1}H-NMR (DMSO-d_6): δ 11.08 (br. s, 1H, OH), 8.61 (s, 1H, H-4 of pyranone ring), 7.69–7.67 (d, J = 7.8, 1H, Ar-H), 6.80–6.78 (d, J = 7.8, 1H, Ar-H), 6.67 (s, 1H, Ar-H), 3.77 (s, 3H, OCH_3) ppm. ^{13}C-NMR (DMSO-d_6): δ 164.67, 163.92 (C═O), 157.59, 156.79 (C─O), 150.15, 132.51, 114.45, 112.51, 110.86, 102.22 (C-aromatic and pyranone ring), 52.53 (OCH_3) ppm.

Ethyl 7-hydroxy-2*H*-benzopyranone-3-carboxylate (**1b**) as colorless, yield: 76%, m.p. 187°C. IR (KBr): cm^{-1} 3523 (br. OH), 1764, 1723 (C═O), 1615, 1587 (C═C), 1125, 1083 (C─O). ^{13}C-NMR (DMSO-d_6): δ 164.51, 163.33 (C═O), 157.52, 156.77 (C─O), 149.78, 132.46, 114.44, 112.08, 110.81, 102.19 (C-aromatic and pyranone ring), 61.23 (OCH_2), 14.54 (CH_3) ppm.

1,2-Bis(2,4-dihydroxybenzaldehyde)hydrazone (2). A mixture of alkyl 7-hydroxy-2*H*-benzopyranone-3-carboxylate (**1**, 0.01 mole) and hydrazine hydrate (0.01 mol) in ethanol (70 mL) was heated under reflux for 4 h. The reaction mixture was cooled, then poured in water and neutralized with dilute HCl (2%). The solid formed was filtered off, washed with water, dried, and the product was crystallized from ethanol to give **2** as yellow crystals, yield 63%, m.p. 225°C. IR (KBr): cm^{-1} 3466, 3221 (br. 2xOH), 1625 (C═N), 1605, 1511 (C═C), 1215, 1121 (C─O). ^{1}H-NMR (DMSO-d_6): δ 6.35 (s, 2H, Ar-H), 6.40–6.42 (d, J = 8.1, 2H, Ar-H), 7.40–7.42 (d, J = 8.1, 2H, Ar-H), 8.76 (s, 2H, 2xCH═N), 10.21 (br. s, 2H, OH), 4.43 (s, 2H, OH) ppm. ^{13}C-NMR (DMSO-d_6): δ 162.54, 162.28 (C─O), 161.17 (CH═N), 133.49, 110.74, 108.69. 102.97 (C-aromatic) ppm. MS (EI) *m/z* (%) = 272 (M^+, 36.5). *Anal.* Calcd for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.41; N, 10.29. Found: C, 61.53; H, 4.24; N, 10.08.

Acetylation reactions. A solution of **2**, **4**, **7**, and **6a** in acetic anhydride (25 mL) was heated under reflux for 2 h, then cooled and poured into ice water. The reaction mixture was left 24 h, and the solid formed was filtered off, washed with water, and dried. Finally, the product was recrystallized from proper solvent to give **3**, **5**, **8**, and **13**. 1,2-Bis(2,4-diacetoxybenzaldehyde)-hydrazone (**3**) as pale yellow crystals, yield 63%, m.p. 156°C. IR (KBr): cm^{-1} 1761 (C═O), 1612 (C═N), 1616, 1585 (C═C), 1142, 1093 (C─O). ^{1}H-NMR (DMSO-d_6): δ 2.30 (s, 3H, $COCH_3$), 2.40 (s, 3H, $COCH_3$), 7.1–7.24 (m, 4H, Ar-H), 8.25 (d, J = 8.0, 2H, Ar-H), 8.79 (s, 2H, 2xCH═N) ppm. ^{13}C-NMR (DMSO-d_6): δ 169.47, 169.18 (C═O), 157.04, 153.58 (C─O), 150.89 (C═N), 129.96, 124.01, 120.68, 117.89 (C-aromatic), 21.51, 21.21 (2x$COCH_3$) ppm. MS

(EI): *m/z* (%) = 440 (M^+, 57.20). *Anal.* Calcd for $C_{22}H_{20}N_2O_8$: C, 60.00; H, 4.55; N, 6.36. Found: C, 59.66; H, 4.32; N, 6.19.

***N*-acetyl-7-acetoxy-2*H*-benzopyranone-3-carboxamide (5)**. As colorless, yield 63%, m.p. 220°C. IR (KBr): cm^{-1} 3250 (NH), 1774 (C═O of ester), 1735–1695 (br. C═O), 1613, 1583 (C═C), 1126, 1083 (C─O). ^{1}H-NMR (DMSO-d_6): δ 2.26 (s, 3H, $COCH_3$), 2.43 (s, 3H, $COCH_3$), 7.10–7.96 (m, 3H, Ar-H), 8.71 (s, 1H, H-4 of pyranone ring), 10.95 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 171.71, 169.11 (C═O), 162.35, 159.88 (C─O), 155.33, 147.83, 131.88, 120.12, 116.63, 110.60, 110.37 (C-aromatic and pyranone ring), 25.60, 21.36 (2x$COCH_3$) ppm. MS (EI): *m/z* (%) = 289 (M^+, 36.71). *Anal.* Calcd for $C_{14}H_{11}NO_6$: C, 58.13; H, 3.81; N, 4.84. Found: C, 58.01; H, 3.58; N, 3.62.

***N*-(P-chlorobenzoyl)methyl-7-acetoxy-2*H*-benzopyranone-3-carboxamide (8)**. As pale yellow, yield 63%, m.p. 183°C. IR (KBr): cm^{-1} 3222 (NH), 1776 (C═O of ester), 1723–1692 (C═O), 1607, 1583 (C═C), 1125, 1095 (C─O). ^{1}H-NMR (DMSO-d_6): δ 2.33 (s, 3H, $COCH_3$), 2.50 (s, 2H, NCH_2CO), 7.02–8.01 (m, 7H, Ar-H), 8.79 (s, 1H, H-4 of pyranone) and 11.03 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 171.71, 169.09, 162.31, 159.90 (C═O), 155.30, 155.10 (C─O), 147.55, 131.32, 130.32, 129.46, 129.31, 120.11, 120.06, 117.97, 116.82, 110.59 (C-aromatic), 25.68 (NCH_2CO) and 21.36 ($COCH_3$) ppm. MS (EI): *m/z* (%) = 401 (M^++2, 6.21), 399 (M^+, 18.63). *Anal.* Calcd for $C_{20}H_{14}NClO_6$: C, 60.15; H, 3.51; N, 3.51. Found: C, 59.98; H, 3.31; N, 3.29.

6,8-Dibromo-7-acetoxy-2*H*-benzopyranone-3-carboxylate (13). As colorless crystals, yield 73%, m.p. 152°C. IR (KBr): cm^{-1} 1767 (CO of acetoxy), 1742, 1703 (C═O), 1617, 1582 (C═C), 1197, 1059 (C─O). ^{1}H-NMR (DMSO-d_6): δ 2.35 (s, 3H, $COCH_3$), 4.29 (s, 3H, OCH_3), 8.30 (s, 1H, Ar-H) and 8.69 (s, 1H, H-4 of pyranone) ppm. ^{13}C-NMR (DMSO-d_6): δ 172.79, 167.48, 162.92 (C═O), 155.20, 152.10, 150.02 (C─O), 147.90, 143.54, 132.82, 130.32, 118.73, 112.58, 106.36 (C-aromatic and pyranone rings), 62.12 (OCH_3) and 21.44 ($COCH_3$) ppm. *Anal.* Calcd for $C_{13}H_8Br_2O_6$: C, 37.32; H, 1.91. Found: C, 37.13; H, 1.69.

7-Hydroxy-2*H*-benzopyranone-3-carboxamide (4). A mixture of alkyl 7-hydroxy-2*H*-benzopyranone-3-carboxylate (**1a**,**b**, 0.01 mol), ammonium acetate (0,1 mol) and/or formamide (25 mL) was fused on a hot plate at 150°C for 2 h. The reaction mixture was cooled and poured into water; and the resulting solid was filtered off, washed with water, and dried. Finally, the product was recrystallized from acetic acid to give **4** as colorless crystals, yield 56%, m.p. 263°C. IR (KBr): cm^{-1} 3510–3105 (br. OH), 3413, 3317 (NH_2), 1725–1693 (br. C═O), 1605, 1583 (C═C), 1125, 1083 (C─O). ^{1}H-NMR (DMSO-d_6): δ 6.78 (s, 2H, NH_2), 6.88–6.89 (d, J = 7.8, 1H, Ar-H), 7.79–7.81 (d, J = 7.8, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 8.79 (s, 1H, H-4 of pyranone ring), 11.02 (br. s, 1H, OH) ppm. MS (EI): *m/z* (%) = 205 (M^+, 100). *Anal.* Calcd for $C_{10}H_7NO_4$: C, 58.54; H, 3.41; N, 6.83. Found: C, 58.35; H, 3.22; N, 6.61.

Alkyl-6,8-dibromo-7-hydroxy-2*H*-benzopyranone-3-carboxylate (6a,b). In 15 mL of glacial acetic acid, alkyl 7-hydroxy-2*H*-benzopyranone-3-carboxylate (**1**, 01 mole) was dissolved. Then, 10 mL of bromine (0.02 mol) in glacial acetic acid was added dropwise to 7-hydroxy-2*H*-benzopyranone solution, while stirring at RT. After 5–10 min. The bromine color was discharged and a yellow solution remained. At this point, an additional 0.5–1 mL of the bromine-AcOH solution with stirring at RT for 30–45 min. The reaction mixture was poured into water, and the resulting product was filtered off, washed with water, and dried. Finally, the product was recrystallized from ethanol to give **6**.

Methyl 6,8-dibromo-7-hydroxy-2*H*-benzopyranone-3-carboxylate (**6a**) as colorless crystals, yielded 71%, m.p. °C. IR (KBr): cm^{-1} 3540 (br. OH), 1758, 1737 (C═O), 1607, 1542 (C═C), 1237, 1217, 1061 (C─O). ^{1}H-NMR (DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 8.21 (s, 1H, Ar-H), 8.65 (s, 1H, H-4 of pyranone ring), 11.03 (s, 1H, OH) ppm. ^{13}C-NMR (DMSO-d_6): δ 163.31, 156.88 (C═O), 155.77, 153.06 (C─O), 149.01, 133.01, 114.71, 113.14, 107.92, 99.05 (C-aromatic and pyranone ring), 52.62 (OCH_3) ppm.

Ethyl 6,8-dibromo-7-hydroxy-2*H*-benzopyranone-3-carboxylate (**6b**) as colorless crystals, yielded 73%, m.p. 205°C. IR (KBr): cm^{-1} 3615 (br. OH), 1764, 1705 (C═O), 1605, 1598 (C═C), 1236, 1019 (C─O). ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, 3H, CH_3), 4.28 (q, 2H, OCH_2), 8.23 (s, 1H, H-aromatic), 8.75 (s, 1H, H-4 of pyranone ring), 11.12 (s, 1H, OH) ppm. ^{13}C-NMR (DMSO-d_6): δ 163.05, 157.35 (C═O), 156.35, 154.23 (C─O), 152.13, 148.71, 132.99, 115.35, 113.86, 108.03, 99.08 (C-aromatic and pyranone ring), 61.60 (OCH_2), 14.56 (CH_3) ppm. MS (EI): *m/z* (%) = 394 (M^++4, 49.50), 392 (M^++2, 86.30), 390 (M^+, 51.03). *Anal.* Calcd for $C_{12}H_8Br2O_5$: C, 36.92; H, 2.05. Found: C, 36.63; H, 1.97.

***N*-(P-chlorobenzoyl)methyl-7-hydroxy-2*H*-benzopyranone-3-carboxamide (7)**. A mixture of compound **4** (0.01 mol) and *p*-chlorophenacyl bromide (0.01 mol) in dimethyl formamide (30 mL) was heated under reflux for 4 h, then cooled and poured into water. The reaction mixture was neutralized with dilute hydrochloric acid and the resulting solid was filtered off, washed with water, and dried. Finally, the product was recrystallized from ethanol to give **7** as pale yellow crystals, yielded 68%, m.p. 210°C. IR (KBr): cm^{-1} 3510 (br. OH), 3210 (NH), 1730–1689 (br. C═O), 1605, 1583 (C═C), 1140, 1093 (C─O). ^{1}H-NMR (DMSO-d_6): δ 2.50 (s, 2H, NCH_2CO), 6.80–8.01 (m, 8H, Ar-H and NH), 8.79 (s, 1H, H-4 of

pyranone ring), 11.05 (s, 1H, OH) ppm. ^{13}C-NMR (DMSO-d_6): δ 164.11, 163.46, 161.44 (C═O), 156.92 (C─O), 148.88, 132.45, 130.54, 130.30, 129.45, 129.32, 129.24, 114.74, 114.41, 111.57, 102.25 (C-aromatic and pyranone ring), 27.19 (NCH_2CO) ppm. MS (EI): *m/z* (%) = 394 (M^++2, 11.20), 357 (M^+, 34.30). *Anal.* Calcd. for $C_{18}H_{12}NClO_5$: C, 60.50; H, 3.36; N, 3.92. Found: C, 60.33; H, 3.17; N, 3.63.

***N*-(β-2-hydroxy-5-bromophenyl-α-p-chlorobenzoyl)vinyl-7-hydroxy-2*H*-benzopyranone-3-carboxamide (9)**. A mixture of compound **7** (0.01 mol) and 5-bromo-2-hydroxybenzaldehyde (0.01 mol) in the presence of piperidine (1 mL) was fused on a hot plate at 2–3 min. The reaction mixture was added ethanol (50 mL) and heated under reflux for 2 h, then cooled and poured into water. The reaction mixture was neutralized with dilute hydrochloric acid (2%), and the resulting solid was filtered, washed with water, and dried. Finally, the product was recrystallized from ethanol to give **9** as orange crystals, yielded 63%, m.p. 160°C. IR (KBr): cm^{-1} 3430 (br. OH), 3210 (NH), 1731–1689 (br. C═O), 1608, 1583 (C═C), 1217, 1014 (C─O). ^{1}H-NMR (DMSO-d_6): δ 6.91–8.03 (m, 11H, Ar-H and olefinic), 8.74 (s, 1H, H-4 of pyranone), 8.78 (s, 1H, NH) 10.20 (s, 1H, OH), 11.05 (br. s, 1H, OH) ppm. MS (EI): *m/z* (%) = 543 (M^++4, 6.30), 541 (M^++2, 18.20), 539 (M^+, 8.20). *Anal.* Calcd. for $C_{25}H_{15}NBrClO_6$: C, 55.66; H, 2.78; N, 2.60. Found: C, 55.28; H, 2.52; N, 2.42.

1,2-Bis(2,4-dihydroxy-3,5-dibromobenzaldehyde) hydrazone (10). A mixture of compound (**6a**,**b**, 0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol was heated under reflux for 4 h, then cooled and poured into water. The reaction mixture was neutralized with dilute HCl (2%), and the resulting product was filtered off, washed with water, and dried. Finally, the product was recrystallized from ethanol to give **10** as yellow crystals, m.p. 228°C, yielded 56%. IR (KBr): cm^{-1} 3335–3285 (br. OH), 1623 (C═N), 1605, 1583 (C═C), 1215, 1093 (C─O). ^{1}H-NMR (DMSO-d_6): δ 7.85 (s, 2H, Ar-H), 8.42 (s, 2H, 2xCH═N), 9.36 (br. s, 2H, 2xOH), 11.05 (s, 2H, 2xOH) ppm. *Anal.* Calcd. for $C_{14}H_8N_2Br_4O_4$: C, 30.22; H, 1.44; N, 5.04. Found: C, 30.03; H, 1.19; N, 4.84.

1-(6,8-Dibromo-7-hydroxycoumarin-3-carbonyl)-4-(*p*-methoxyphenyl) thiosemicarbazide (11). A mixture of (**6a**, **b**, 0.01 mol) and 4-*N*-(*p*-methoxyphenyl) thiosemicarbazide (0.01 mol) in dimethyl formamide (25 mL) was heated under reflux for 4 h, then cooled and poured into water. The reaction mixture was neutralized with dilute HCl (2%), and the resulting solid was filtered, washed with water, and dried. Finally, the product was recrystallized from acetic acid to give yellow crystals, yield 64%, m.p. 220°C. IR (KBr): cm^{-1} 3450–3120 (br. OH), 1729–1695 (br. C═O), 3225, 3202 (NH), 1605, 1588 (C═C), 1210, 1096 (C─O). ^{1}H-NMR (DMSO-d_6): δ 3.71 (s, 3H, OCH_3), 6.87–7.46 (m, 6H, Ar-H and NH), 8.15 (s, 1H, Ar-H), 8.65 (s, 1H, H-4 of pyranone ring), 9.57 (br. s, 1H, NH), 11.93 (br. s, 1H, OH) ppm. MS (EI): *m/z* (%) = 545 (M^++4, 11.31), 543 (M^++2, 23.20), 541 (M^+, 13.30). *Anal.* Calcd for $C_{18}H_{13}N_3Br_2O_5S$: C, 39.93; H, 2.40; N, 7.76. Found: C, 39.69; H, 2.22; N, 7.48.

1-(6,8-Dibromo-7-methoxycoumarin-3-carbonyl)-4-(*p*-methoxyphenyl) thiosemicarbazide (12). A mixture of compound **11** (0.01 mol), methyl iodide (0.01 mol), and anhydrous potassium carbonate (0.03 mol) in dry acetone (50 mL) was heated under reflux for 6 h, then cooled and poured into water. The reaction mixture was neutralized with dilute HCl (2%), and the resulting product was filtered, washed with water, and dried. Finally, the product was recrystallized from ethanol to give **12** as pale yellow crystals, yield 61%, m.p. 185°C. IR (KBr): cm^{-1} 3286, 3225 (NH), 1730–1689 (br. C═O), 1605, 1592 (C═C), 1215, 1178, 1084 (C─O). ^{1}H-NMR (DMSO-d_6): δ 3.73 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 6.87–7.46 (m, 6H, Ar-H and NH), 8.21 (s, 1H, Ar-H), 8.68 (s, 1H, H-4 of pyran ring), 9.57 (br. s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6): δ 174.6 (C═S), 162.58, 160.09 (C═O), 156.14, 153.35 (C─O), 148.35, 148.11, 134.82, 133.99, 133.40, 129.52, 118.98, 118.33, 114.71, 114.41, 112.42, 106.65, 100.44 (C-aromatic and pyranone ring), 57.43, 55.19 (2xOCH_3) ppm. MS (EI): *m/z* (%) = 559 (M^++4, 7.23), 557 (M^++2, 15.30), 555 (M^+, 8.31). *Anal.* Calcd. for $C_{19}H_{15}N_3Br_2O_5S$: C, 41.08; H, 2.70; N, 7.57. Found: C, 40.87; H, 2.53; N, 7.47.

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