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Abha Kathuria
University of Delhi

Sarah Jalal
University of Delhi

Rakesh Tiwari
Chapman University, tiwari@chapman.edu

Amir Nasrolahi Shirazi
Chapman University, shirazi@chapman.edu

Shilpi Gupta
University of Delhi

See next page for additional authors

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Substituted Coumarin Derivatives: Synthesis and Evaluation of Antiproliferative and Src Kinase Inhibitory Activities

Authors
Abha Kathuria, Sarah Jalal, Rakesh Tiwari, Amir Nasrolahi Shirazi, Shilpi Gupta, Shiv Kumar, Keykavous Parang, and Sunil K. Sharma

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Research Article

Substituted coumarin derivatives: Synthesis and evaluation of antiproliferative and Src kinase inhibitory activities

Abha Kathuria,[a] Sarah Jalal,[a] Rakesh Tiwari,[b] Amir Nasrolahi Shirazi,[b] Shilpi Gupta,[a] Shiv Kumar,[a] Keykavous Parang,[b,*] Sunil K. Sharma[a,*]

[a] *Department of Chemistry, University of Delhi, Delhi 110007, India*
[b] *Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA,*
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Abstract: Six classes of coumarin derivatives (i.e. 3-alkyl-4-methylcoumarins, pyranocoumarins, coumarin carboxamides, quaternary ammonium coumarins, 7-aminocoumarins, and 4-aminocoumarins) were synthesized and evaluated for inhibition of cell proliferation of colon adenocarcinoma (HT-29), breast carcinoma (MDA-MB-468 or MCF-7), and human ovarian adenocarcinoma (SK-OV-3) cells. C-3-Alkyl substituted analogs of 4-methylcoumarins and pyranocoumarins, **5** and **6**, inhibited the cell proliferation of MDA-MB-468 and SK-OV-3 cells by 53-74%, while 3-decyl substituted pyranocoumarin **10** and triethyl substituted quaternary ammonium coumarin derivative **29** inhibited the cell proliferation of HT-29 and SK-OV-3 cells by 63-72% at a concentration of 50 µM. Among all the compounds studied, C-3 decyl substituted quaternary ammonium coumarin derivative **25** exhibited the highest Src kinase inhibition with an IC_{50} value of 21.6 µM.

Introduction

Cancer is the second leading cause of death worldwide, following heart diseases. World Health Organization has estimated over 11 million global deaths due to cancer in 2030 [1]. According to the American Cancer Society, about 1.5 million new cancer cases and more than 500,000 deaths have occurred due to cancer in USA alone in 2009 [2].

The reduction in mortality and morbidity has been achieved among cancer patients through use of current chemotherapeutic agents. Most drugs currently available for treatment of cancer are mechanistically based on inhibition of cell proliferation and induction of apoptosis. However, the application of many antiproliferative drugs is associated to high toxicity due to their mechanisms of action and non-specific targeting. For example, cardiovascular side effects are common with antimetabolites and the anti-angiogenic agents. Furthermore, multi-drug resistance has become one of the major challenges to encounter in cancer chemotherapy. Statistically half of all cancer patients either fail to respond or will relapse from the initial response and ultimately die from their metastatic disease [3]. Thus, the

*Corresponding author. Sunil K. Sharma, E-mail:sk.sharma90@gmail.com; Fax: (+91-11)-2766 7206; Tel: (+91-11) 27666646 Extn.-191. / Keykavous Parang, E-mail: kparang@uri.edu; Fax: +1- 401-874-5787; Tel.: +1-401-874-4471.

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continued commitment to the arduous tasks involved in the discovery of new antiproliferative agents with less toxicity, higher efficacy, and better selectivity remains critically important.

Extensive research during the past decade has focused on targeting tumors at molecular level and processes that are prerequisites for tumor growth. For example, angiogenesis has spurred an intensive interest in the last decade of the twentieth century. Metastasis is a closely related process to angiogenesis, and thus has also become a focus of contemporary cancer drug discovery. Recent major advances in cancer therapy have been seen in the area of protein kinases, which represent one of the largest protein families [4]. Phosphorylation of many protein substrates occurs in the presence of protein tyrosine kinases (PTKs) that catalyze the transfer of γ-phosphate group from ATP to specific tyrosine residues. PTKs have critical roles in the signal transduction pathways. Extensive knowledge on PTKs has offered some of the most important targets such as HER-2, Flk-1/KDR (VEGFR-2), Bcr-Abl, EGFR, and Src kinases for antiproliferative drug design [4].

Src kinase (Src), a prototype member of the Src family of kinases (SFKs) is over-expressed in several types of human tumors including colon, breast, ovary, prostate, lung, and pancreas, and increased Src activity is observed in metastatic tumors [5,6]. Another key role of Src is the regulation of specific angiogenic factors that promote tumor progression. Studies demonstrated that this tyrosine kinase regulates both constitutive and growth factor-induced VEGF and IL-8 expression [7]. Recently our group studied the Src kinase inhibitory and antiproliferative activity of 3-phenylpyrazolopyrimidine-1,2,3-triazole conjugates [8], 1-substituted 3-(*N*-alkyl-*N*-phenylamino)propane-2-ols [9], and 4-aryl-4*H*-chromene-3-carbonitrile derivatives [10]. Furthermore, owing to the importance of Src kinase in cancer cell invasion and metastasis, there is a greater need to identify other small-molecules, scaffolds, or pharmacophore fragments as inhibitors.

Coumarins comprise a vast array of biologically active compounds ubiquitous in plants, many of which have been used in traditional medicine since ancient times. The antitumor activity of coumarin against human tumor cell lines was first noted by Weber *et al*. [11]. The selective tumor cell-specific cytotoxicity of coumarins has been well documented by Riveiro *et al*. [12]. However, the use of coumarin in cancer chemotherapy was first established by the successful application of Warfarin sodium on V2 cancer cell, granulocytes, lymphocytes and macrophages in different animal models. Later, clinical trials demonstrated activity of coumarins in many different cancers, including prostate cancer, malignant melanoma and metastatic renal cell carcinoma [13]. In continuation of our efforts to design new antiproliferative agents [4,8-10,14-19], herein we report the synthesis of an array of six classes of coumarin derivatives (Figure 1) and evaluation of antiproliferative activities for establishing the structure-activity relationships. Further, to decipher the mechanism of antiproliferative activities of the compounds, the Src kinase inhibitory activities of the compounds were investigated.

Materials and methods

Chemistry

The organic solvents were dried and distilled prior to their use. Reactions were monitored by precoated TLC plates (Merck silica gel $60F_{254}$); the spots were visualized either by UV light, or by spraying with 5% alcoholic $FeCl_3$ solution. Silica gel (100-200 mesh) was used for column chromatography. All the chemicals and

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

reagents were procured from Spectrochem Pvt. Ltd., India and Sigma-Aldrich Chemicals Pvt. Ltd., USA. Melting points were recorded in capillaries in suphuric acid bath and are uncorrected. Infrared spectra were recorded on Perkin-Elmer FT-IR model 9 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on Jeol-400 (400 MHz, 100.6 MHz) NMR spectrometer and Avance-300 (300 MHz, 75.5 MHz) spectrometer using TMS as internal standard. The chemical shift values are on δ scale and the coupling constant values (*J*) are in Hz. The HRMS data were recorded on Agilent-6210 ES-TOF, JEOL JMX-SX-102A and Waters LCT Micromass-KC455.

General procedure for the synthesis of 10-hydroxy-4,8,8-trimethyl-3-alkyl-7,8-dihydropyrano[3,2-g]chromen-2(6*H*)-ones (6-10). A mixture of 7,8-dihydroxy-3-alkyl-4-methylcoumarin (1.0 g), *p*-toluenesulfonic acid monohydrate (1 equivalent), and 1.5 equivalents of 2-methyl-3-buten-2-ol were taken in a round bottom flask along with toluene as a solvent and refluxed for 24 h. The progress of the reaction was monitored using TLC. On completion of the reaction, the solvent was removed in *vacuo* and the solid obtained was dissolved in chloroform (50 mL). The chloroform layer was washed first with sodium hydroxide solution (1N, 2 × 50 mL) and then with brine solution (20 mL). The organic layer was then dried over anhydrous sodium sulfate. The solvent was removed in *vacuo* in a rotary evaporator to give an oily residue, which was then crystallized from hexane.

3-Ethyl-10-hydroxy-4,8,8-trimethyl-7,8-dihydro-6*H*-pyrano[3,2-g]chromen-2-one (6). Melting point = 175-176 ^{o}C; UV (acetonitrile) λ_{max}: 261 and 318 nm; IR (KBr) ν_{max}: 3404 (OH), 1710 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.10 (t, 3H, *J* = 7.3 Hz, -CH_2CH_3), 1.41 (s, 6H, 2 x H-1′), 1.87 (t, 2H, *J* = 6.4 Hz, H-6), 2.36 (s, 3H, C-4 CH_3), 2.66 (q, 2H, *J* = 7.3 Hz, -CH_2CH_3), 2.85 (t, 2H, *J* = 6.4 Hz, H-7), 5.65 (*brs*, 1H, OH), 6.87 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 13.26 (-CH_2CH_3), 14.66 (C-4 CH_3), 20.96 (-CH_2CH_3), 22.22 (C-6), 26.91 and 27.18 (2 x C-1′), 32.78 (C-7), 76.68 (C-8), 113.98 and 114.80 (C-5 and C-12), 117.58 and 124.92 (C-3 and C-14), 131.87 and 138.87 (C-10 and C-11), 143.36 and 146.21 (C-4 and C-13), 161.39 (C-2); HRMS: Calculated for $C_{17}H_{20}O_4$ $[M + H]^+$ 289.1395, found 289.1524.

10-Hydroxy-4,8,8-trimethyl-3-propyl-7,8-dihydro pyrano[3,2-g]chromen-2(6*H*)-one (7). Melting point = 132-134 ^{o}C; UV (MeOH) λ_{max}: 264 and 328 nm; IR (KBr) ν_{max}: IR (KBr) ν_{max}: 3388 (OH), 1690 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) : δ 0.98 (t, 3H, *J* = 7.4 Hz, -$CH_2CH_2CH_3$), 1.41 (s, 6H, 2 x H-1′),1.49-1.62 (m, 2H, -$CH_2CH_2CH_3$), 1.87 (t, 2H, *J* = 6.6 Hz, H-6), 2.35 (s, 3H, C-4 CH_3), 2.61 (t, 2H, *J* = 7.8 Hz, -$CH_2CH_2CH_3$), 2.85 (t, 2H, *J* = 6.6 Hz, H-7), 5.62 (*brs*, 1H, OH), 6.87 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.13 (-$CH_2CH_2CH_3$), 14.78 (C-4 CH_3), 22.18 and 22.65 (-$CH_2CH_2CH_3$ and C-6), 24.32 (-$CH_2CH_2CH_3$), 26.87 (2 x C-1′), 32.54 (C-7), 76.61 (C-8), 113.96 and 114.44 (C-5 and C-12), 119.12 and 123.67 (C-3 and C-14), 131.77 and 138.82 (C-10 and C-11), 143.22 and 146.25 (C-4 and C-13), 161.40 (C-2); HRMS: Calculated for $C_{18}H_{22}O_4$ $[M + H]^+$ 303.1552, found 303.1812.

3-Hexyl-10-hydroxy-4,8,8-trimethyl-7,8-dihydro-6*H*-pyrano[3,2-g]chromen-2-one (8). Melting point = 140-141 ^{o}C; UV (MeOH) λ_{max}: 263 and 320 nm; IR (KBr) ν_{max}: 3436 (OH), 1672 (CO) cm^{-1}; ^{1}H NMR (Acetone-d_6, 500 MHz): δ 0.90 (t, 3H, *J* = 6.9 Hz, -$CH_2(CH_2)_4CH_3$), 1.32-1.52 (m, 14H, -$CH_2(CH_2)_4CH_3$ and 2 x H-1′), 1.88 (t, 2H, *J* = 6.6 Hz, H-6), 2.39 (s, 3H, C-4 CH_3), 2.62 (t, 2H, *J* = 7.7 Hz, -$CH_2(CH_2)_4CH_3$), 2.87 (t, 2H, *J* = 6.6 Hz, H-7), 6.99 (s, 1H, H-5), 7.81 (*brs*, 1H, OH); ^{13}C NMR (Acetone-d_6, 125.7 MHz): δ 19.02 (-$CH_2(CH_2)_4CH_3$), 19.68 (C-4 CH_3), 22.98 (C-6), 26.79 (2 x C-1′), 27.44, 31.68, 34.05, 34.21, 37.16 (-$(CH_2)_5CH_3$), 38.05 (C-7),

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

81.04 (C-8), 119.60 and 120.42 (C-5 and C-12), 123.04 and 128.72 (C-3 and C-14), 138.29 (C-10), 144.99, 149.48 and 151.74 (C-4, C-11 and C-13), 166.40 (C-2); HRMS: Calculated for $C_{21}H_{28}O_4$ $[M + H]^+$ 345.1988, found 345.2100.

3-Heptyl-10-hydroxy-4,8,8-trimethyl-7,8-dihydro-6*H*-pyrano[3,2-g]chromen-2-one (9). Melting point = 120-122 ^oC; UV (MeOH) λ_{max}: 264 and 328 nm; IR (KBr) ν_{max}: 3409 (OH), 1687 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, 3H, *J* = 6.3 Hz, -$CH_2(CH_2)_5CH_3$), 1.27-1.50 (m, 10H, -$CH_2(CH_2)_5CH_3$), 1.41 (s, 6H, 2 x H-1′), 1.87 (t, 2H, *J* = 6.6 Hz, H-6), 2.34 (s, 3H, C-4 CH_3), 2.62 (t, 2H, *J* = 7.2 Hz, -$CH_2(CH_2)_5CH_3$), 2.85 (t, 2H, *J* = 6.6 Hz, H-7), 5.64 (*brs*, 1H, OH), 6.87 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.13 (-$CH_2(CH_2)_5CH_3$), 14.92 (C-4 CH_3), 22.18 (C-6), 26.87 (2 x C-1′), 22.67 27.68, 28.92, 29.24, 29.69, 31.88 (-$(CH_2)_6CH_3$), 33.74 (C-7), 80.61 (C-8), 114.12 and 117.44 (C-5 and C-12), 123.82 and 124.23 (C-3 and C-14), 131.77 (C-10), 138.82, 143.23 and 146.24 (C-4, C-11 and C-13), 161.40 (C-2); HRMS: Calculated for $C_{22}H_{30}O_4$ $[M + H]^+$ 359.2144, found 359.1708.

3-Decyl-10-hydroxy-4,8,8-trimethyl-7,8-dihydro-6H-pyrano[3,2-g]chromen-2-one (10)

Melting point = 147-148 ^oC; UV (MeOH) λ_{max}: 263 and 321 nm; IR (KBr) ν_{max}: 3429 (OH), 1672 (CO) cm^{-1}; ^1H NMR (Acetone-d_6, 500 MHz): δ 0.87 (t, 3H, *J* = 7.2 Hz, -$CH_2(CH_2)_8CH_3$), 1.30-1.54 (m, 22H, -$CH_2(CH_2)_8CH_3$ and 2 x H-1′), 2.06 (t, 2H, *J* = 6.6 Hz, H-6), 2.41 (s, 3H, C-4 CH_3), 2.62-2.64 (m, 4H, -$CH_2(CH_2)_8CH_3$ and H-7), 6.86 (*brs*, 1H, OH), 7.15 (s, 1H, H-5); ^{13}C NMR (Acetone-d_6, 125.7 MHz): δ 13.83 (-$CH_2(CH_2)_8CH_3$), 14.75 (C-4 CH_3), 22.81 (C-6), 29.01 (2 x C-1′), 27.53, 28.58, 29.16, 29.32, 29.47, 29.54, 29.72, 29.78 and 29.82 (-$(CH_2)_9CH_3$), 32.11 (C-7), 76.71 (C-8), 112.12 and 114.39 (C-5 and C-12), 115.89 and 122.87 (C-3 and C-14), 131.93 (C-10), 142.22, 147.07 and 147.98 (C-4, C-11 and C-13), 161.05 (C-2); HRMS: Calculated for $C_{25}H_{36}O_4$ $[M + H]^+$ 401.2647, found 401.2701.

General procedure for the synthesis of amino acid derivatives of coumarin-3-carboxamide (14-17). To a stirred solution of carboxy coumarin (1.0 g, 31 mmol) in 100 mL acetone, triethylamine (4.2 equiv.) and ethyl chloroformate (4 equiv.) were added at 0 ^oC in about 15 minutes. The reaction mixture was stirred for 1 h and then filtered. To the resulting filtrate, protected amino acid (1.1 equiv.) was added and then the reaction mixture was stirred for 24 h at room temperature. The acetone was distilled off and remaining mixture was dissolved in ethyl acetate, washed with sodium bicarbonate solution. The solvent was distilled off and the resultant compound was recrystallized with chloroform and ether.

Methyl 2-(2-oxo-2*H*-chromene-3-carboxamido)-3-phenylpropanoate (14). Melting point = 108-110 ^oC (Literature value = 105-107 ^oC) [20]; UV (MeOH) λ_{max}: 292 nm; IR (KBr) ν_{max}: 3312 (NH), 2951, 2923, 2868, 1750 (COO), 1726 (CO), 1656 (CONH) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 3.13-3.31 (m, 2H, -CH_2Ph), 3.74 (s, 3H, OCH_3), 4.98 (q, 1H, *J* = 7.35 Hz, H-2), 7.22-7.34 (m, 5H, C_6H_5), 7.36-7.40 (m, 2H, H-6' and H-8'), 7.63-7.68 (m, 2H, H-5' and H-7'), 8.84 (s, 1H, H-4'), 9.23 (d, 1H, *J* = 7.2 Hz, N*H*); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 37.79 (-CH_2Ph), 52.29 (OCH_3), 54.19 (C-2), 116.53 (C-8'), 117.79 and 118.35 (C-3' and C-10'), 125.19 (C-7'), 127.08 (C_6H_5), 128.56 (C_6H_5), 129.14 (C_6H_5), 129.74 (C-6'), 134.14 (C-5'), 135.82 (C_6H_5), 148.48 (C-4'), 154.36 (C-9'), 160.97 and 161.20 (C-2' and C-11'), 171.39 (C-1); HRMS: Calculated for $C_{20}H_{17}NO_5$ $[M]^+$ 351.1107, found 351.7543.

Methyl 2-(2-oxo-2*H*-chromene-3-carboxamido)acetate (15). Melting point = 195 ^oC (Literature value = 193-195 ^oC) [20]; UV (MeOH) λ_{max}: 292 nm; IR (KBr) ν_{max}: 3326 (NH), 2936, 2739, 1748 (COO), 1709 (CO), 1654 (CONH) cm^{-1}; ^1H NMR

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

(DMSO-d_6, 400 MHz): δ 3.68 (s, 3H, OCH_3), 4.15 (d, 2H, *J* = 4.5 Hz, H-2), 7.41-7.47 (m, 1H, H-6'), 7.51 (d, 1H, *J* = 6.7 Hz, H-8'), 7.77 (t, 1H, *J* = 6.2 Hz, H-7'), 7.96 (d, 1H, *J* = 6.3 Hz, H-5'), 8.91(s, 1H, H-4'), 9.08 (t, 1H, *J* = 4.1 Hz, N*H*); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 45.31 (O*C*H_3), 51.91 (C-2), 116.19 (C-8'), 118.10 and 118.39 (C-3' and C-10'), 125.21 (C-7'), 130.45 (C-6'), 134.40 (C-5'), 148.24 (C-4'), 154.02 (C-9'), 160.31 and 161.46 (C-2' and C-11'), 169.98 (C-1); HRMS: Calculated for $C_{13}H_{11}NO_5$ $[M+H]^+$ 262.0637, found 262.0307.

Methyl 4-methyl-2-(2-oxo-2*H*-chromen-3-carboxamido)pentanoate (16). Melting point = 124-126 ^oC (Literature value =120-122 ^oC) [20]; UV (MeOH) λ_{max}: 292 nm; IR (KBr) ν_{max}: 3353 (NH), 3052, 2928, 2854, 1734 (COO), 1705 (CO), 1652 (CONH) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.91-0.94 (m, 6H, -CH_2C*H*(CH_3)$_2$), 1.66-1.76 (m, 3H, -CH_2C*H*(CH_3)$_2$), 3.69 (s, 3H, OCH_3), 4.58-4.62 (m, 1H, H-2), 7.44-7.47 (m, 1H, H-6'), 7.52 (d, 1H, *J* = 6.7 Hz, H-8'), 7.69-7.78 (m, 1H, H-7'), 7.99 (dd, 1H, *J* = 0.90 and 6.2 Hz, H-5'), 8.87 (s, 1H, H-4'), 8.98 (d, 1H, *J* = 6.1 Hz, N*H*); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 21.63 and 22.72 (-CH_2C*H*(CH_3)$_2$), 24.50 (-CH_2*C*H(CH_3)$_2$), 40.33 (-*C*H_2CH(CH_3)$_2$), 50.85 (O*C*H_3), 52.23 (C-2), 116.27 (C-8'), 118.33 and 118.43 (C-3' and C-10'), 125.29 (C-7'), 130.44 (C-6'), 134.44 (C-5'), 148.13 (C-4'), 154.01 (C-9'), 160.58 and 161.21 (C-2' and C-11'), 172.41 (C-1); HRMS: Calculated for $C_{17}H_{19}NO_5$ $[M]^+$ 317.1263, found 317.8474.

Methyl 3-(2-oxo-2*H*-chromen-3-carboxamido)propanoate (17). Melting point = 152-154 ^oC (Literature value =150-152 ^oC) [20]; UV (MeOH) λ_{max}: 290 nm; IR (KBr) ν_{max}: 3352 (NH), 3052, 2927, 1733 (COO), 1703 (CO), 1650 (CONH) cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.62 (t, 2H, *J* = 5.2 Hz, H-2), 3.57 (q, 2H, *J* = 5.0 Hz, H-3), 3.63 (s, 3H, OCH_3), 7.44 (t, 1H, *J* = 6.0 Hz, H-6'), 7.49 (d, 1H, *J* = 6.6 Hz, H-8'), 7.75 (t, 1H, *J* = 6.0 Hz, H-7'), 7.98 (d, 1H, *J* = 6.0 Hz, H-5'), 8.87 (*brs*, 2H, H-4' and N*H*); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 33.37 (C-2), 35.02 (C-3), 51.45 (O*C*H_3), 116.08 (C-8'), 118.39 and 118.54 (C-3' and C-10'), 125.09 (C-7'), 130.27 (C-6'), 134.11 (C-5'), 147.65 (C-4'), 153.85 (C-9'), 160.32 and 161.06 (C-2' and C-11'), 171.94 (C-1); HRMS: Calculated for $C_{14}H_{13}NO_5$ $[M + H]^+$ 276.0794, found 276.1454.

General procedure for the synthesis of amino alkylamino derivatives of coumarin-3-carboxamide (18-21). To a solution of coumarin-3-carboxylic acids (1.0 g), BOP reagent (1.0 equivalent) in acetonitrile (20 ml) was added 1.3 equivalents of triethylamine. This was followed by the addition of a solution of *t*-butyl aminoalkylcarbamates (1.0 equiv.) dissolved in chloroform (15 ml). The resulting mixture was stirred at room temperature for 10-12 h. The progress of reaction was monitored on TLC. On completion of the reaction saturated brine solution was added to quench the reaction followed by extraction with ethyl acetate (3 x 20 ml). The ethyl acetate layer was then successively washed with 4% citric acid, water, 4% sodium bicarbonate solution, and water. The organic layer was dried over anhydrous sodium sulfate, and solvent was removed in vacuo at 40 °C to provide crude product. The crude product obtained was crystallized in chloroform-petroleum ether (1:10) to give Boc protected alkyl amino coumarin carboxamides. Deprotection of the Boc group was carried out by treating the coumarin carbamate with a mixture of TFA/DCM (1:1, 2 ml) at room temperature. The progress of reaction was monitored on TLC. On completion of the reaction the solvent was evaporated under reduced pressure. Further the residue was treated with sodium hydroxide solution (1N) to neutralize the excess of trifluoroacetic acid and to generate a free amino group. This was followed by extraction with dichloromethane. The organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

obtained residue was crystallized in methanol to give free amino analogs of coumarin carboxamide.

***N*-(2-Aminoethyl)-2-oxo-2*H*-1-benzopyran-3-carboxamide (18).** Melting point = 320 ^{o}C [21]; UV (acetonitrile) λ_{max}: 298 and 332 nm; IR (KBr) ν_{max}: 3328 (NH), 1694 (CO), 1654 (CONH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.14 (brm, 2H, H-2″), 3.74 (t, 2H, *J* = 6.6 Hz, H-1″), 7.36-7.41 (m, 2H, H-6 and H-8), 7.64-7.71 (m, 2H, H-5 and H-7), 8.92 (brs, 1H, N*H*CO), 9.07 (s, 1H, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C 29.79 (C-2″), 37.58 (C-1″), 116.73, 118.55 and 118.73 (C-3, C-8 and C-10), 125.33, 129.84 and 134.03 (C-5, C-6 and C-7), 148.39 (C-4), 154.50 (C-9), 161.50 and 162.02 (C-1′ and C-2); HRMS: Calculated for $C_{12}H_{12}N_2O_3$ $[M]^{+\cdot}$ 232.0848, found 232.7881.

***N*-(4-Aminobutyl)-2-oxo-2*H*-1-benzopyran-3-carboxamide (19).** Melting point = 249 ^{o}C; UV (acetonitrile) λ_{max}: 292 and 326 nm; IR (KBr) ν_{max}: 3313 (NH), 1722 (CO), 1658 (CONH) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.62 (*brs*, 2H, H-3″), 3.24 (*brs*, 4H, H-2″ and H-4″), 3.39 (*brs*, 2H, H-1″), 7.43-7.49 (m, 2H, H-6 and H-8), 7.73 (t, 1H, *J* = 6.0 Hz, H-7), 7.95 (d, 1H, *J* = 6.9 Hz, H-5), 8.66 (*brs*, 1H, N*H*CO), 8.82 (s, 1H, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 29.52 (C-3″), 30.20 (C-2″), 32.02 (C-4″), 37.31 (C-1″), 112.92, 115.43 and 119.32 (C-3, C-8 and C-10), 120.99 and 124.88 (C-5 and C-6), 146.85, 148.14 and 148.52 (C-4, C-7 and C-9), 160.89 and 162.13 (C-1′ and C-2); HRMS: Calculated for $C_{14}H_{16}N_2O_3$ $[M]^{+\cdot}$ 260.1161, found 260.6169.

***N*-(2-Aminoethyl)-8-methoxy-2-oxo-2*H*-1-benzopyran-3-carboxamide (20).** Melting point = > 320 ^{o}C; UV (acetonitrile) λ_{max}: 311 nm; IR (KBr) ν_{max}: 3332 (NH), 1715 (CO), 1657 (CONH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.18 (t, 2H, *J* = 5.5 Hz, H-2″), 3.71 (t, 2H, *J* = 5.5 Hz, H-1″), 3.96 (s, 3H, OC*H*$_3$), 7.34-7.40 (m, 3H, H-5, H-6 and H-7), 8.83 (s, 1H, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 38.56 (C-2″), 40.86 (C-1″), 56.92 (O*C*H$_3$), 117.35, 118.41 and 119.22 (C-3, C-5 and C-7), 122.17 and 126.48 (C-6 and C-10), 145.29, 148.37 and 150.01 (C-4, C-8 and C-9), 162.19 and 165.08 (C-1′ and C-2); HRMS: Calculated for $C_{13}H_{14}N_2O_4$ $[M]^{+\cdot}$ 262.0954, found 262.4382.

***N*-(4-Aminobutyl)-8-methoxy-2-oxo-2*H*-1-benzopyran-3-carboxamide (21).** Melting point = 304 ^{o}C; UV (acetonitrile) λ_{max}: 310 nm; IR (KBr) ν_{max}: 3422 (NH), 2932, 1706 (CO), 1658 (CONH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.60 (*brs*, 2H, H-3″), 3.35-3.36 (m, 4H, H-2″ and H-4″), 3.83 (*brs*, 2H, H-1″), 3.92 (s, 3H, OC*H*$_3$), 7.31-7.41 (m, 2H, H-6 and H-7), 7.47 (d, 1H, *J* = 7.5 Hz, H-5), 8.64 (*brs*, 1H, N*H*CO), 8.76 (s, 1H, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 25.92 (C-3″), 27.35 (C-2″), 35.34 (C-4″), 38.81 (C-1″), 56.82 (O*C*H$_3$), 116.08, 117.24 and 121.20 (C-3, C-5 and C-7), 122.17 and 126.24 (C-6 and C-10), 144.46, 148.42 and 149.59 (C-4, C-8 and C-9), 162.22 and 163.87 (C-1′ and C-2); HRMS: Calculated for $C_{15}H_{18}N_2O_4$ $[M]^{+\cdot}$ 290.1267, found 290.9057.

General procedure for the synthesis of 7-aminocoumarins (30-34). Ethyl chloroformate (10.0 g, 92 mmol) was added in one portion to a stirred suspension of *m*-aminophenol (10.0 g, 92 mmol) in 400 mL of anhydrous diethyl ether. A white precipitate (amine hydrochloride) formed immediately. The reaction mixture was stirred for additional 2 h at room temperature. The hydrochloride was removed by filtration. The filtrate was then evaporated to give grey colored solid. Further crystallization from petroleum ether (200 mL) gave upon cooling (0 ^{o}C) 3-hydroxyphenylurethane as white solid. A solution of 3-hydroxyphenylurethane (7.0 g) and substituted ethyl acetoacetate (1.2 equivalent) suspended in 88 mL of 70% ethanolic H_2SO_4 was stirred at room temperature for 4-6 h. On completion of the reaction the clear yellow solution was

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

poured into 400 mL of ice cold water, giving a voluminous brown crystalline precipitate. The solid was filtered and then crystallized from ethanol to give carbethoxy/urethane protected aminocoumarin. The protected aminocoumarin (5.0 g) were refluxed for 4 h in a mixture of concentrated H_2SO_4 and glacial acetic acid (1:1, 10 ml). On cooling a yellow precipitate was deposited. The mixture was poured over 100 mL of ice cold water and allowed to stand overnight. The resulting suspension was made slightly alkaline with 50% aqueous NaOH under cold conditions. The brown precipitate formed was then filtered and washed with ice cold water (3 × 50 mL). Crystallization from ethanol yielded light brown colored crystals to give 7-amino coumarin (**30**-**34**).

7-Amino-3-hexyl-4-methylcoumarin (32). Melting point = 198-200 ^oC; UV (acetonitrile) λ_{max}: 348 nm; IR (KBr) ν_{max}: 3447.78, 3356.61 (NH_2), 1678.16 (COO) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 0.81 (brs, 3H, -$CH_2(CH_2)_4CH_3$), 1.23-1.34 (m, 8H, -$CH_2(CH_2)_4CH_3$), 2.24 (s, 3H, C-4 CH_3), 2.42 (*brs*, 2H, -$CH_2(CH_2)_4CH_3$), 5.90 (*brs*, 2H, NH_2), 6.34 (s, 1H, H-8), 6.51 (d, 1H, *J* = 8.4 Hz, H-6), 7.36 (d, 1H, *J* = 8.4 Hz, H-5); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 14.49 and 14.88 (-$CH_2(CH_2)_4CH_3$ and C-4 CH_3), 22.62, 27.19, 28.97, 29.21, 31.69 (-$(CH_2)_5CH_3$), 98.94 (C-8), 109.96 and 111.75 (C-6 and C-10), 119.34 (C-3), 126.51 (C-5), 147.80 (C-4), 152.45 and 154.32 (C-7 and C-9), 161.90 (C-2); HRMS: Calculated for $C_{16}H_{21}NO_2$ $[M + H]^+$ 260.1572, found 260.1652.

7-Amino-3-decyl-4-methylcoumarin (33). Melting point = 172-174 ^oC; UV (acetonitrile) λ_{max}: 339 nm; IR (KBr) ν_{max}: 3451.51, 3358.51 (NH_2), 1676.82 (COO) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 0.85 (*brs*, 3H, -$CH_2(CH_2)_8CH_3$), 1.23-1.39 (m, 16H, -$CH_2(CH_2)_8CH_3$), 2.28 (s, 3H, C-4 CH_3), 2.45-2.47 (m, 2H, -$CH_2(CH_2)_8CH_3$), 5.93 (*brs*, 2H, NH_2), 6.39 (s, 1H, H-8), 6.56 (d, 1H, *J* = 8.4 Hz, H-6), 7.40 (d, 1H, *J* = 8.4 Hz, H-5); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 13.86 and 14.27 (-$CH_2(CH_2)_8CH_3$ and C-4 CH_3), 22.02, 22.02, 26.58, 28.37, 28.63, 28.84, 28.92, 28.92, 31.22 (-$(CH_2)_9CH_3$), 98.39 (C-8), 109.41 and 111.17 (C-6 and C-10), 118.80 (C-3), 126.89 (C-5), 147.17 (C-4), 151.85 and 153.74 (C-7 and C-9), 161.37 (C-2); HRMS: Calculated for $C_{20}H_{29}NO_2$ $[M + Na]^+$ 338.2096, found 338.2071.

General procedure of synthesis of 4-*N*-aminoalkylamino-2*H*-1-benzopyran-2-ones (35-40). 4-Chlorocoumarin (1.0 g) and *t*-butyl aminoalkylcarbamate (1 equivalent) were taken in a round bottom flask along with 20 ml of ethanol. The mixture was refluxed for 4 hrs and the progress of reaction was monitored on TLC (5% methanol-chloroform). On completion of the reaction the solvent was evaporated under reduced pressure. Water (50 ml) was added to the crude mixture followed by extraction with methylene chloride. The organic layer was then dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue obtained was crystallized in chloroform-petroleum ether (1:10) to give Boc protected 4-*N*-alkylaminocoumarin. Deprotection of the Boc group was carried out by treating the coumarin carbamate with a mixture of TFA/DCM (1:1, 2 ml) at room temperature. The progress of the reaction was monitored on TLC (10% methanol-chloroform). On completion of deprotection the solvent was evaporated under reduced pressure. Further the residue was treated with sodium hydroxide solution (1N) to neutralize the excess of trifluoroacetic acid and to generate a free amino group. This was followed by extraction with dichloromethane. The organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue obtained was crystallized in methanol to give the corresponding unprotected analogs of 4-*N*-alkylaminobenzopyran-2-one.

4-(2-Amino-ethylamino)-2*H*-1-benzopyran-2-one (35). Melting point =

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

223 °C; UV (acetonitrile) λ_{max}: 290 and 308 nm; IR (KBr) ν_{max}: 3347, 3291 (NH), 1694 (CO) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 2.06 (*brs*, 2H, NH_2), 2.48 (*brs*, 2H, H-2′), 2.77-2.79 (m, 2H, H-1′), 5.17 (s, 1H, H-3), 7.26-7.29 (m, 2H, H-6 and H-8), 7.56 (t, 1H, *J* = 7.0 Hz, H-7), 8.05 (d, 1H, *J* = 7.5 Hz, H-5); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 40.26 and 45.68 (C-1′ and C-2′), 81.22 (C-3), 114.43 and 116.89 (C-8 and C-10), 122.47 and 123.20 (C-5 and C-6), 131.82 (C-7), 153.01 and 153.33 (C-4 and C-9), 161.61 (C-2); HRMS: Calculated for $C_{11}H_{12}N_2O_2$ $[M + H]^+$ 205.0932, found 205.1016.

4-(4-Amino-butylamino)-2*H*-1-benzopyran-2-one (36). Melting point = 156 °C; UV (acetonitrile) λ_{max}: 288 and 310 nm; IR (KBr) ν_{max}: 3383, 3346 (NH), 1671 (CO) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 1.65 (*brm*, 4H, H-2′ and H-3′), 3.00-3.04 (m, 2H, H-4′), 3.28-3.30 (m, 2H, H-1′), 5.20 (s, 1H, H-3), 7.30-7.35 (m, 2H, H-6 and H-8), 7.59 (t, 1H, *J* = 7.4 Hz, H-7), 7.71-7.73 (*brm*, 3H, N*H* and NH_2), 8.05 (d, 1H, J = 8.1 Hz, H-5); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 24.06 and 24.72 (C-2′ and C-3′), 38.60 and 41.61 (C-1′ and C-4′), 81.42 (C-3), 114.48 and 116.98 (C-8 and C-10), 122.45 and 123.28 (C-5 and C-6), 131.91 (C-7), 153.12 and 158.61 (C-4 and C-9), 161.62 (C-2); HRMS: Calculated for $C_{13}H_{16}N_2O_2$ $[M]^{+\cdot}$ 232.1212, found 232.1290.

4-(2-Amino-ethylamino)-6-methyl-2*H*-1-benzopyran-2-one (37). Melting point = 212 °C at atmospheric pressure. (Literature value = 192-194 °C at 0.5 torr) [22]; UV (acetonitrile) λ_{max}: 291, 312 and 322; IR (KBr) ν_{max}: 3364, 3325 (NH), 1675 (CO) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 1.89 (*brm*, 2H, H-2′), 2.36 (s, 3H, CH_3), 2.91 (*brm*, 2H, H-1′), 5.20 (s, 1H, H-3), 7.20 (d, 1H, *J* = 8.1 Hz, H-8), 7.40 (d, 1H, *J* = 7.8 Hz, H-7), 7.66 (*brs*, 1H, N*H*), 7.83 (*brs*, 3H, H-5 and NH_2); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 20.95 (*C*H_3), 38.70 and 41.10 (C-1′ and C-2′), 83.23 (C-3), 115.26 and 118.14 (C-8 and H-10), 122.95 (C-7), 134.43 and 135.24 (C-5 and C-6), 152.66 and 156.04 (C-4 and C-9), 166.32 (C-2); HRMS: Calculated for $C_{12}H_{14}N_2O_2$ $[M]^{+\cdot}$ 218.2518, found 218.2734.

4-(4-Amino-butylamino)-6-methyl-2*H*-1-benzopyran-2-one (38), Melting point = 175 °C; UV (acetonitrile) λ_{max}: 292, 308 and 322 nm; IR (KBr) ν_{max}: 3348, 3310 (NH), 1671 (CO) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 1.66 (*brm*, 4H, H-2′ and H-3′), 2.35 (s, 3H, CH_3), 2.84 (*brm*, 2H, H-4′), 3.26 (*brm*, 2H, H-1′), 5.14 (s, 1H, H-3), 7.17 (d, 1H, *J* = 7.5 Hz, H-8), 7.38 (d, 1H, *J* = 7.2 Hz, H-7), 7.66 (*brs*, 1H, N*H*), 7.88 (*brs*, 3H, H-5 and NH_2); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 25.54 (*C*H_3), 26.02 and 27.17 (C-2′ and C-3′), 40.41 and 43.16 (C-1′ and C-4′), 81.98 (C-3), 115.79 and 118.47 (C-8 and C-10), 122.99 and 125.16 (C-5 and C-7), 133.39 (C-6), 154.60 and 156.10 (C-4 and C-9), 166.42 (C-2); HRMS: Calculated for $C_{14}H_{18}N_2O_2$ $[M - H]^+$ 245.1368, found 245.1410.

General procedure for the synthesis of 4-(3-(N^3,N^3-dialkylamino)propylamino)-2*H*-1-benzopyran-2-ones (39-40). 4-Chloro-2*H*-1-benzopyran-2-one (1.0 g, 5 mmol) and N^3, N^3-dialkylpropane-1,3-diamine were mixed in 1:2 ratio in a round bottom flask along with 20 ml of ethanol. The mixture was refluxed for 4 h and the progress of reaction was monitored on TLC. On completion of the reaction the solvent was evaporated under reduced pressure. Water (15 ml) was added to the crude mixture followed by extraction with dichloromethane (3 x 10 ml). Then, the organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue obtained was crystallized in chloroform-petroleum ether (1:10) to give the 4-*N*,*N*-dialkylaminobenzopyran-2-one (**39**–**40**).

4-(3-(N^3,N^3-Dimethylamino) propylamino)-2*H*-1-benzopyran-2-one (39). Melting point = 84-85 °C.; UV (acetonitrile) λ_{max}: 293 and

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

308 nm; IR (KBr) ν_{max}: 3343 (NH), 2926, 2827, 1669 (CO) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 1.72-1.76 (m, 2H, H-2′), 2.14 (s, 6H, 2 x CH_3), 2.30 (t, 2H, *J* = 6.5 Hz, H-3′), 3.24-3.28 (m, 2H, H-1′), 5.12 (s, 1H, H-3), 7.27-7.32 (m, 2H, H-6 and H-8), 7.54-7.56 (m, 1H, H-7), 7.93 (*brs*, 1H, N*H*), 7.94 (d, 1H, *J* = 7.5 Hz, H-5); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 23.60 (C-2′), 44.23 (C-3′), 45.35 (2 x *C*H_3), 59.46 (C-1′), 82.25 (C-3), 115.11 and 117.77 (C-8 and C-10), 120.72 and 123.31 (C-5 and C-6), 131.36 (C-7), 153.71 and 153.71 (C-4 and C-9), 163.51 (C-2); HRMS: Calculated for $C_{14}H_{18}N_2O_2$ $[M]^{+\cdot}$ 246.1368, found 246.2602.

4-(3-(N^3,N^3-Diethylamino)propylamino)-2*H*-1-benzopyran-2-one (40). Melting point = 99-100 ^oC; UV (acetonitrile) λ_{max}: 293 and 308 nm; IR (KBr) ν_{max}: 3326 (NH), 1702 (CO) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 0.96 (t, 6H, *J* = 7.0 Hz, 2 x CH_2CH_3), 1.72-1.77 (m, 2H, H-2′), 2.45-2.52 (m, 6H, H-3′ and 2 x CH_2CH_3), 3.26-3.28 (m, 2H, H-1′), 5.14 (s, 1H, H-3), 7.29-7.34 (m, 2H, H-6 and H-8), 7.56-7.58 (m, 1H, H-7), 7.88 (*brs*, 1H, N*H*), 7.95 (d, 1H, *J* = 8.1 Hz, H-5); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 11.36 (2 x CH_2CH_3), 23.55 (C-2′), 44.66 (C-3′), 46.99 (2 x CH_2CH_3), 53.49 (C-1′), 82.23 (C-3), 115.02 and 117.80 (C-8 and C-10), 121.10 and 122.98 (C-5 and C-6), 131.35 (C-7), 153.58 and 153.70 (C-4 and C-9), 163.49 (C-2); HRMS: Calculated for $C_{16}H_{22}N_2O_2$ $[M + H]^+$ 275.1715, found 275.1691.

Pharmacology

Cell culture. Human ovarian adenocarcinoma cell line SKOV3 (ATCC no. HTB-77), human breast carcinoma MCF-7 (ATCC no. HTB-22), human breast carcinomaMDA-MB-468 (ATCC no. HTB-27), and human colon adenocarcinoma HT-29 (ATCC no. HTB-38) were obtained from American Type Culture Collection. The cells were grown on 75 cm^2 cell culture flasks with EMEM (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 ºC.

Cell proliferation assay. Cell proliferation assay was carried out using Cell Titer 96 aqueous one solution cell proliferation assay kit (Promega, USA) as described previously [8,9]. Briefly, upon reaching about 75-80% confluency, 5000 cells/well were plated in 96-well microplate in 100 µL media. After seeding for 72 h, the cells were treated with 50 µM compound in triplicate. Doxorubicin (10 µM) was used as the positive control. At the end of the sample exposure period (72 h), 20 µL Cell Titer 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 Cell Titer 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® ADP^2 FI Assay, from Bell Brook Labs, Madison, WI, (catalogue no. 3013-1K) according to manufacturer's protocol as described previously [8,9,19]. 384-well Low volume Black non-binding surface round bottom microplate was purchased from Corning (#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 His_6-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 microplate shaker. The reaction cocktail was made using the kinase buffer HEPES (200 mM, pH 7.5), $MgCl_2$

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

(16 mM), EGTA (8 mM), DMSO (4%), Brij-35 (0.04%), and 2-mercaptoethanol (43 mM). Kinase reaction was started by adding 5 μL of ATP/substrate (40 μM/600μM) cocktail and incubated for 30 min at room temperature on microplate shaker. 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 ADP[2] Antibody-IRDyeR QC-1 (10 μg/mL) and ADP Alexa594 Tracer (8 nM) to Stop and Detect Buffer B(1X). Fluorescence Intensity measurements were performed using fluorescence intensity optical module using the excitation of 580 nm and emission of 630 nm with band widths of 10 nm by Optima, BMG Labtech microplate reader. IC_{50} of the compounds were calculated using ORIGIN 6.0 (origin lab) software. IC_{50} is the concentration of the compound that inhibited enzyme activity by 50%. All the experiments were carried out in triplicate.

Results and Discussion

Chemistry

Six classes of coumarin derivatives (i.e. C-3 alkylated-4-methylcoumarins, pyranocoumarins, coumarin carboxamides, quaternary ammonium coumarins, 7-aminocoumarins, and 4-aminocoumarins) were synthesized.

Synthesis of *C*-3 alkyl-4-methylcoumarins

Scheme 1 shows the synthesis of *C*-3 alkyl-4-methylcoumarins (Class I). 7-Hydroxy-4-methylcoumarins (**1** and **2**) and 7,8-dihydroxy-4-methylcoumarins (**3-5**) were synthesized in quantitative yields by Pechmann condensation of resorcinol or pyrogallol with alkylated ethyl acetoacetates in the presence of sulphuric acid. Synthesis of alkylated β-ketoester in turn was carried out according to the earlier published procedure from our group [23,24].

Synthesis of pyranocoumarins

The synthesis of pyranocoumarins (Class II) is shown in Scheme 2. Nuclear prenylation of 3-alkyl-7,8-dihydroxy-4-methylcoumarins was carried out using 2-methyl-3-buten-2-ol in the presence of *p*-toluene sulfonic acid monohydrate ($PTSA.H_2O$) in toluene, which undergoes *in situ* cyclization to afford corresponding pyranocoumarin in a moderate yield (35%) (Scheme 2).

Synthesis of coumarin carboxamides

Coumarin carboxamides (Class III) were synthesized starting from 3-substituted carboxycoumarins (**11**,**12**), which were in turn prepared from salicylaldehydes and Meldrum's acid *via* Knovaenegel reaction [25]. However, 3-carboxy-4-methylcoumarin **13** was synthesized according to the previously reported procedure [26].

Coumarin-3-carboxamides (**14–17**) were obtained via coupling of 3-carboxycoumarin with methyl 2-amino alkanoates in the presence of ethyl chloroformate and triethlyamine in acetone (Scheme 3). Methyl 2-amino alkanoates were in turn synthesized by esterification of various amino acids using thionyl chloride and methanol [27,28]. In another route, coumarin carboxamides (**18**-**21**) having diamino alkyl groups were synthesized by amidation of 3-carboxycoumarins with mono-Boc protected diamino alkanes using BOP as a coupling agent. Finally, deprotection of the Boc group using a mixture of trifluoroacetic acid (TFA)/dichloromethane (DCM) gave the corresponding coumarin carboxamides (**18–21**, Scheme 3). The mono-Boc

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

protected diamino alkanes were synthesized by the reaction of the corresponding daimio alkane with di-tertbutyl dicarbonate ($(Boc)_2O$) (0.12 equivalent) [29-31].

Synthesis of quaternary ammonium coumarin derivatives

The quaternary ammonium ester (**22-25**) and ether (**26-29**) derivatives of coumarins (Class IV) were synthesized as shown in Schemes 4 and 5. The physical and spectral data (^1H, ^{13}C NMR, UV, IR, HRMS) for this class of compounds have already been reported [32].

Synthesis of 7-aminocoumarins

7-Aminocoumarins, the Class V compounds i.e. C-3 unsubstituted/C-3 alkyl substituted 7-amino-4-methylcoumarins (**30-33**) and 7-amino-4-trifluoromethylcoumarin (**34**) were synthesized as per literature method [33] and characterized completely. First, the urethane protected *m*-aminophenol (3-hydroxyphenylurethane) was prepared to react with alkylated ethyl acetoacetate or 4,4,4-trifluoro ethyl acetoacetate in the presence of 70% H_2SO_4-C_2H_5OH to obtain 3-alkyl-7-carbethoxy-4-methyl/ trifluoromethyl coumarin quantitatively *via* Pechmann condensation. The deprotection of corresponding 7-carbethoxy-4-methyl/ trifluoromethyl coumarins was then carried out with a mixture of sulphuric acid and acetic acid (1:1) to yield 3-alkyl-7-aminocoumarins (**30-34**, Scheme 6).

Synthesis of 4-aminocoumarin derivatives

The synthesis of 4-aminocoumarin derivatives (**35–40**, Class VI) is outlined in Scheme 7. They were synthesized in two steps i.e. first the 4-chlorocoumarins were treated with mono-Boc protected diamino alkanes in ethanol. In the next step, deprotection of the Boc group using a mixture of TFA / DCM gave the corresponding 4-*N*-alkylaminocoumarins (**35-40**, Scheme 7). The 4-*N*,*N*-dialkylaminocoumarins (**39-40**, Scheme 7), on the other hand were synthesized by coupling of 4-chlorocoumarin with *N*,*N*-dialkylpropane-1,3-diamine.

Biological Activity

Antiproliferative activities

The effect of forty diversely substituted coumarins from six classes was evaluated on the cell proliferation of cancer cells, colon adenocarcinoma (HT-29), breast carcinoma (MDA-MB-468/MCF-7), and human ovarian adenocarcinoma (SK-OV-3) cells, at the concentration of 50 µM (Figure 2). Compounds **10** and **29** showed modest antiproliferative activity (63-72%) against HT-29 and SK-OV-3 cells. Furthermore, compounds **5** and **6** inhibited the cell proliferation of MDA-MB-468 and SK-OV-3 cells by 53-74%.

On further analyzing the effect of these compounds on proliferation of cancer cells, it was observed that the proliferation activity gets reduced by incorporating the chroman ring, i.e. the pyranocoumarins **7** and **9** were generally less active than their hydroxyl-substituted precursors **3** and **4**. However, in chromano coumarins the substitutions of smaller (C_2) and larger (C_9/C_{10}) alkyl chains at C-3 position exhibited better cell proliferation inhibitory activity as compared to compounds having intermediate size alkyl chains.

The substitution of C-3 alkyl group by carboxylic group in coumarins **11** and **13** drastically reduced the activity. The C-3 carboxy coumarin **13** containing a methyl group at the C-4 position was less active than the C-3 alkyl 4-methylcoumarins (**1-5**). However, the conversion of carboxylic to amidic linkage in compounds **14** and **16** slightly improved the antiproliferative activity against HT-29 cells in comparison with compounds **11** and **13**. The amino acid conjugates (**14**, **16**, and **17**) also exhibited

ISSN: 2249 –4820
Chemistry & Biology Interface, 2011, 1, 2, 279-296

better activity as compared to amides formed from diamino alkanes (**18**-**21**) against HT-29 and MDA-MB-468/MCF cells. A comparison of quaternary amino acyloxy (**22**-**25**) and alkoxy (**28** and **29**) coumarin derivatives revealed that the latter are more active against all cells and the activity enhances with increase in hydrophobicity of compounds. Among the aminocoumarin derivatives, 7-amino-4-methylcoumarins (**31** and **32**) showed slightly better activity in comparison to the 4-aminocoumarin derivatives **35**-**40** against HT-29 and SK-OV-3 cells.

Src kinase inhibitory activities

The mechanism of antiproliferative activities of these compounds is currently under investigation. HT-29, SK-OV-3, and MDA-MB-468 cell lines express highly activated Src [34,35]. Thus, preliminary studies were carried out to investigate the inhibitory activities of forty diversely substituted coumarins from six classes against Src kinase. Table 1 shows the inhibitory potency of the synthesized compounds compared to a general protein kinase inhibitor, staurosporine, and a Src kinase inhibitor, PP2. In general, most of the coumarin derivatives were weak Src kinase inhibitors (IC_{50} > 150 µM). The data suggest that among all the coumarin derivatives the C-3 alkyl-substituted coumarins showed improved activity in comparison to the unsubstituted analogs. Also it has been observed that among all the compounds, the quaternary ammonium derivatives substituted at C-3 position with hexyl or decyl chains (**24** and **25**), exhibited higher Src inhibitory activity (IC_{50} = 21.6-36.0 µM). 7-Aminocoumarins **33** and **34** also showed modest Src kinase inhibition (IC_{50} = 30.9-73.9 µM).

From the enzyme inhibition studies, it can be inferred that there was poor correlation between Src kinase inhibitory potency and the growth inhibition of cancer cells. Compounds **10** and **29** showed modest antiproliferative activity (63-72%) against HT-29 and SK-OV-3 cells. Furthermore, compounds **5** and **6** inhibited the cell proliferation of MDA-MB-468 and SK-OV-3 cells by 53-74%. However, these compounds did not exhibit any significant Src kinase inhibition. On the other hand, compounds with modest Src kinase inhibitory activities, such as **25**, demonstrated weak antiproliferative activities. The data suggest that other mechanisms may be involved in the relatively modest antiproliferative activities of these compounds. Various substituted 4-aryl-4*H*-chromen-3-carbonitrile have previously reported to exhibit antiproliferative activities through apoptosis-inducing effect [36-38]. It remains to be investigated whether the active compounds in these six classes of coumarin derivatives have any apoptosis effect.

Conclusions

In summary, six classes of coumarin derivatives including fourteen novel compounds (**6**-**10**, **19**-**21**, **32**-**33**, **35**-**36**, **38** and **40**) were synthesized and fully characterized by ^{1}H, ^{13}C NMR, UV, IR, and high resolution mass spectroscopy (HRMS). The spectral data for all the novel compounds and those that are not previously reported (**14**-**18**, **37 a**nd **39**) is given as supplementary material.

The coumarin derivatives were evaluated for Src kinase inhibitory and antiproliferative activities. To the best of our knowledge, this is the first report of the evaluation of these six classes of coumarin derivatives as Src kinase inhibitors. Structure-activity relationships revealed that a number of *C*-3 alkyl-substituted quaternary ammonium (**24** and **25**) and 7-aminocoumarins (**33** and **34**) showed modest Src kinase inhibitor activities. Among all compounds, *C*-3 alkyl-substituted pyranocoumarins **6** and **10** exhibited 63-74% antiproliferative activity in SK-OV-3 cells. A structure activity relationship for coumarin derivatives in

ISSN: 2249 –4820

Chemistry & Biology Interface, 2011, 1, 2, 279-296

terms of Src kinase inhibitory and antiproliferative activities has been reported. Further studies are underway for structural optimization of *C*-3 alkyl-substituted coumarin derivatives to generate compounds with more potential antiproliferative activities.

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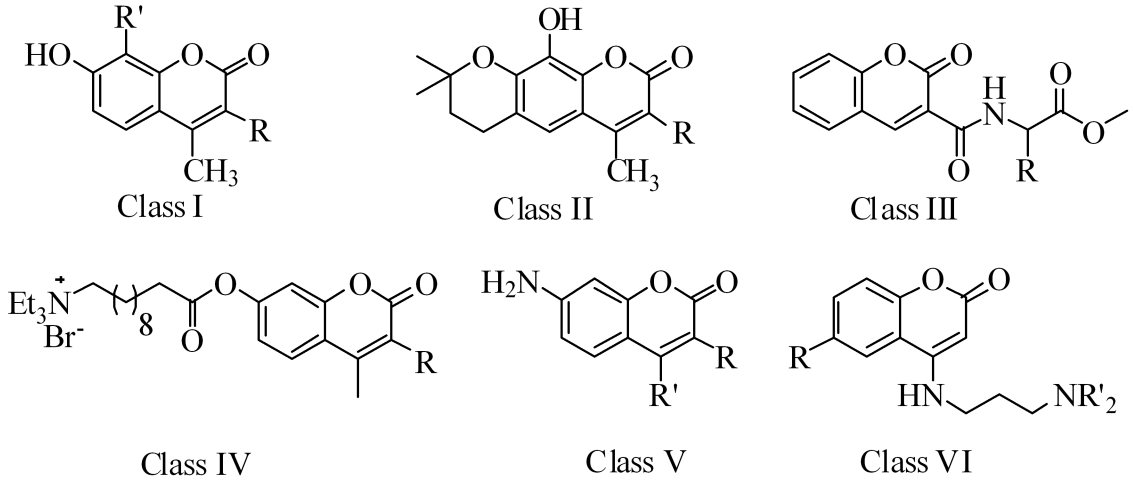
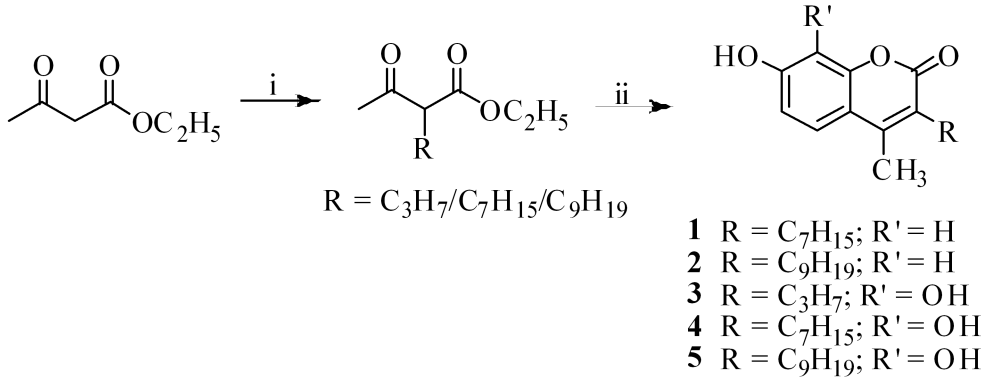


Chemical structures of six classes of synthesized coumarin derivatives: I: C-3 alkylated-4-methylcoumarins; II: pyranocoumarins; III: coumarin carboxamides; IV: quaternary ammonium coumarins; V: 7-aminocoumarins; and VI: 4-aminocoumarin derivatives.

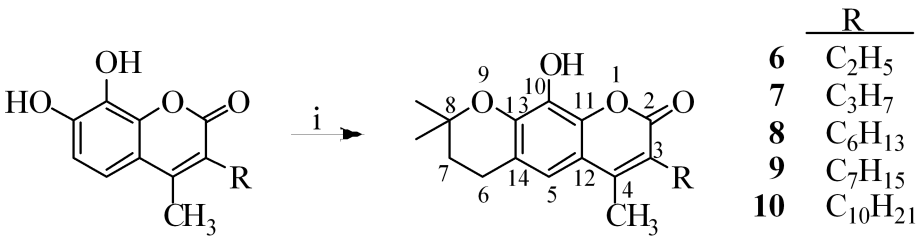
Figure 1.



Scheme 1. Synthesis of *C*-3 alkylated-4-methylcoumarins: i. NaH, RBr, THF; ii. resorcinol/pyrogallol, H_2SO_4.

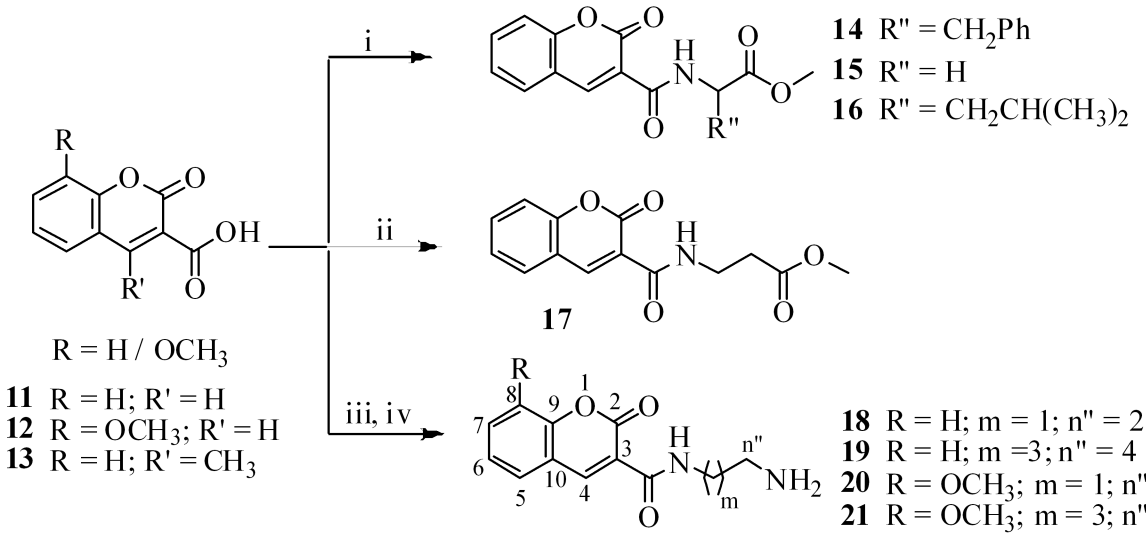
ISSN: 2249 –4820

Chemistry & Biology Interface, 2011, 1, 2, 279-296



	R
6	C_2H_5
7	C_3H_7
8	C_6H_{13}
9	C_7H_{15}
10	$C_{10}H_{21}$

Scheme 2. Synthesis of pyranocoumarins. i. 2-methyl-3-buten-2-ol, *P*-TSA.H_2O, toluene, reflux.



14 R" = CH_2Ph
15 R" = H
16 R" = $CH_2CH(CH_3)_2$

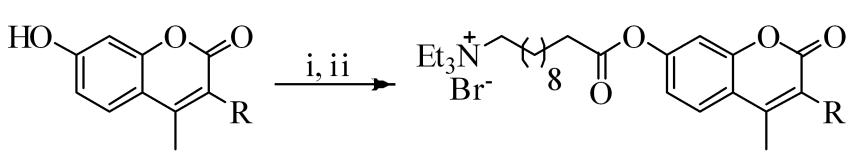
17

R = H / OCH_3

11 R = H; R' = H
12 R = OCH_3; R' = H
13 R = H; R' = CH_3

18 R = H; m = 1; n" = 2
19 R = H; m =3; n" = 4
20 R = OCH_3; m = 1; n" = 2
21 R = OCH_3; m = 3; n" = 4

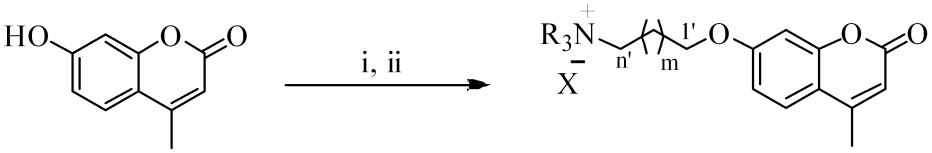
Scheme 3. Synthesis of coumarin carboxamides: i. Ethyl chloroformate, Et_3N, $NH_2CH(R'')COOMe$, acetone, 37 °C; ii. Ethyl chloroformate, Et_3N, $NH_2(CH_2)_2COOMe$, acetone, 37 °C; iii. $BocNHCH_2(CH_2)_nNH_2$, BOP reagent, CH_3CN, Et_3N, DCM, 37 °C; iv. TFA-DCM (1:1), 37 °C.



	R
22	H
23	C_2H_5
24	C_6H_{13}
25	$C_{10}H_{21}$

R = $H/C_2H_5/C_6H_{13}/C_{10}H_{21}$

Scheme 4. Synthesis of quaternary ammonium coumarin derivatives: i. $Br(CH_2)_{10}COCl$, Et_3N (1 equiv.), CH_3CN, 37 °C; ii. Et_3N (5 equivalent), CH_3CN, 60 °C, 96 h.

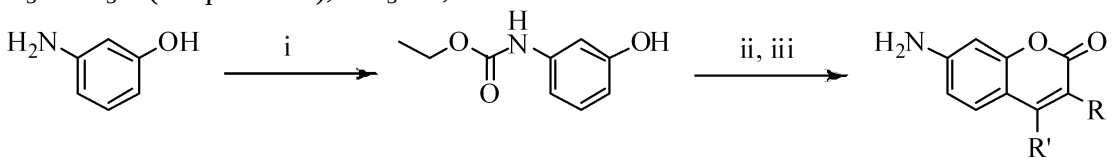


26 R = C_2H_5; m = 1; n' = 3; X = Cl
27 R = C_2H_5; m = 4; n' = 6; X = Br
28 R = C_4H_9; m = 4; n' = 6; X = Br
29 R = C_2H_5; m = 8; n' = 10; X = Br

ISSN: 2249 –4820

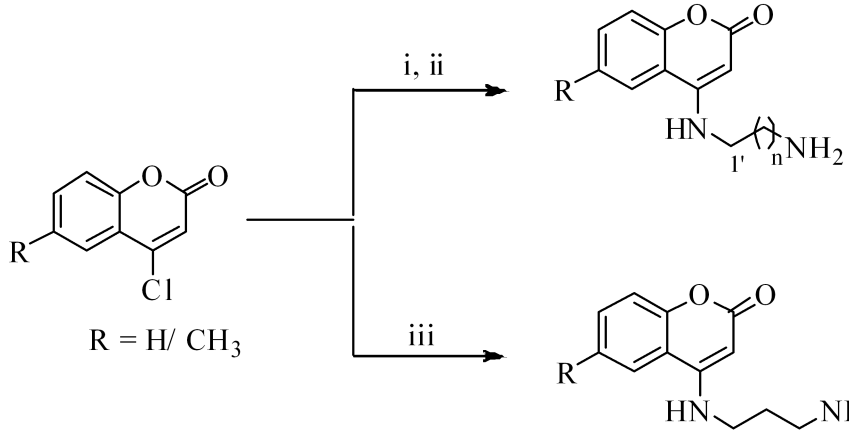
Chemistry & Biology Interface, 2011, 1, 2, 279-296

Scheme 5. Synthesis of quaternary ammonium derivatives: i. $XCH_2(CH_2)_mCH_2X$, K_2CO_3, acetone; ii. Et_3N/Bu_3N (5 equivalent), CH_3CN, 60 °C.



30 R = H; R' = CH_3
31 R = C_2H_5; R' = CH_3
32 R = C_6H_{13}; R' = CH_3
33 R = $C_{10}H_{21}$; R' = CH_3
34 R = H; R' = CF_3

Scheme 6. Synthesis of 7-aminocoumarins: i. C_2H_5OCOCl, Et_2O, 37 °C; ii. $R'COCHRCOOC_2H_5$, H_2SO_4-C_2H_5OH (7:3), 37 °C; iii. H_2SO_4-CH_3COOH (1:1), reflux.



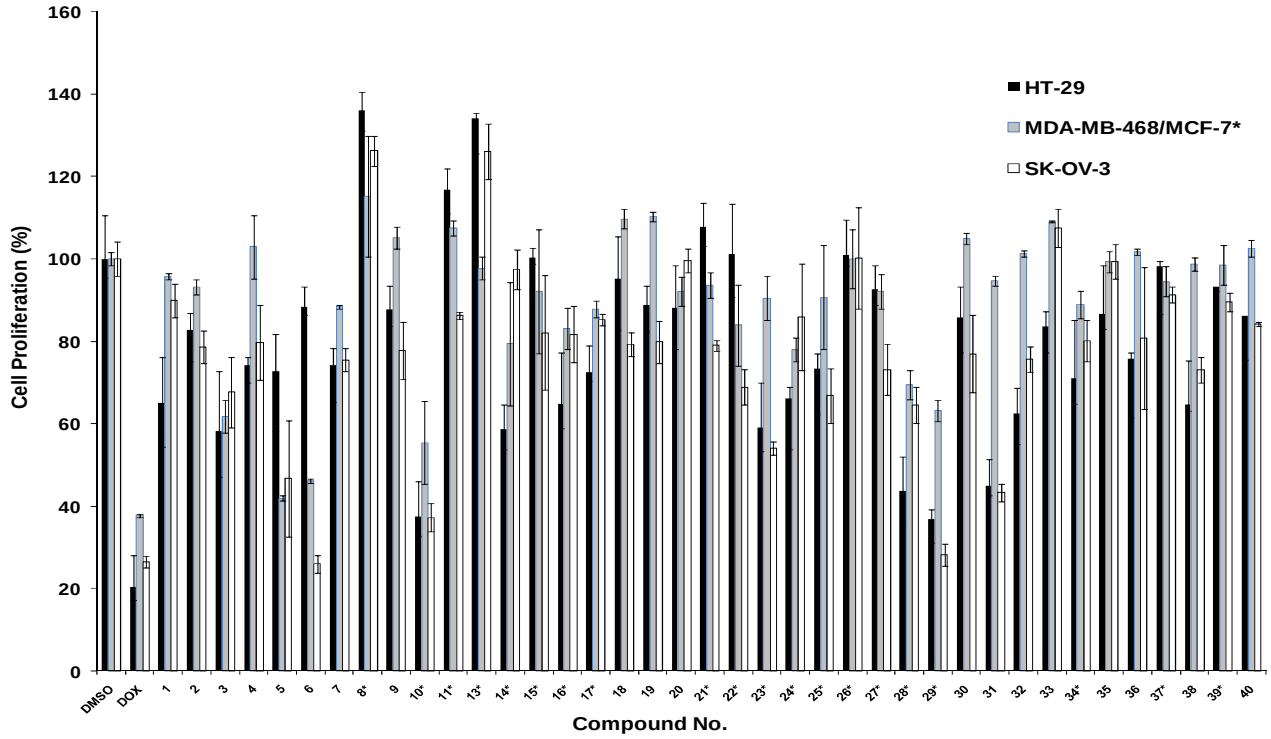
35 R = H; n = 1
36 R = H; n =3
37 R = CH_3; n = 1
38 R = CH_3; n = 3

39 R = H; R' = CH_3
40 R = H; R' = C_2H_5

Scheme 7. Synthesis of 4-aminocoumarin derivatives: i. $BocNHCH_2(CH_2)_nNH_2$, ethanol; ii. TFA-DCM (1:1), 25 °C; iii. $R'_2NCH_2CH_2CH_2NH_2$, ethanol.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2011, 1, 2, 279-296



*Compound (**8**, **10**-**11**, **13**-**17**, **21**-**29**, **34**, **37**, and **39**) were tested against breast carcinoma MCF-7 cell line.

Figure 2. Cell proliferation of HT-29, MDA-MB-468/ MCF-7*, and SK-OV-3 cell lines by coumarin derivatives.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2011, 1, 2, 279-296

Table 1. Src kinase inhibitory activity of coumarin derivatives (**1**-**40**).

Compound	IC_{50} (µM)[a]	Compound	IC_{50} (µM)[a]
1	> 300	**23**	> 300
2	> 300	**24**	36.0
3	90.4	**25**	21.6
4	> 300	**26**	> 300
5	> 300	**27**	> 300
6	> 300	**28**	> 300
7	> 300	**29**	> 300
8	87.8	**30**	78.5
9	> 300	**31**	> 300
10	> 150	**32**	> 300
11	> 300	**33**	73.9
13	> 150	**34**	30.9
14	> 300	**35**	> 300
15	> 300	**36**	> 300
16	> 300	**37**	> 300
17	> 300	**38**	> 300
18	> 300	**39**	> 300
19	> 300	**40**	> 250
20	> 300	**Staurosporine**	0.6
21	> 300	**PP2**	0.5
22	62.6		

[a]The concentration at which the enzyme activity is inhibited by 50%.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2011, 1, 2, 279-296

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