Synthesis, Antiproliferative, and c-Src Kinase Inhibitory Activities of 4-Oxo-4*H*-1-benzopyran Derivatives

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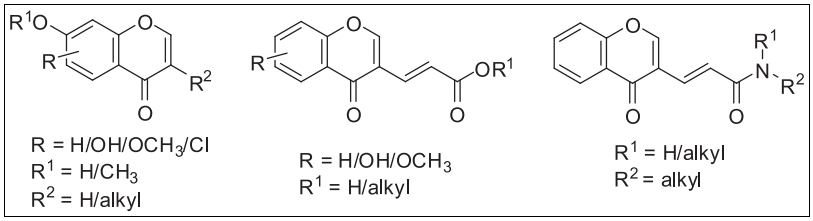

A new class of 4-oxo-4*H*-1-benzopyran derivatives were synthesized and their antiproliferative activity examined against a panel of three human cancer cell lines, that is, breast carcinoma (MDA-MB-468), ovarian adenocarcinoma (SK-OV-3), and colorectal adenocarcinoma (HT-29). Two compounds, that is, 3-hexyl-7,8-dihydroxy-4-oxo-4*H*-1-benzopyran and (*E*)-ethyl 3-(7-methoxy-4-oxo-4*H*-1-benzopyran-3-yl)acrylate were found to be potent against all three cancer cell lines studied at 50 μM concentration. Also, the inhibitory potency of the compounds was evaluated against active Src kinase. A few of these compounds exhibited modest Src kinase inhibitory activity (IC_{50} = 52–57 μM). Structure-activity relationship studies with respect to the nature and position of substituents on the lead compounds could be further exploited for the design and development of more potent antiproliferative agents and/or Src kinase inhibitors.

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INTRODUCTION

Although enormous efforts have been made to develop new anticancer drugs, there is a need to introduce new drugs with less side effects and broad spectrum resistance profile in cancer chemotherapy. The c-Src proto-oncogene is known to be responsible for the development, growth, progression, and metastasis of a wide variety of human cancers [1]. Thus, there is an opportunity to design novel alternative anticancer agents through developing Src kinase inhibitors.

Chromones are known as an important class of naturally occurring compounds containing 4-oxo-4*H*-1-benzopyran skeleton. Furthermore, compounds with a chromone scaffold possess a wide spectrum of biological activities, such as antiproliferative, antioxidant, antiviral, antiinflammatory, kinase inhibitory, antihypertensive, antiallergic, and antifungal properties [2–5]. Molecular mechanisms of anticancer activity mediated by chromones and their derivatives could be attributed to antiproliferation, induction of apoptosis, cell cycle arrest, promotion of differentiation, inhibition of angiogenesis and various enzymes involved in the signaling pathway, and modulation of multidrug resistance [6].

Among the 4-oxo-4*H*-1-benzopyran derivatives, flavopiridol (**1**) (Fig. 1) has been identified as a cyclin-dependent kinase inhibitor. Flavopiridol significantly inhibited the growth of xenografted rhabdoid tumors, and its effect was correlated with the induction of p21 and down regulation of cyclin D_1 [7]. This compound is under investigation in phase II clinical trials [8,9]. Recent studies of another chromone derivative, SF1126 (**2**) (Fig. 1), a water soluble prodrug of LY294002, are currently nearing completion in two adults phase I clinical trial in the treatment of recurrent neuroblastoma. SF1126 is a pan PI-3 kinase inhibitor that acts as a competitive ATP binding site inhibitor and induces apoptosis in multiple neuroblastoma cell lines [10]. PD98059 (**3**) (Fig. 1) was shown to inhibit the proliferation in acute myelogenous leukemia cell lines and caused G1 cell-cycle arrest through blocking p53-dependent p21 induction [11]. Moreover, several other chromone derivatives, for example, formylchromone [12], and its copper complexes [13,14], chromone gallates [13], and styryl chromones [15,16] have been reported to show anticancer activity.

We have previously explored the chemical diversity space around benzopyran-2-one (coumarin) scaffold and reported the synthesis and antiproliferative activities of a series of

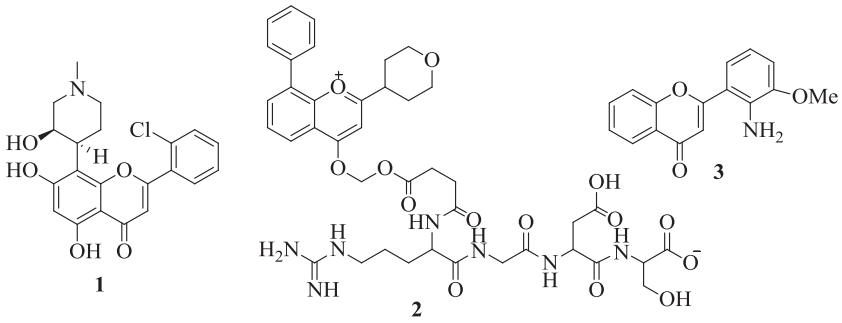


Figure 1. Chemical structures of potent biological active 4-oxo-4*H*-1-benzopyran derivatives.

coumarin derivatives against human colon adenocarcinoma (HT-29), breast carcinoma (MDA-MB-468 or MCF-7), ovarian adenocarcinoma (SK-OV-3) cells, and Src kinase inhibitory activity [17]. In continuation of our efforts to design antiproliferative agents and/or Src kinase inhibitors, herein we report the synthesis of novel differently substituted 4-oxo-4 *H*-1-benzopyran (chromone) derivatives, evaluation of their antiproliferative activity, and Src kinase inhibitory potency.

RESULTS AND DISCUSSION

A number of approaches have been developed for the synthesis of 4-oxo-4*H*-1-benzopyran derivatives by various groups in recent time. However, the most frequent classical methods used for the synthesis of chromones include Baker–Venkataraman rearrangement [18] and Vilsmeir–Haack formylation reaction [19]. Recently, the role of various acid catalysts in chromone ring closure has been meticulously reviewed by Li *et al*. [20]. Herein, we have also utilized a few acid catalysts and Vilsmeir–Haack formylation reaction for the synthesis of chromone derivatives with various substituents (alkyl, alkoxy, chloro, and acrylyl acid/ester/amide) at C-3, C-6, C-7, and C-8 - positions of 4-oxo-4*H*-1-benzopyran skeleton.

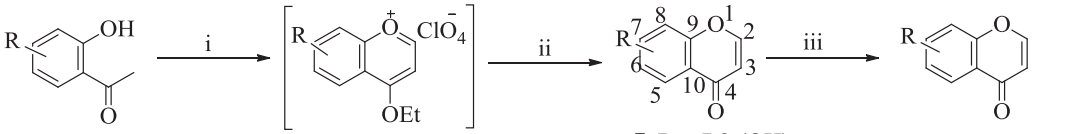
Synthesis of hydroxy/methoxy substituted 4-oxo-4H-1-benzopyrans 7-12. The hydroxychromones **7-9** were prepared in 55–60% yield from the corresponding hydroxyacetophenones **4-6** by treatment with triethyl orthoformate and perchloric acid (70%) followed by aqueous hydrolysis of the intermediate perchlorate salts (Scheme 1). Methylation of compounds **7-9** with methyl iodide afforded the corresponding methoxy chromones **10-12**.

Synthesis of 3-alkyl-4-oxo-4H-1-benzopyrans 15-21. A series of 3-alkyl-4-oxo-4*H*-1-benzopyran derivatives, viz. **15-21** were synthesized according to the previously reported procedure [21]. First, the precursors aryl alkyl ketones **14a-g** were synthesized from the corresponding substituted phenols **13a-c** by reacting them with alkanoic acids in the presence of a catalytic amount of zinc chloride. Compounds **14a-g** were then cyclized to 3-alkyl-4-oxo-4*H*-1-benzopyrans **15-21** using BF_3-etherate and methanesulphonyl chloride (Scheme 2).

Synthesis of (E)-alkyl-3-(4-oxo-4H-1-benzopyran-3-yl)acrylates 31-48. A series of (*E*)-alkyl-3-(4-oxo-4*H*-1-benzopyran-3-yl)acrylates **31-48** were synthesized starting from the corresponding hydroxyacetophenones according to the our previously reported procedure [22]. While using dihydroxyacetophenone (**5/6**), its monoacetylation was carried out first in the presence of acetic anhydride and pyridine. 4-oxo-4*H*-1-benzopyran-3-yl-carbaldehydes **25-27** were then synthesized using Vilsmeir–Haack formylation reaction, followed by the Knoevenagel condensation with malonic acid to yield the respective (4-oxo-4*H*-1-benzopyran-3-yl)acrylic acids **28-30**. The desired (*E*)-alkyl-3-(4-oxo-4*H*-1-benzopyran-3-yl)acrylates **31-46** were obtained by esterification of acrylic acids **28-30** with the corresponding alcohols under acidic conditions (Scheme 3). The methylation of compounds **37** and **42** by using methyl iodide under basic conditions afforded the corresponding (*E*)-ethyl 3-(methoxy-4-oxo-4*H*-1-benzopyran-3-yl)acrylates **47** and **48**. All of the products were fully characterized from their physical and spectral data. The structural data for compounds **32-34** and **37-39** have been previously reported by our group [22].

Synthesis of (E)-3-(4-oxo-4H-1-benzopyran-3-yl)-acrylamides 49-54. (*E*)-3-(4-Oxo-4*H*-1-benzopyran-3-yl)-*N*-alkylacrylamides

Scheme 1. Synthesis of hydroxy/methoxy-4-oxo-4*H*-1-benzopyrans. Reagents and conditions: i. $HC(OEt)_3$, 70% $HClO_4$, 2 h; ii. H_2O, 100°C, 10 min; iii. CH_3I, K_2CO_3, anhyd. acetone, 8 h.

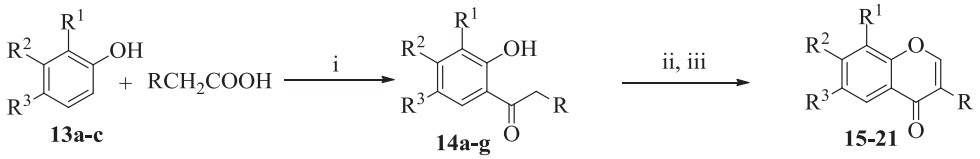


4 R = 3,4-$(OH)_2$
5 R = 4-OH
6 R = 5-OH

7 R = 7,8-$(OH)_2$
8 R = 7-OH
9 R = 6-OH

10 R = 7,8-$(OMe)_2$
11 R = 7-OMe
12 R = 6-OMe

Scheme 2. Synthesis of 3-alkyl-4-oxo-4*H*-1-benzopyrans. Reagents and conditions: i. $ZnCl_2$; ii. $BF_3.Et_2O$, dimethylformamide; iii. $MeSO_2Cl$, dimethylformamide.

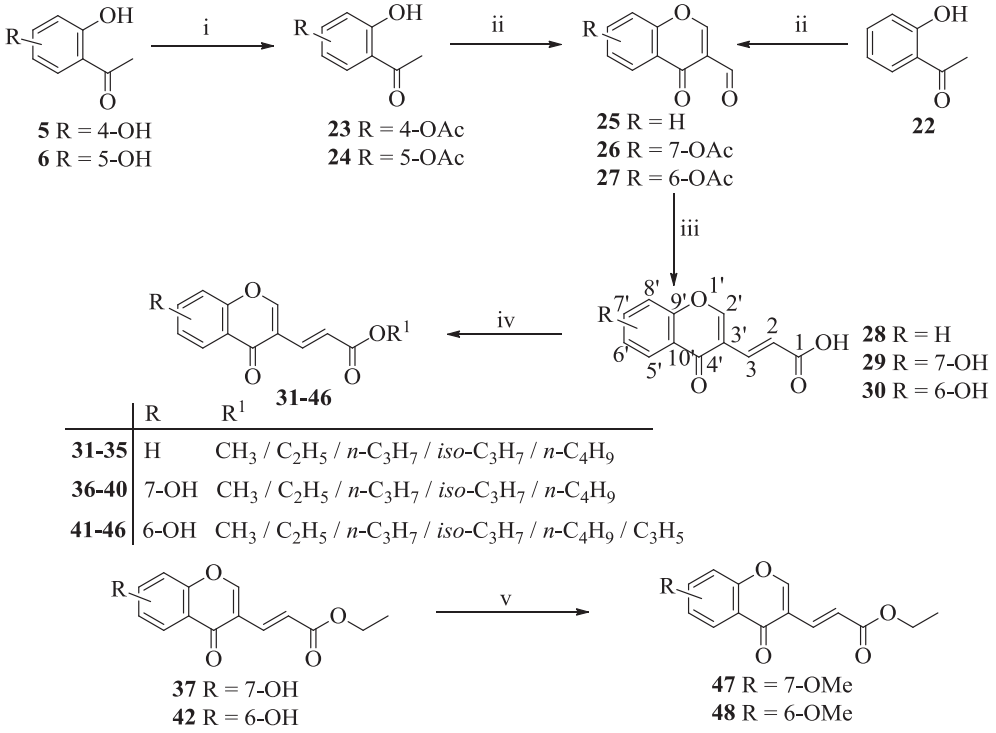


13a-c **14a-g** **15-21**

13	R^1	R^2	R^3
a	H	OH	H
b	OH	OH	H
c	H	OH	Cl

	R^1	R^2	R^3	R
14a / 15	H	OH	H	CH_3
14b / 16	H	OH	H	*n*-C_3H_7
14c / 17	H	OH	H	*n*-C_6H_{13}
14d / 18	OH	OH	H	CH_3
14e / 19	OH	OH	H	*n*-C_3H_7
14f / 20	OH	OH	H	*n*-C_6H_{13}
14g / 21	H	OH	Cl	*n*-C_3H_7

Scheme 3. Synthesis of (*E*)-alkyl 3-(4-oxo-4*H*-1-benzopyran-3-yl)acrylates. Reagents and conditions: i. $(CH_3CO)_2O$, pyridine, 6 h; ii. $POCl_3$, dimethylformamide, 50°C, 13 h; iii. $CH_2(COOH)_2$, pyridine, 1.5 h; iv. R^1OH, concentrated H_2SO_4 (1–2 drops), 12 h; v. CH_3I, K_2CO_3, anhydr. acetone, reflux, 12 h.



5 R = 4-OH
6 R = 5-OH

23 R = 4-OAc
24 R = 5-OAc

25 R = H
26 R = 7-OAc
27 R = 6-OAc

22

28 R = H
29 R = 7-OH
30 R = 6-OH

31-46

	R	R^1
31-35	H	CH_3 / C_2H_5 / *n*-C_3H_7 / *iso*-C_3H_7 / *n*-C_4H_9
36-40	7-OH	CH_3 / C_2H_5 / *n*-C_3H_7 / *iso*-C_3H_7 / *n*-C_4H_9
41-46	6-OH	CH_3 / C_2H_5 / *n*-C_3H_7 / *iso*-C_3H_7 / *n*-C_4H_9 / C_3H_5

37 R = 7-OH
42 R = 6-OH

47 R = 7-OMe
48 R = 6-OMe

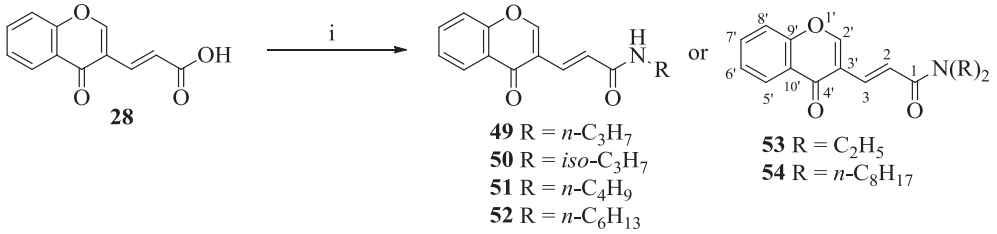
49-54 were synthesized by reacting (*E*)-3-(4-oxo-4*H*-1-benzopyran-3-yl)acrylic acid **28** with appropriate amines in the presence of 1-hydroxybenzotriazole (HOBt) and dicyclohexyl carbodiimide (DCC) (Scheme 4).

Synthesis of quaternary ammonium derivatives of 4-oxo-4H-1-benzopyran. The quaternary ammonium derivatives **58** and **59** were synthesized according to the previously reported procedure (Scheme 5) [23].

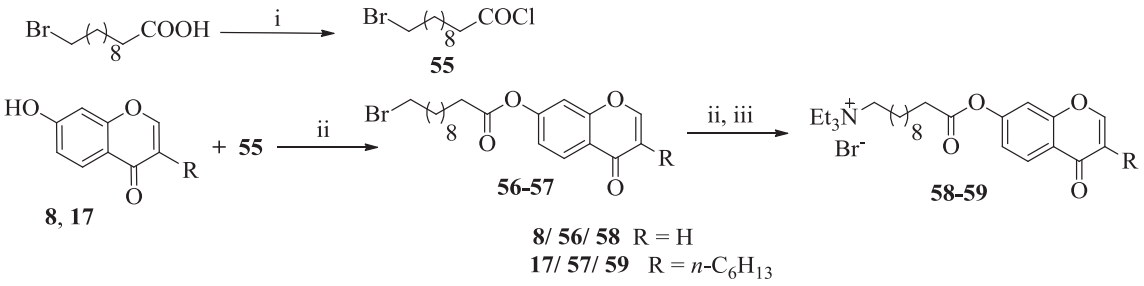
Antiproliferative activity. 4-Oxo-4*H*-1-benzopyran derivatives were evaluated against three human cancer cell lines, colon adenocarcinoma (HT-29), breast carcinoma (MDA-68), and ovarian adenocarcinoma (SK-OV-3) cell lines at 50 μM concentration (Figs. 2 and 3).

Antiproliferative assay results showed that among the simple chromones **7-12** and 3-alkyl chromones **15-21**, only one compound 3-hexyl-7,8-dihydroxy-4-oxo-4*H*-1-benzopyran (**20**) exhibits significant antiproliferative activity against all of the three cell lines studied. The antiproliferative activity of 3-acrylyl acid **(28-30)**, ester **(31-48)**, and amide **(49-59)** derivatives of 4-oxo-4*H*-1-benzopyran was also compared (Figs. 2 and 3). The acids and amides were found to have insignificant antiproliferative activity; however, the acrylyl

Scheme 4. Synthesis of (*E*)-3-(4-oxo-4*H*-1-benzopyran-3-yl)-*N*-alkylacrylamide and (*E*)-*N*,*N*-dialkyl-3-(4-oxo-4*H*-1-benzopyran-3-yl)acrylamide. Reagents and conditions: i. RNH_2/$(R)_2NH$, dimethylformamide, HOBt, DCC.



Scheme 5. Synthesis of quaternary ammonium derivatives of 4-oxo-4*H*-1-benzopyran. Reagents and conditions: i. oxalyl chloride, dichloromethane; ii. triethylamine (1.0 eq), acetonitrile; iii. triethylamine (excess), acetonitrile, 60°C, 48 h.



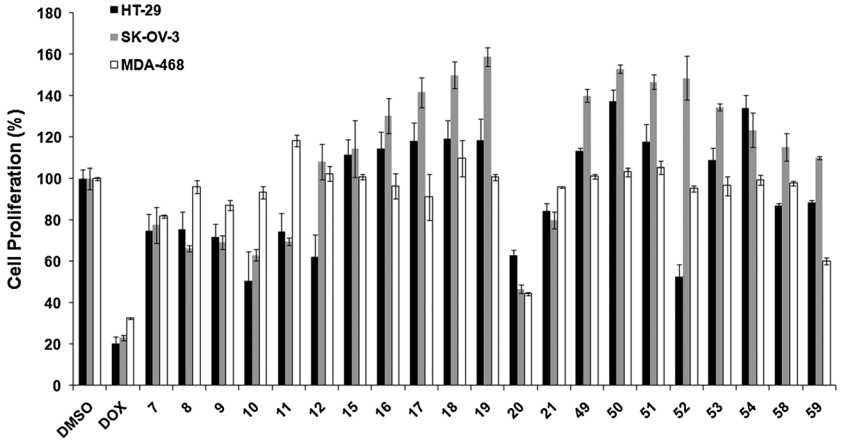


Figure 2. Inhibition of HT-29, SK-OV-3, and MDA-468 cells by compounds **7-12**, **15-21**, and **49-54** and **58-59** (50 μM) after 72 h incubation. The results are shown as the percentage of the control that has no compound (set at 100%). All the experiments were performed in triplicate.

ester derivatives **31-48** were observed to possess low to moderate activity. Out of eighteen 4-oxo-4*H*-1-benzopyran-3yl acrylyl esters (**31-48**), six compounds, that is, **31-32**, **37-38**, **40**, and **47** exhibited high to moderate antiproliferative activity against at least one cell line. In these ester derivatives, the presence of a hydroxy or methoxy group at the C-7 position was found to enhance the antiproliferative activity. However, the presence of such substituent at the C-6 position decreased the antiproliferative potency. An analysis of the activity of various esters revealed that ethyl esters exhibited optimal activity. The ethyl ester derivative **47** [(*E*)-ethyl 3-(7-methoxy-4-oxo-4*H*-1-benzopyran-3-yl)acrylate] was found to be the most potent compound to inhibit the cell proliferation in all three cell lines screened. However, the corresponding 7-hydroxy analog **37** showed antiproliferative activity against SK-OV-3 cells only (Fig. 3). These preliminary results can be used for the further development of lead compounds with improved antiproliferative activity.

Src kinase inhibitory activity. To get a better understanding of the mechanism of the antiproliferative activity of these compounds, the Src kinase inhibitory potency of all 4-oxo-4*H*-1-benzopyran derivatives were tested. Table 1 shows the inhibitory potency results of the synthesized compounds compared with a standard protein

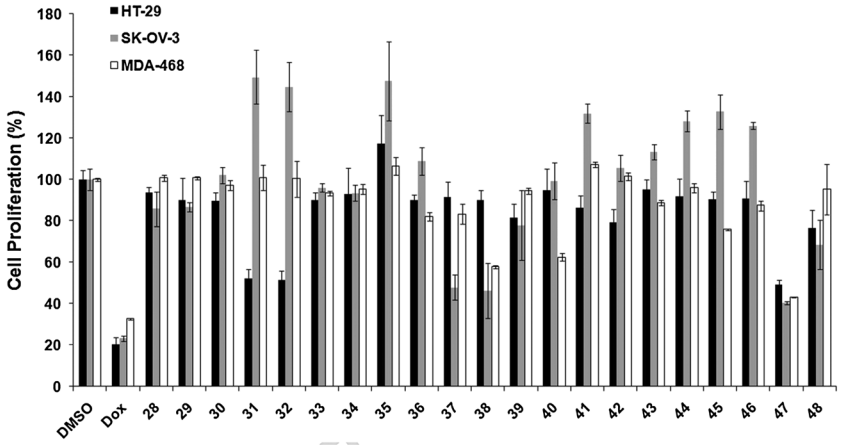


Figure 3. Inhibition of HT-29, SK-OV-3, and MDA-468 cells by compounds **28-48** (50 μM) after 72 h incubation. The results are shown as the percentage of the control that has no compound (set at 100%). All the experiments were performed in triplicate.

kinase inhibitor, staurosporine, and an Src kinase inhibitor, PP2. Among all of the 42 compounds, only three, **31**, **32**, and **53**, showed moderate Src kinase inhibitory potency with IC_{50} values in the range of 50 μM. Compounds **31** and **32** possessed antiproliferative activity (48–49%) against HT-29 cells. On comparing the antiproliferative results with Src kinase inhibitory data, a correlation pattern could not be established, suggesting that these compounds exhibit antiproliferative activity by other mechanisms.

Table 1

Src kinase inhibitory activity of different substituted derivatives of 4-oxo-4*H*-1-benzopyran (**7-12**, **15-21**, **28-54**, **58**, and **59**).

Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)[a]
7	<300	**37**	ND[b]
8	<250	**38**	<300
9	<300	**39**	<300
10	<300	**40**	<300
11	<300	**41**	<300
12	<300	**42**	<300
15	>150	**43**	<300
16	100.0	**44**	<300
17	>150	**45**	<300
18	>150	**46**	<300
19	109.6	**47**	<300
20	<300	**48**	ND[b]
21	<300	**49**	>150
28	<300	**50**	>150
29	<300	**51**	>150
30	<300	**52**	>150
31	54.8	**53**	52.1
32	56.7	**54**	>150
33	<300	**58**	<250
34	<300	**59**	<300
35	<300	**Staurosporine**	0.6
36	>150	**PP2**	0.5

[a]The concentration at which the enzyme activity is inhibited by 50%. IC_{50} value is calculated from Graph Prism software. [b]Not determined, insoluble under assay conditions.

CONCLUSION

A series of 42 differently substituted 4-oxo-4*H*-1-benzopyran derivatives were synthesized and evaluated for their antiproliferative activity against a panel of three human cancer cell lines and Src kinase inhibitory activity. The structures of all the compounds were confirmed by ^{1}H NMR, ^{13}C NMR, HRMS, UV, and FT-IR spectral techniques. Among the compounds reported here, 18 are novel, that is, the compounds **16-17**, **19-21**, **36**, **40-46**, **49-52**, and **54**. Although the compounds **7-12**, **15**, **18**, **28-31**, **47-48**, and **53** were previously reported in literature, their complete spectral data were not reported. Herein, we have provided the spectral data of the compounds in the experimental section. The antiproliferative activity data showed that seven compounds were active against HT-29 cell lines, six against SK-OV-3 cell lines, whereas only four compounds were found to have modest activity against MDA-468 cell lines. Only three compounds were found to have noticeable Src kinase inhibitory activity. Compound **47** showed 51–60% antiproliferative activity against all three cancer cell lines (HT-29, SK-OV-3, and MDA-468) studied but failed to exhibit any significant Src kinase inhibitory activity. Structure-activity relationship studies provide insights for designing the next generation of 4-oxo-4*H*-1-benzopyran derivatives and development of new leads as anticancer agents. These preliminary results can be further explored for the development of compounds with improved antiproliferative activity.

EXPERIMENTAL

The organic solvents were dried and distilled prior to their use. Reactions were monitored by using precoated TLC plates (Merck silica gel 60 F254); the spots were visualized either by UV light or by spraying of alcoholic $FeCl_3$ solution (5%). Silica gel (100–200 mesh) was used for column chromatography. All of the chemicals and reagents were procured from Spectrochem Pvt. Ltd., India and Sigma-Aldrich Chemicals Pvt. Ltd., USA. Melting points were measured on Buchi M-560 apparatus and

are uncorrected. Infrared spectra were recorded on a PerkinElmer FT-IR model 9 spectrophotometer. The ^{1}H & ^{13}C NMR spectra were recorded on a Jeol-400 (400 MHz, 100.5 MHz) NMR spectrometer, Bruker Avance-300 (300 MHz, 75.5 MHz), and Bruker-500 (500 MHz, 125.5 MHz) spectrometer using tetramethylsilane as the internal standard. The chemical shift values are on a δ scale, and the coupling constant values (*J*) are in hertz. The HRMS data were recorded on Agilent-6210 ES-TOF, JEOL JMX-SX-102A, and Waters LCT Micromass-KC455 instruments.

General procedure for the synthesis of hydroxy-4-oxo-4H-1-benzopyrans 7-9. A mixture of dihydroxyacetophenone or trihydroxyacetophenone (10 g) in triethyl orthoformate (58 mL) was placed into a 500 mL beaker. While the suspension was stirred on a magnetic stirrer, 70% perchloric acid (6.6 mL) was then slowly added. The resulting warm and thick dark solution was stirred until it cooled to room temperature (about 1 h). Anhydrous diethyl ether (250 mL) was added to precipitate out the brown-colored intermediate of the oxonium perchlorate salt. The salt was taken in water (250 mL) and hydrolyzed by heating to reflux for 5 min. The solution was then allowed to cool at room temperature over night. A dark brown solid precipitated out, which was filtered, dried under vacuum, and purified by column chromatography using 20–30% ethyl acetate/petroleum ether as eluents to give the desired hydroxy-4*H*-chromen-4-ones **7-9** in 55–60% yield.

7,8-Dihydroxy-4-oxo-4H-1-benzopyran (7) [24]. Compound **7** was obtained as a light yellow solid in 55% yield. mp: 269–270°C (*d*); UV (MeOH) λ_{max}: 320 and 220 nm; IR (KBr) υ_{max}: 3435 (OH), 2978, 1628 (CO), 1570, 1476, 1420, 1333, 1236, 1137, 1146, and 982 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.18 (d, J = 5.9 Hz, 1H, H-3), 6.93 (d, 1H, J = 8.8 Hz, H-6), 7.38 (d, J = 8.8 Hz, 1H, H-5), 8.17 (d, J = 5.9 Hz, 1H, H-2), 9.45 (s, 1H, OH), and 10.18 (s, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 111.48, 114.14, 115.23, 117.94, 133.16, 147.03, 150.23, 155.96, and 176.29; HRMS, *m/z*: $[M+H]^+$ calculated for $C_9H_6O_4$ 178.0266, found 179.0164.

7-Hydroxy-4-oxo-4H-1-benzopyran (8). Compound **8** was obtained as a light yellow solid in 60% yield. mp: 214–216°C (lit. value: 215–216°C) [25]; UV (MeOH) λ_{max}: 300, 248, and 240 nm; IR (KBr) υ_{max}: 3100 (OH), 2928, 1629 (CO), 1592, 1560, 1508, 1407, 1313, 1270, 1078, 948, and 860 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.35 (d, J = 5.9 Hz, 1H, H-3), 6.80 (s, 1H, H-8), 6.89 (d, 1H, J = 8.8 Hz, H-6), 7.85 (d, J = 8.8 Hz, 1H, H-5), 8.08 (d, J = 5.9 Hz, 1H, H-2), and 10.76 (brs, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 102.42, 111.98, 115.09, 117.13, 126.76, 156.06, 157.82, 162.69, and 175.76; HRMS, *m/z*: $[M+H]^+$ calculated for $C_9H_6O_3$ 163.0317, found 163.0338.

6-Hydroxy-4-oxo-4H-1-benzopyran (9). Compound **9** was obtained as a light yellow solid in 58% yield. mp: 244–245°C (lit. value: 244°C) [25]; UV (MeOH) λ_{max}: 325 and 230 nm; IR (KBr) υ_{max}: 3447 (OH), 3069, 1629 (CO), 1612, 1570, 1475, 1420, 1333, 1236, 1137, 867, and 826 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.24 (d, J = 5.9 Hz, 1H, H-3), 6.20 (dd, J = 3.0 and 8.8 Hz, 1H, H-7), 7.30 (d, 1H, J = 3.0 Hz, H-5), 7.46 (d, J = 8.8 Hz, 1H, H-8), 8.18 (d, J = 5.9 Hz, 1H, H-2), and 10.01 (brs, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 107.57, 111.15, 119.84, 123.14, 125.19, 149.76, 154.86, 156.53, and 176.38; HRMS, *m/z*: $[M+Na]^+$ calculated for $C_9H_6O_3$ 185.0215, found 185.0227.

General procedure for the synthesis of methoxy-4-oxo-4*H*-1-benzopyrans (10-12). A mixture of mono/dihydroxy-4-oxo-4*H*-1-benzopyran (0.5 g), anhydrous potassium carbonate (0.5/1.0 g) and methyl iodide (0.5/1.0 mL) in anhydrous acetone (50 mL) was refluxed for 12–14 h, and the progress of the reaction was monitored on TLC (2% methanol-chloroform). On completion of the reaction, the mixture was cooled to room temperature and the solvent was filtered, and the resulting filtrate was evaporated under reduced pressure. The resulting precipitate was then washed with water and crystallized from acetone to give the desired methoxy-4-oxo-4*H*-1-benzopyrans **10-12** in quantitative yield.

7,8-Dimethoxy-4-oxo-4H-1-benzopyran (10) [26]. Compound **10** was obtained as a light yellow solid in 96% yield. mp:122–123°C; UV (MeOH) λ_{max}: 317 and 220 nm; IR (KBr) υ_{max}: 2940, 1661 (CO), 1620, 1599, 1405, 1325, 1292, 1253, 1186, 1080, and 811 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.17 (d, J = 5.5 Hz, 1H, H-3), 6.94 (d, 1H, J = 8.8 Hz, H-6), 7.79 (d, J = 8.8 Hz, 1H, H-5), and 7.82 (d, J = 5.5 Hz, 1H, H-2); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 56.21, 61.33, 109.83, 112.25, 119.30, 120.94, 136.49, 150.63, 154.87, 156.31, and 176.88; HRMS, *m/z*: calculated for $C_{11}H_{10}O_4$ $[M+H]^+$ and $[M+Na]^+$ 207.0579, 229.0477 found 207.0414 and 229.0292, respectively.

7-Methoxy-4-oxo-4H-1-benzopyran (11). Compound **11** was obtained as a light yellow solid in 94% yield. mp: 57–58°C (lit. value: 56–57°C) [27]; UV(MeOH) λ_{max}: 300, 248, and 242 nm; IR (KBr) υ_{max}: 2925, 1624 (CO), 1594, 1438, 1310, 1271, 1225, 1035, 857, and 828 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.87 (s, 3H, OCH_3), 6.28 (d, J–5.9 Hz, 1H, H-3), 6.81 (d, J = 2.2 Hz, 1H, H-8), 6.94 (dd, J = 2.2 and 8.8 Hz, 1H, H-6), 7.76 (d, J = 5.9 Hz, 1H, H-2), and 8.08 (d, J = 8.8 Hz, 1H, H-5); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 55.77, 100.28, 112.29, 114.49, 118.71, 127.14, 154.81, 158.19, 164.03, and 176.99; HRMS, *m/z*: Calculated for $C_{10}H_8O_3$ $[M+H]^+$ and $[M+Na]^+$ 177.0473, 199.0371, found 177.0364 and 199.0197, respectively.

6-Methoxy-4-oxo-4H-1-benzopyran (12). Compound **12** was obtained as a light yellow solid in 92% yield. mp: 82–83°C (lit. value: 82–83°C) [28]; UV(MeOH) λ_{max}: 320 and 225 nm, IR (KBr) υ_{max}: 2982, 1636 (CO), 1484, 1312, 1227, 1021, and 818 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.86 (s, 3H, OCH_3), 6.29 (d, J = 5.9 Hz, 1H, H-3), 7.22 (dd, J = 2.9 and 8.8 Hz, 1H, H-7), 7.36 (d, 1H, J = 8.8 Hz, H-8), 7.53 (d, J = 2.9 Hz, 1H, H-5), and 7.82 (d, J = 5.9 Hz, 1H, H-2); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 55.77, 104.64, 111.97, 119.49, 123.73, 125.32, 151.23, 154.95, 156.79, and 177.36; HRMS, *m/z*: calculated for $C_{10}H_8O_3$ $[M+Na]^+$ 199.0371, found 199.0358.

General procedure for the synthesis of 3-alkyl-4-oxo-4H-1-benzopyrans 15-21. Alkylaryl ketone (**14a-g**) was first prepared from the appropriate phenol (**13a-c**) by following the literature procedure [29]. Then, to the solution of **14a-g** (11 mmol) in DMF (7 mL), BF_3^-/etherate (2 mL) was added dropwise, and the mixture was stirred for 1 h followed by addition of methanesulphonyl chloride (1.5 mL) in DMF (5 mL) at 50°C. The reaction mixture was stirred at 100°C for 90 min, cooled, and poured into ice-cold water. The resulting precipitate was filtered and washed with water. The crude product was recrystallized from acetone to afford the desired 3-alkyl substituted 4-oxo-4*H*-1-benzopyrans as brown crystals in 80–90% yield (Scheme 2).

7-Hydroxy-3-methyl-4-oxo-4H-1-benzopyran (15). Compound **15** was obtained as a brown solid in 82% yield by following the general procedure. mp: 228–229°C (lit. value: 223°C) [21]; UV (MeOH) λ_{max}: 295 nm; IR (KBr) υ_{max}: 3401 (OH), 3216, 1637 (CO), 1588, 1471, 1309, 1252, 1180, 844, 771, and 691 cm^{-1};

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.86 (s, 3H, H-1′), 6.80 (d, J = 2.2 Hz, 1H, H-8), 6.89 (dd, J = 2.2 and 8.8 Hz, 1H, H-6), 7.89 (d, J = 8.8 Hz, 1H, H-5), 8.12 (s, 1H, H-2), and 10.68 (s, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 11.65, 102.93, 115.74, 116.75, 120.02, 127.64, 153.09, 158.77, 163.16, and 177.18; HRMS, *m/z*: calculated for $C_{10}H_8O_3$ $[M+H]^+$ 177.0552, found 177.0570.

7-Hydroxy-3-propyl-4-oxo-4H-1-benzopyran (16). Compound **16** was obtained as a brown solid in 87% yield. mp: 186–187°C; UV (MeOH) λ_{max}: 297 nm; IR (KBr) υ_{max}: 3226 (OH), 2964, 1633 (CO), 1586, 1403, 1241, 1164, 855, and 726 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, J = 7.4 Hz, 3H, H-3′), 1.45–1.54 (m, 2H, H-2′), 2.29 (t, J = 7.4 Hz, 2H, H-1′), 6.80 (d, J = 2.2 Hz, 1H, H-8), 6.89 (dd, J = 2.2, 8.8 Hz, 1H, H-6), 7.88 (d, J = 8.8 Hz, 1H, H-5), 8.08 (s, 1H, H-2), and 10.67 (s, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 14.55, 22.12, 27.87, 102.92, 115.71, 117.01, 123.73, 127.73, 153.46, 158.59, 163.17, and 176.71; HRMS, *m/z*: calculated for $C_{12}H_{12}O_3$ $[M+Na]^+$ 227.0679, found 227.0672.

3-Hexyl-7-hydroxy-4-oxo-4H-1-benzopyran (17). Compound **17** was obtained as a brown solid in 89% yield. mp: 113–114°C; UV (MeOH) λ_{max}: 296 nm; IR (KBr) υ_{max}: 3242 (OH), 2930, 1635 (CO), 1574, 1404, 1242, 1166, 857, and 719 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84 (d, J = 6.6 Hz, 3H, H-6′), 1.23–1.30 (m, 6H, H-3′, H-4′, H-5′), 1.42–1.49 (m, 2H, H-2′), 2.30 (t, J = 7.7 Hz, 2H, H-1′), 6.80 (d, J = 2.2 Hz, 1H, H-8), 6.88 (dd, J = 2.2 and 8.8 Hz, 1H, H-6), 7.88 (d, J = 8.8 Hz, 1H, H-5), 8.07 (s, 1H, H-2), and 10.77 (s, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 14.78, 22.90, 25.85, 28.89, 29.27, 31.90, 102.91, 115.70, 117.00, 124.00, 127.00, 153.00, 158.58, 163.17, and 176.69; HRMS, *m/z*: calculated for $C_{15}H_{18}O_3$ $[M+H]^+$ 247.1334, found 247.1329.

7,8-Dihydroxy-3-methyl-4-oxo-4H-1-benzopyran (18). Compound **18** was obtained as a brown solid in 85% yield. mp: 274–275°C (lit. value: 203°C) [21]; UV (MeOH) λ_{max}: 301 nm; IR (KBr) υ_{max}: 3319 (OH), 1635 (CO), 1554, 1459, 1352, 1205, 1063, 928, and 689 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.87 (s, 3H, H-1′), 6.90 (d, J = 8.7 Hz, 1H, H-6), 7.38 (d, J = 8.7 Hz, 1H, H-5), 8.17 (s, 1H, H-2), 9.40 (s, 1H, OH), and 10.26 (s, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 11.69, 114.80, 115.98, 117.54, 119.42, 133.65, 148.04, 150.53, 152.93, and 177.63; HRMS, *m/z*: calculated for $C_{10}H_8O_4$ $[M+H]^+$ 193.0501, found 193.0514.

7,8-Dihydroxy-3-propyl-4-oxo-4H-1-benzopyran (19). Compound **19** was obtained as a brown solid in 90% yield. mp: 144–145°C; UV (MeOH) λ_{max}: 262 and 302 nm; IR (KBr) υ_{max}: 3512 (OH), 2928, 1632 (CO), 1597, 1414, 1305, 1160, 1027, 923, and 768 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, J = 7.5 Hz, 3H, H-3′), 1.45–1.54 (m, 2H, H-2′), 2.39 (t, J = 7.5 Hz, 2H, H-1′), 6.91 (d, J = 8.7 Hz, 1H, H-6), 7.38 (d, J = 8.7 Hz, 1H, H-5), 8.13 (s, 1H, H-2), 9.40 (s, 1H, OH), and 10.27 (s, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 14.46, 22.18, 27.62, 114.76, 116.09, 117.81, 123.14, 133.65, 147.88, 150.55, 153.29, and 177.17; HRMS, *m/z*: calculated for $C_{12}H_{12}O_4$ $[M+Na]^+$ 243.0628, found 243.0627.

3-Hexyl-7,8-dihydroxy-4-oxo-4H-1-benzopyran (20). Compound **20** was obtained as a brown solid in 88% yield. mp: 73–74 °C; UV (MeOH) λ_{max}: 269 and 352 nm; IR (KBr) υ_{max}: 3411 (OH), 3196, 2928, 2855, 1631 (CO), 1594, 1463, 1332, 1175, 925, and 782 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.82 (t, J = 6.5 Hz, 3H, H-6′), 1.22-1.27 (m, 6H, H-3′, H-4′ and H-5′), 1.42–1.49 (m, 2H, H-2′), 2.30 (t, J = 7.6 Hz, 2H, H-1′), 6.90 (d, J = 8.7 Hz, 1H, H-6), 7.38 (d, J = 8.7 Hz, 1H, H-5), 8.12 (s, 1H, H-2), 9.39 (s, 1H, OH), and 10.26 (s, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 13.27, 21.38, 24.35, 27.42, 27.76, 30.38, 113.24, 114.46, 116.27, 121.87, 132.12, 146.33, 149.00, 151.62, and 175.68; HRMS, *m/z*: calculated for $C_{15}H_{18}O_4$ $[M+H]^+$ 263.1278, found 263.1277.

6-Chloro-7-hydroxy-3-propyl-4-oxo-4H-1-benzopyran (21). Compound **21** was obtained as an off-white solid in 90% yield. mp: 236–237 °C; UV (MeOH) λ_{max}: 224, 318 and 340 nm; IR (KBr) υ_{max}: 3422 (OH), 3180, 2954, 1637 (CO), 1582, 1566, 1499, 1396, 1246, 1155, and 721 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, J = 7.3 Hz, 3H, H-3′), 1.46–1.54 (m, 2H, H-2′), 2.32 (t, J = 7.3 Hz, 2H, H-1′), 6.91 (s, 1H, H-8), 7.57 (s, 1H, H-5), 8.06 (s, 1H, H-2), and 10.08 (s, 1H, OH); ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 13.70, 21.23, 27.05, 103.59, 116.59, 119.35, 122.97, 125.69, 152.85, 155.91, 157.72, and 175.07; HRMS, *m/z*: calculated for $C_{12}H_{11}ClO_3$ $[M+H]^+$ 239.0475, found 261.0692.

General procedure for synthesis of (E)-3-(4-oxo-4H-1-benzopyran-3-yl)acrylic acids 28-30. A mixture of 4-oxo-4*H*-1-benzopyran-3-yl-carbaldehyde (**25-27**, 20 mmol), malonic acid (20 mmol), and pyridine (50 mL) was heated at 110°C; additional malonic acid (5 mmol) was added to the reaction mixture after 1 h, and the contents were stirred for 30 min. The solvent was evaporated under reduced pressure. The resulting precipitate was filtered, washed with water, and purified by column chromatography using 4–5 % methanol-chloroform as eluents to afford the desired (*E*)-3-(4-oxo-4*H*-1-benzopyran-3-yl)acrylic acids **28-30** as yellow needles in 78–87% yield.

(E)-3-(4-oxo-4H-1-benzopyran-3-yl)acrylic acid (28). Compound **28** was obtained as yellow crystals in 87% yield. mp: 251–253°C (lit. value: 253–254°C), (*d*) [30,31]; UV (MeOH) λ_{max}: 217, 264, and 287 nm; IR (KBr) υ_{max}: 3422 (CO*OH*), 3065, 2926, 2851, 1660 (*CO*OH), 1611 (CO), 1561 (C═C), 1465, 1359, 1300, 1279, 988, and 877 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.10 (d, 1H, J = 15.9 Hz, H-2), 7.40 (d, 1H, J = 15.9 Hz, H-3), 7.52 (t, J = 6.0, 1H, H-6'), 7.67 (d, 1H, J = 6.8 Hz, H-8'), 7.82 (t, 1H, J = 7.2, H-7'), 8.10 (d, 1H, J = 6.9 Hz, H-5'), 8.84 (s, 1H, H-2'), and 12.25 (brs, 1H, COOH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 119.51, 122.51, 124.62, 126.53, 127.05, 135.51, 136.86, 156.19, 160.68, 168.86, and 176.34; HRMS, *m/z*: calculated for $C_{12}H_8O_4$ $[M]^{+\cdot}$ 216.1895, found 216.8750.

(E)-3-(7-Hydroxy-4-oxo-4H-1-benzopyran-3-yl)acrylic acid (29). Compound **29** was obtained as yellow crystals in 81% yield. mp: 287–288°C (*d*), (lit. value: 288–290°C) [30,31]; UV (MeOH) λ_{max}: 278 and 233 nm; IR (KBr) υ_{max}: 3264 (CO*OH*), 3079, 1716 (*CO*OH), 1671 (CO), 1613 (C═C), 1467, 1336, 1268, 1247, 1157, and 978 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14 (d, 1H, J = 16.2 Hz, H-2), 7.22 (dd, 1H, J = 2.9 and 8.8 Hz, H-6'), 7.36 (d, 1H, J = 2.9 Hz, H-8'), 7.43 (d, 1H, J = 16.2 Hz, H-3), 7.53 (d, 1H, J = 8.8 Hz, H-5'), 8.79 (s, 1H, H-2'), and 10.10 (brs, 1H, COO*H*). ^{13}C NMR (100.5 MHz, DMSO-d_6,): δ 102.50, 115.74, 116.30, 117.87, 121.14, 127.32, 136.24, 157.01, 159.11, 163.11, 168.03, and 174.64; HRMS, *m/z*: calculated for $C_{12}H_8O_5$ $[M+H]^+$ 233.0372, found 233.0382.

(E)-3-(6-Hydroxy-4-oxo-4H-1-benzopyran-3-yl)acrylic acid (30) [31]. Compound **30** was obtained as yellow crystals in 78% yield. mp: 264°C (*d*); UV(MeOH) λ_{max}: 267 and 213 nm; IR (KBr) υ_{max}: 3253 (CO*OH*), 3075, 1704 (*CO*OH), 1662 (CO), 1603 (C═C), 1473, 1346, 1284, 1247, 1146, and 982 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.05(d, 1H, J = 15.8 Hz, H-2), 7.20–7.24 (dd, 1H, J = 2.5 and 8.9 Hz, H-7'),

7.34–7.39 (m, 2H, H-3, H-5'), 7.50 (d, 1H, J = 8.9 Hz, H-8'), and 8.71 (s, 1H, H-2'); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 108.87, 117.87, 120.67, 121.72, 124.27, 125.25, 137.07, 149.70, 156.18, 160.21, 168.76, and 176.02; HRMS, *m/z*: calculated for $C_{12}H_8O_5$ $[M + Na]^+$ 255.0269, found 255.0325.

General procedure for the synthesis of (E)-3-(4-oxo-4H-1-benzopyran-3-yl)acrylates 31-46. A mixture of (*E*)-3-(4-oxo-4*H*-1-benzopyran-3-yl)acrylic acid (**28-30**, 1 g), appropriate alcohol (50 mL), and conc. sulfuric acid (2–3 drops) was refluxed. The esterification reaction was monitored by TLC. On completion of reaction, the reaction mixture was allowed to cool to room temperature. Then alcohol was evaporated under reduced pressure, and the resulting product was poured in ice-cold water with continuous stirring. The precipitated solid was filtered, washed with water, and purified by column chromatography using 20–40 % ethyl acetate/petroleum ether as eluents to give the desired acrylates **31-46** in 90–96% yield (Scheme 3).

(E) Methyl 3-(4-oxo-4H-1-benzopyran-3-yl)acrylate (31). Compound **31** was obtained as yellow crystals in 95% yield. mp: 95–96°C (lit. value: 92°C) [32]; UV (MeOH) λ_{max}: 290, 266, and 218 nm; IR (KBr) υ_{max}: 3085, 2953, 1723 (*CO*O), 1647 (CO), 1614 (C═C), 1560, 1467, 1365, 1266, 1041, 990, and 863 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.81 (s, 3H, H-1"), 7.29 (d, 1H, J = 15.8 Hz, H-2), 7.40–7.51 (m, 3H, H-3, H-6', and H-8'), 7.72 (t, 1H, J = 7.6 Hz, H-7'), 8.13 (s, 1H, H-2'), and 8.30 (d, 1H, J = 7.8 Hz, H-5'); ^{13}C NMR (125.5 MHz, $CDCl_3$): δ 52.06, 118.50, 119.77, 122.24, 124.66, 126.24, 126.78, 134.39, 135.99, 155.97, 157.72, 169.11, and 178.78; HRMS, *m/z*: calculated for $C_{13}H_{10}O_4$ $[M + Na]^+$ 253.0477, found 253.0464.

(E) Butyl 3-(4-oxo-4H-1-benzopyran-3-yl)acrylate (35) [33]. Compound **35** was obtained as yellow crystals in 98% yield. mp: 129–130°C; UV (MeOH) λ_{max}: 289, 265, and 217 nm; IR (KBr) υ_{max}: 3063, 2958, 2932, 2871, 1706 (*CO*O), 1657 (CO), 1613 (C═C), 1561, 1469, 1356, 1292, 1265, 1162, 1033, 997, and 857 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$,): δ 0.96 (t, J = 6.4 Hz, 3H, H-4"), 1.39–1.47 (m, 2H, H-3"), 1.65–1.71 (m, 2H, H-2"), 4.20 (t, J = 6.8 Hz, 2H, H-1"), 7.28 (d, 1H, J = 15.8 Hz, H-2), 7.38–7.48 (m, 3H, H-3, H-6' and H-8'), 7.68 (t, 1H, J = 7.6 Hz, H-7'), 8.11 (s, 1H, H-2'), and 8.26 (d, 1H, J = 7.9 Hz, H-5'); ^{13}C NMR (125.5 MHz, $CDCl_3$): δ 14.08, 19.56, 31.15, 64.76, 118.48, 119.77, 122.67, 124.61, 125.52, 126.18, 134.35, 135.62, 155.97, 157.65, 167.79, and 176.23; EIMS, *m/z*: 272.11 ($M^{+\cdot}$), 215.04 ($M^{+\cdot} - C_4H_9$), 199.04 ($M^{+\cdot} - OC_4H_9$), 189.90, 171.04 ($M^{+\cdot} - OC_4H_9$-CO), 149.95, 115.06, 82.93, and 57.02 ($C_4H_9^+$).

(E) Methyl 3-(7-hydroxy-4-oxo-4H-1-benzopyran-3-yl) acrylate (36). Compound **36** was obtained as yellow crystals in 83% yield. mp: 249–250°C; UV (MeOH) λ_{max}: 263 and 255 nm; IR (KBr) υ_{max}: 3083 (OH), 1718.34 (*CO*O), 1654.39 (CO), 1630.29 (C═C), 1457.49, 1437.10, 1289.47, 1170.35, 1099.79, 841.47, and 783.93 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.22 (s, 3H, H-1"), 6.31 (d, 1H, J = 2.2 Hz, H-8'), 6.39 (d, 1H, J = 8.8 Hz, H-6'), 6.68 (d, 1H, J = 16.1 Hz, H-2), 6.88 (d, 1H, J = 16.1 Hz, H-3), 7.46 (d, 1H, J = 8.8 Hz, H-5'), 7.79 (s, 1H, H-2'), 9.98 (brs, 1H, OH); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 50.26, 101.45, 114.57, 115.51, 117.14, 119.22, 126.11, 133.37,156.05, 156.90, 162.01, 166.29, and 173.91; HRMS, *m/z*: calculated for $C_{13}H_{10}O_5$ $[M + Na]^+$ 269.0426, found 269.0437.

(E) Butyl 3-(7-hydroxy-4-oxo-4H-1-benzopyran-3-yl)acrylate (40). Compound **40** was obtained as yellow crystals in 83% yield following the general procedure; mp: 149–150°C; UV (MeOH) λ_{max}: 272, 246, and 216 nm; IR (KBr) υ_{max}: 3152.11 (OH), 2955.91, 1707.66 (*CO*O), 1648.31 (CO), 1623.63 (C═C), 1571.93, 1412.46, 1287.20, 1243.87, 1169.71, and 849.49 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (t, 3H, J = 7.3 Hz, H-4"), 1.13–1.18 (m, 2H, H-3"), 1.37–1.44 (m, 2H, H-2"), 3.89 (t, 2H, J = 6.6 Hz, H-1"), 6.58 (d, 1H, J = 2.2 Hz, H-8'), 6.68 (dd, 1H, J = 2.2 and 8.8 Hz, H-6'), 6.97 (d, 1H, J = 16.1 Hz, H-2), 7.12 (d, 1H, J = 16.1 Hz, H-3), 7.77 (d, 1H, J = 8.8 Hz, H-5'), 7.88 (s, 1H, H-2'), and 9.51 (brs, 1H, OH); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 13.13, 18.49, 30.04, 63.55, 102.08, 115.19, 116.24, 117.99, 120.61, 126.84, 135.40, 156.71, 156.76, 162.54, 166.76, and 174.73; HRMS, *m/z*: calculated for $C_{16}H_{16}O_5$ $[M + H]^+$ 289.1076, found 289.1081.

(E) Methyl 3-(6-hydroxy-4-oxo-4H-1-benzopyran-3-yl) acrylate (41). Compound **41** was obtained as yellow crystals in 92% yield. mp: 246–247°C; UV (MeOH) λ_{max}: 278, 269, 245, and 218 nm; IR (KBr) υ_{max}: 3386 (OH), 1699 (*CO*O), 1643 (CO), 1619 (C═C), 1473, 1344, 1316, 1298, 1229, 981, and 864 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.72 (s, 3H, H-1"), 7.20 (d, 1H, J = 16.0 Hz, H-2), 7.26 (dd, 1H, J = 3.0 and 9.0 Hz, H-7'), 7.39 (d, 1H, J = 3.0 Hz, H-5'), 7.48 (d, 1H, J = 16.0 Hz, H-3), 7.58 (d, 1H, J = 9.0 Hz, H-8'), 8.84 (s, 1H, H-2'), and 10.15 (s, 1H, OH); ^{13}C NMR (125.5 MHz, DMSO-d_6): δ 51.43, 107.95, 116.81, 119.33, 119.90, 123.41, 124.40, 136.90, 148.81, 155.34, 160.00, 166.98, and 175.09; HRMS, *m/z*: calculated for $C_{13}H_{10}O_5$ $[M + Na]^+$ 269.0426, found 269.0433.

(E) Ethyl 3-(6-hydroxy-4-oxo-4H-1-benzopyran-3-yl)acrylate (42). Compound **42** was obtained as yellow crystals in 96% yield. mp: 179–180°C; UV (MeOH) λ_{max}: 278, 246, and 216 nm; IR (KBr) υ_{max}: 3342 (OH), 3050, 2985, 1687 (*CO*O), 1655 (CO), 1604 (C═C), 1470, 1342, 1294, 1227, 985, and 867 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25 (t, J = 7.0 Hz, 3H, H-2"), 4.17 (q, J = 7.0 Hz, 2H, H-1"), 7.16 (d, 1H, J = 16.0 Hz, H-2), 7.22–7.26 (dd, 1H, J = 3.0 and 9.0 Hz, H-7'), 7.38 (d, 1H, J = 3.0 Hz, H-5'), 7.45 (d, 1H, J = 16.0 Hz, H-3), 7.55 (d, 1H, J = 9.0 Hz, H-8'), 8.81 (s, 1H, H-2'), and 10.10 (s, 1H, -OH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 17.03, 62.78, 110.86, 119.75, 122.63, 122.72, 126.25, 127.28, 139.44, 151.69, 158.21, 162.63, 169.34, and 177.93; HRMS, *m/z*: calculated for $C_{14}H_{12}O_5$ $[M + Na]^+$ 283.0582, found 283.0591.

(E) Propyl 3-(6-hydroxy-4-oxo-4H-1-benzopyran-3-yl)acrylate (43). Compound **43** was obtained as yellow crystals in 94% yield. mp: 177–178°C; UV (MeOH) λ_{max}: 278, 269, 247, and 217 nm; IR (KBr) υ_{max}: 3369 (OH), 3071, 2967, 2880, 1687 (*CO*O), 1654 (CO), 1605 (C═C), 1471, 1316, 1292, 1223, 1186, 984, and 866 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.94 (t, J = 7.4, 3H, H-3"), 1.63–1.70 (m, 2H, H-2"), 4.09 (t, J = 6.6, 2H, H-1"), 7.13 (d, 1H, J = 15.9 Hz, H-2), 7.23–7.27 (m, 1H, H-7'), 7.39 (d, 1H, J = 3.0 Hz, H-5'), 7.47 (d, 1H, J = 15.9 Hz, H-3), 7.55 (d, 1H, J = 9.0 Hz, H-8'), 8.82 (s, 1H, H-2'), and 10.15 (s, 1H, OH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 10.65, 21.98, 65.75, 108.37, 117.26, 120.09, 120.17, 123.73, 124.79, 136.97, 149.18, 155.71, 160.15, 166.93, and 175.44; HRMS, *m/z*: calculated for $C_{15}H_{14}O_5$ $[M + Na]^+$ 297.0739, found 297.0723.

(E) Isopropyl 3-(6-hydroxy-4-oxo-4H-1-benzopyran-3-yl) acrylate (44). The title compound **44** was obtained as yellow crystals in 95% yield. mp: 183–184°C; UV (MeOH) λ_{max}: 279, 270, and 217 nm; IR (KBr) υ_{max}: 3371 (OH), 2979, 1702 (*CO*O), 1655 (CO), 1605 (C═C), 1470, 1340, 1291, 1224, 1187, 1106, 985, and 868 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.24 (d, 6H, J = 6.2 Hz, H-2"), 4.97–5.01 (m, 1H, H-1"), 7.13 (d, 1H, J = 16.0 Hz, H-2), 7.22–7.26 (dd, 1H, J = 3.0 and 9.0 Hz, H-7'),

7.38 (d, 1H, *J* = 3.0 Hz, H-5'), 7.43 (d, 1H, *J* = 16.0 Hz, H-3), 7.56 (d, 1H, *J* = 9.0 Hz, H-8'), 8.82 (s, 1H, H-2'), and 10.20 (s, 1H, OH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 21.84, 22.02, 67.54, 108.32, 117.26, 120.22, 120.52, 123.74, 124.75, 136.72, 149.17, 155.69, 160.06, 166.33, and 175.42; HRMS, *m/z*: calculated for $C_{15}H_{14}O_5$ $[M+Na]^+$ 297.0739, found 297.0730.

(E) Butyl 3-(6-hydroxy-4-oxo-4H-1-benzopyran-3-yl)acrylate (45). Compound **45** was obtained as yellow crystals in 90% yield. mp: 174–175°C; UV (MeOH) λ_{max}: 279, 270, 245, and 217 nm; IR (KBr) υ_{max}: 3329 (OH), 3052, 2929, 2871, 1686 (*CO*O), 1658 (CO), 1606 (C═C), 1468, 1249, 1219, 1192, 986, and 867 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.91 (t, *J* = 7.5, 3H, H-4"), 1.32–1.44 (m, 2H, H-3"), 1.59–1.64 (m, 2H, H-2"), 4.13 (t, 2H, *J* = 6.5 Hz, H-1"), 7.18 (d, 1H, *J* = 15.9 Hz, H-2), 7.25–7.27 (m, 1H, H-7'), 7.39 (d, 1H, *J* = 3.0 Hz, H-5'), 7.46 (d, 1H, *J* = 15.9 Hz, H-3), 7.58 (d, 1H, *J* = 9.0 Hz, H-8'), 8.84 (s, 1H, H-2'), and 10.17 (s, 1H, OH); ^{13}C NMR (125.5 MHz, DMSO-d_6): δ 13.56, 18.65, 30.24, 63.64, 107.92, 116.82, 119.66, 119.90, 123.40, 124.38, 136.67, 148.80, 155.33, 159.92, 166.57, and 175.09; HRMS, *m/z*: calculated for $C_{16}H_{16}O_5$ $[M+Na]^+$ 311.0895, found 311.0870.

(E) Allyl 3-(6-hydroxy-4-oxo-4H-1-benzopyran-3-yl)acrylate (46). Compound **46** was obtained as yellow crystals in 90% yield. mp: 173–174°C; UV (MeOH) λ_{max}: 279, 270, 246, and 216 nm; IR (KBr) υ_{max}: 3384 (OH), 3081, 2934, 1687 (*CO*O), 1648 (CO), 1606 (C═C), 1491, 1471, 1343, 1292, 1223, 1183, 1150, 985, and 866 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.66 (d, *J* = 5.1, 2H, H-1"), 5.27–5.37 (m, 2H, H-3"), 5.85–6.15 (m, 1H, H-2"), 7.21 (d, 1H, *J* = 15.9 Hz, H-2), 7.38 (d, 1H, *J* = 3.0 Hz, H-5'), 7.49 (d, 1H, *J* = 15.9 Hz, H-3), 7.57 (m, 2H, H-7' and H-8'), 8.84 (s, 1H, H-2'), and 10.13 (s, 1H, OH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 64.78, 108.33, 117.18, 118.12, 120.22, 119.68, 123.75, 124.77, 133.12, 137.45, 149.16, 155.72, 160.36, 166.47, and 175.43; HRMS, *m/z*: calculated for $C_{15}H_{12}O_5$ $[M+Na]^+$ 295.0582, found 295.0573.

General procedure for the synthesis of (E) ethyl 3-(7/6-methoxy-4-oxo-4H-1-benzopyran-3-yl)acrylate 47 and 48. A mixture of (*E*) ethyl 3-(7/6-hydroxy-4-oxo-4*H*-1-benzopyran-3-yl) acrylate (**37** or **42**, 1 g), anhydrous potassium carbonate (0.6 g), and methyl iodide (0.5 mL) in anhydrous acetone was refluxed for 12–14 h, and the progress of the reaction was monitored on TLC (2% MeOH-$CHCl_3$). On completion of the reaction, the mixture was cooled to room temperature, and the solvent was filtered and evaporated under reduced pressure. The resulting precipitate was then washed with water and crystallized from acetone to give the desired (*E*)-ethyl 3-(7/6-methoxy-4-oxo-4*H*-1-benzopyran-3-yl) acrylate **47** and **48** in quantitative yield (Scheme 3).

(E) Ethyl 3-(7-methoxy-4-oxo-4H-1-benzopyran-3-yl)acrylate (47). Compound **47** was obtained as a yellow solid in 97% yield. mp: 129–131°C; UV ($CHCl_3$) λ_{max}: 273.49 nm; IR (KBr) υ_{max}: 3000, 2975, 2944, 1712 (*CO*O), 1640 (CO), 1583 (C═C), 1506, 1454, 1313, 1279, 1244, 1180, 1122, 996, and 824 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, 3H, *J* = 7.3 Hz, H-2''), 3.85 (s, 1H, OC*H*$_3$), 4.19 (q, 2H, *J* = 7.3 Hz, H-1''), 6.77 (d, 1H, *J* = 2.2 Hz, H-8'), 6.92 (dd, 1H, *J* = 2.2 and 8.8 Hz, H-6'), 7.19 (d, 1H, *J* = 16.1 Hz, H-2), 7.32 (d, 1H, *J* = 16.1 Hz, H-3), 7.97(s, 1H, H-2'), and 8.07 (d, 1H, *J* = 8.8 Hz, H-5'); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.25, 55.86, 60.41, 100.26, 114.99, 117.99, 119.21, 121.98, 127.64, 136.68, 156.83, 157.23, 164.24, 167.37, and 175.22; HRMS, *m/z*: calculated for $C_{15}H_{14}O_5$ $[M+H]^+$ 275.0919, found 275.0952.

(E) Ethyl 3-(6-methoxy-4-oxo-4H-1-benzopyran-3-yl)acrylate (48). Compound **48** was obtained as yellow solid in 97% yield. mp: 130–132°C; UV ($CHCl_3$) λ_{max}: 273 nm; IR (KBr) υ_{max}: 3067, 1700 (*CO*O), 1615 (CO), 1593 (C═C), 1463, 1417, 1361, 1288, 1173, 1097, 1030, 874, and 842 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (t, 3H, *J* = 7.3 Hz, H-2''), 3.87 (s, 1H, OCH_3), 4.22 (q, 2H, *J* = 7.3 Hz, H-1''), 7.21–7.25 (m, 2H, H-2 and H-7'), 7.37 (d, 1H, *J* = 9.5 Hz, H-8'), 7.38 (d, 1H, *J* = 16.1 Hz, H-3) 7.57 (d, 1H, *J* = 2.9 Hz, H-5'), and 8.06 (s, 1H, H-2'); ^{13}C NMR (100.5 MHz, $CDCl_3$): δ 14.24, 55.89, 60.42, 105.28, 118.39, 119.46, 121.84, 123.92, 124.78, 135.49, 150.28, 157.06, 157.33, 167.37, and 175.66; HRMS, *m/z*: calculated for $C_{15}H_{14}O_5$ $[M+H]^+$ and $[M+Na]^+$ 275.0919, 297.0739, found 275.0782 and 297.0647, respectively.

General procedure for the synthesis of (*E*) 3-(4-oxo-4*H*-1-benzopyran-3-yl)-acrylamides 49-54. To a solution of **28** (5.0 mmol) in DMF (10 mL) was added HOBt (6.0 mmol) and DCC (6.0 mmol). The appropriate amine (5.1 mmol) was then added, and the reaction mixture was stirred overnight at room temperature. The progress of the reaction was monitored by TLC, and on completion of the reaction; the reaction mixture was cooled to 0°C and filtered. The filtrate was evaporated and to the solid so obtained was added DCM (10 mL) and filtered again to remove traces of dicyclohexylurea. The DCM layer was evaporated, and the crude product so obtained was purified by column chromatography using ethyl acetate/petroleum ether (3:7 v/v) as eluents to obtain the desired amide in 72–83% yield.

(E) 3-(4-oxo-4H-1-benzopyran-3-yl)-N-propyl-acrylamide (49). Compound **49** was obtained as a yellow solid in 72% yield. mp: 148°C; UV (MeOH) λ_{max}: 290, 266, and 219 nm; IR (KBr) υ_{max}: 3290 (CO*NH*), 3069, 2956, 2931, 2870, 1654 (*CO*NH), 1637 (CO), 1614 (C═C), 1565, 1539, 1468, 1402, 1362, 1341, 1324, 1259, 1182, 1146, 1100, 1028, 972, 953, and 850 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.96 (t, *J* = 7.4 Hz, 3H, H-3"), 1.56–1.63 (m, 2H, H-2"), 3.34–3.38 (m, 2H, H-1"), 5.91 (brs, 1H, N*H*), 7.29 (d, 1H, *J* = 15.2 Hz, H-2), 7.42–7.48 (m, 2H, H-6' and H-8'), 7.49 (d, 1H, *J* = 15.2 Hz, H-3), 7.69 (t, 1H, *J* = 7.0 Hz, H-7'), 8.12 (s, 1H, H-2'), and 8.26 (d, 1H, *J* = 7.9 Hz, H-5'); ^{13}C NMR (125.5 MHz $CDCl_3$): δ 11.80, 23.29, 41.97, 118.40, 119.73, 124.62, 125.54, 125.75, 125.98, 30.71, 134.41, 155.86, 158.16, 166.72, and 176.92; HRMS, *m/z*: calculated $[M+H]^+$ 258.1125, found 258.1125.

(E) N-iso-Propyl-3-(4-oxo-4H-1-benzopyran-3-yl)acrylamide (50). Compound **50** was obtained as a yellow solid in 74% yield. mp: 149°C; UV (MeOH) λ_{max}: 290 and 267 nm; IR (KBr) υ_{max}: 3291 (CO*NH*), 3071, 2972, 2861, 1653 (*CO*NH), 1613 (CO), 1547, 1464, 1401, 1345, 1283, 1255, 1223, 1182, 979, and 844 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (d, *J* = 6.5, 6H, H-2"), 4.20–4.24 (m, 1H, H-1"), 5.62 (*brs*, 1H, N*H*), 7.29 (d, 1H, *J* = 15.2 Hz, H-2), 7.43–7.49 (m, 3H, H-3, H-6' and H-8'), 7.69 (t, 1H, *J* = 8.4 Hz, H-7'), 8.10 (s, 1H, H-2'), and 8.28 (d, 1H, *J* = 7.9 Hz, H-5'); ^{13}C NMR (125.5 MHz, $CDCl_3$): δ 23.17, 42.07, 118.41, 118.61, 119.72, 126.53, 126.68, 131.99, 134.38, 155.84, 158.24, 165.77, and 176.85; HRMS, *m/z*: calculated $[M+H]^+$ 258.1125, found 258.1128.

(E) N-Butyl-3-(4-oxo-4H-1-benzopyran-3-yl)acrylamide (51). Compound **51** was obtained as a yellow solid in 74% yield. mp: 140°C; UV (MeOH) λ_{max}: 290, 267, and 220 nm; IR (KBr) υ_{max}: 3307 (CO*NH*), 3054, 2954, 2932, 2868, 1661 (*CO*NH), 1613 (CO), 1541, 1464, 1401, 1321, 1294, 964, and 857 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.95 (t, *J* = 7.3 Hz,

3H, H-4"), 1.38–1.44 (m, 2H, H-3"), 1.53–1.76 (m, 2H, H-2"), 3.38–3.42 (m, 2H, H-1"), 5.78 (brs, 1H, N*H*), 7.30 (d, 1H, *J* = 15.2 Hz, H-2), 7.44–7.52 (m, 3H, H-3, H-6' and H-8'), 7.68 (t, 1H, *J* = 7.3 Hz, H-7'), 8.12 (s, 1H, H-2'), and 8.27 (d, 1H, *J* = 7.9 Hz, H-5'); ^{13}C NMR (125.5 MHz, $CDCl_3$): δ 14.17, 20.48, 32.08, 39.84, 118.41, 118.61, 124.61, 125.58, 125.76, 126.49, 131.96, 134.38, 155.84, 158.35, 168.00, and 176.92; HRMS, *m/z*: calculated $[M+H]^+$ 272.1281, found 278.1279.

(E) N-Hexyl-3-(4-oxo-4H-1-benzopyran-3-yl)acrylamide (52). Compound **52** was obtained as a yellow solid in 76% yield. mp: 121°C; UV (MeOH) λ_{max}: 290 and 266 nm; IR (KBr) υ_{max}: 3324 (CO*NH*), 2929, 2855, 1659 (*CO*NH), 1612 (CO), 1561, 1533, 1462, 1321, 1295, and 969 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, *J* = 6.6 Hz, 3H, H-6"), 1.31–1.36 (m, 6H, H-3", H-4", H-5"), 1.53–1.57 (m, 2H, H-2"), 3.36–3.40 (m, 2H, H-1"), 5.87 (brs, 1H, N*H*), 7.30 (d, 1H, *J* = 15.4 Hz, H-2), 7.42–7.51 (m, 3H, H-3, H-6', H-8'), 7.68 (t, 1H, *J* = 7.3 Hz, H-7'), 8.11 (s, 1H, H-2'), and 8.26 (d, 1H, *J* = 7.9 Hz, H-5'); ^{13}C NMR (125.5 MHz, $CDCl_3$): δ 14.17, 22.96, 25.40, 29.98, 31.90, 40.17, 118.41, 118.61, 119.74, 124.63, 126.17, 126.67, 132.13, 134.41, 155.86, 158.16, 166.52, and 177.35; HRMS, *m/z*: calculated for $C_{18}H_{21}NO_3$ $[M+Na]^+$ 322.1419, found 322.1407.

(E) N,N-Diethyl-3-(4-oxo-4H-1-benzopyran-3-yl)acrylamide (53). Compound **53** was obtained as yellow solid in 80% yield. mp: 89–90°C, (lit. value: 88°C) [34]; UV (MeOH) λ_{max}: 292, 268, and 220 nm; IR (KBr) υ_{max}: 3448 (CO*NH*), 3050, 2975, 2931, 1657 (*CO*NH), 1597 (CO), 1562, 1462, 1428, 1356, 1292, 971, and 863 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (t, *J* = 6.8, 3H, H-2"), 1.31 (t, *J* = 6.7, 3H, H-2"'), 3.52–3.54 (m, 4H, H-1"and H-1"'), 7.34 (d, 1H, *J* = 15.0 Hz, H-2), 7.45–7.51 (m, 2H, H-6', H-8'), 7.71 (t, 1H, *J* = 7.9 Hz, H-7'), 8.03 (d, 1H, *J* = 15.0 Hz, H-3), 8.11 (s, 1H, H-2'), and 8.30 (d, 1H, *J* = 7.9 Hz, H-5'); ^{13}C NMR (125.5 MHz, $CDCl_3$): δ 13.61, 15.47, 41.45, 42.78, 118.46, 118.64, 120.07, 122.85, 125.99, 126.58, 133.40, 134.37, 155.84, 158.38, 166.61, and 177.02; HRMS, *m/z*: calculated $[M+Na]^+$ 294.1101, found 294.1099.

(E) N,N-Dioctyl-3-(4-oxo-4H-1-benzopyran-3-yl)acrylamide (54). Compound **54** was obtained as a yellow solid in 83% yield. mp: 66–67°C; UV (MeOH) λ_{max}: 292, 269, and 220 nm; IR (KBr) υ_{max}: 3449 (CO*NH*), 3043, 2926, 2854, 1657 (*CO*NH), 1601 (CO), 1562, 1466, 1422, 1355, 1297, 1197, 999, and 872 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.7 Hz, 6H, H-8" and H-8"'), 1.27–1.35 (m, 20H, H-7", H-7"', H-6", H-6"', H-5", H-5"', H-4", H-4"', H-3" and H-3"'), 1.59–1.65 (m, 4H, H-2", 2"'), 3.44 (t, *J* = 7.0 Hz, 4H, H-1", H-1"'), 7.32 (d, 1H, *J* = 15.0 Hz, H-2), 7.42–7.48 (m, 2H, H-6' and H-8'), 7.67 (t, 1H, *J* = 7.1 Hz, H-7'), 8.04 (d, 1H, *J* = 15.0 Hz, H-3), 8.08 (s, 1H, H-2'), and 8.28 (d, 1H, *J* = 7.7 Hz, H-5'); ^{13}C NMR (125.5 MHz, $CDCl_3$): δ 14.42, 22.99, 27.25, 27.51, 28.33, 29.60, 29.68, 29.80, 30.19, 32.17, 32.19, 47.25, 48.56, 118.48,120.20, 123.02, 124.76, 125.96, 126.58, 133.21, 134.12, 155.84, 158.00, 166.93, and 176.78; EIMS, *m/z*: 440.32 $M^{+\cdot}$, 382.24 ($M^{+\cdot}$ – $(C_2H_5)_2$), 368.23, 354.21, 340.19, 312.20, 280.27, 270.12, 240.27, 225.09, 199.04 ($M^{+\cdot}$ – $NH(C_8H_{17})_2$), 182.19, 171.05 ($M^{+\cdot}$ – $NH(C_8H_{17})_2$- CO), 142.16, 121.03, and 115.05.

BIOLOGY

Cell culture and antiproliferative assay [35,36]

Cell culture. Human colon adenocarcinoma HT-29 (ATCC no. HTB-38), human ovarian adenocarcinoma cell line SK-OV-3 (ATCC no. HTB-77), and human breast carcinoma MDA-68 (ATCC no. HTB-27) were obtained from American Type Culture Collection. The cells were grown on 75 cm^2 cell culture flasks with Eagle's minimum essential medium, supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin solution (10 000 units of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO_2, 95% air at 37°C.

Cell proliferation assay. A cell proliferation assay was carried out using a CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, upon reaching about 75–80% confluency, 5000 cells/well were plated in a 96-well microplate in 100 µL of medium. After seeding for 72 h, the cells were treated with 50 µM compound in triplicate. Doxorubicin (Dox, 10 µM) was used as the positive control. At the end of the sample exposure period (24 h), 20 µL CellTiter 96 aqueous solution was added. The plate was returned to the incubator for 1 h in a humidified atmosphere at 37°C. The absorbance of the formazan product was measured at 490 nm using a microplate reader. The blank control was recorded by measuring the absorbance at 490 nm with wells containing medium mixed with CellTiter 96 aqueous solution but no cells. Results were expressed as the percentage of the control (without compound set at 100%).

c-Src kinase activity assay. The effect of synthesized compounds on the activity of c-Src kinase was assessed by Transcreener ADP2 FI assay (Bell Brook Labs, Madison, Wisconsin; catalogue no. 3013-1 K) according to manufacturer's protocol. A 384-well low-volume black nonbinding surface round bottom microplate was purchased from Corning (No. 3676). In summary, the kinase reaction was started in 384-well low-volume black microplate with the incubation of the 2.5 µL of the reaction cocktail (0.7 nM of His6-Src kinase domain in kinase buffer) with 2.5 µL of prediluted compounds (dissolved in 10% DMSO, 4X target concentration) for 10 min at room temperature using a microplate shaker. The reaction cocktail was made using the kinase buffer HEPES (200 mM, pH 7.5), $MgCl_2$ (16 mM), EGTA (8 mM), DMSO (4%), Brij-35 (0.04%), and 2-mercaptoethanol (43 mM). The kinase reaction was started by adding 5 µL of ATP (40 µmol/L)/substrate (600 µmol/L) cocktail and incubated for 30 min at room temperature on a microplate shaker. An Src optimal peptide (AEEEIYGEFEAKKKK) was used as the substrate for the kinase reaction. The kinase reaction was stopped by adding 10 µL of the 1X ADP detection mixture to the enzyme reaction mixture and mixed using a plate shaker. The mixture was incubated at room temperature for 1 h, and the fluorescence intensity was measured. The 1X ADP detection mixture was prepared by adding ADP2 Antibody-IRDyeR QC-1 (10 µg/mL) and ADP Alexa594 Tracer (8 nmol/L) to Stop

& Detect Buffer B (1X). Fluorescence intensity measurements were performed using a fluorescence intensity optical module using an excitation of 580 nm and an emission of 630 nm with bandwidths of 10 nm by an Optima BMG Labtech microplate reader. IC_{50} values of the compounds were calculated using ORIGIN 6.0 (Origin Lab, USA) software. IC_{50} is the concentration of the compound that inhibited enzyme activity by 50%. All of the experiments were carried out in triplicate.

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