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REGULAR ARTICLE

Cytotoxic effects of coumarin substituted benzimidazolium salts against human prostate and ovarian cancer cells

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Abstract. Coumarin and benzimidazole derivatives have individual biological activities including anticancer. In this study, we aimed to synthesize coumarin-benzimidazole hybrids in order to investigate their anticancer properties. For this purpose, six 6-substituted-4-chloromethylene coumarin derivatives were synthesized. Sixteen coumarin substituted benzimidazolium chlorides were synthesized by the reaction of 4-chloromethylene coumarin and *N*-benzylbenzimidazole derivatives. All of the synthesized compounds were characterized by ^{1}H and ^{13}C NMR, IR spectroscopic techniques and elemental analyses. Cytotoxicities of all compounds were tested by [3-(4,5-dimethylthiazole)-2-yl]-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay against human prostate (PC-3) and ovarian (A2780) cancer cells. All compounds performed significant cytotoxicities at 100 μM against both cancer cell lines. Moreover, some compounds performed significant activities at 1 μM against both cancer cell lines and the obtained results suggest that this type of compounds are promising candidates for the treatment of human prostate and ovarian cancers.

Keywords. Benzimidazole; benzimidazolium salt; coumarin; cytotoxicity.

1. Introduction

Cancer is one of the biggest health problems that threaten humanity and the fight against cancer continues with different methods across the world. Chemotherapy is a method that uses chemical compounds including organic, inorganic, and organometallic monomers or polymers and nanoparticles to kill cancer cells. However, drug resistance of cancer cells and side effects of drugs are compelling problems encountered in chemotherapy. Therefore, the development of novel compounds for the treatment of cancer with low toxicity and high selectivity is an important challenge for scientists. Hybridization of some biologically active scaffolds in a structure is one of the newest strategies for drug design. This strategy aims to achieve higher activity and lower toxicity due to synergistic effects. In this study, we aimed to combine the coumarin and benzimidazole groups which have individual biological activities in an ionic structure in order to investigate their cytotoxicity properties.

Coumarin is a bicyclic compound which consists of the fusion of α-pyron and benzene rings. The most known coumarin derivative is *Warfarin* which has a significant anticoagulant effect.[1] In addition, *Khellactone* and *Calanolide* derivatives are well known coumarin-based anti-HIV agents.[2] Coumarin derivatives were also reported as carbonic anhydrase inhibitors by Supuran and co-workers.[3] *Novobiocin* is another coumarin-based compound and was marketed as an antibacterial drug. In a study, Zhao *et al.,* optimized the structure of *Novobiocin* as a highly active antiproliferative agent.[4] Additionally, simple coumarin, 7-hydroxyoumarin, *Esculetin* (6,7-dihydroxycoumarin), and *Scopoletin* (6-methoxy-7-hydroxycoumarin) performed strong cytotoxic effects against cancer cell lines.[5] Coumarin derived compounds have also a wide spectrum of biological activities which were reviewed by expert researchers.[6,7]

Benzimidazole is an important member of nitrogen heterocycles and consist of the fusion of the imidazole and benzene rings *via* 4- and 5- positions of the imidazole ring. The most known benzimidazole derivative is

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N-ribosyl-5,6-dimethylbenzimidazole which serves as an axial ligand to cobalt in the structure of vitamin B12.[8] The first used benzimidazole derivatives are *Thiabendazole* and *Albendazole* which have anti-helminthic properties in agriculture.[9] Due to the cytotoxic properties of some benzimidazole-based compounds, commercially available anticancer drugs were developed.[10] Another important usage of benzimidazole derivatives is in the treatment of ulcer and some benzimidazole-based anti-ulcer drugs were marketed.[9] In addition, antimicrobial, anti-coagulant, anti-oxidant, and other biological properties of benzimidazole derivatives were reported.[11]

Benzimidazolium salts are 1,3-disubstituted ionic benzimidazole derivatives that contain acidic hydrogen at 2-position. Although they were mainly used as *N*-heterocyclic carbene (NHC) precursors, in recent years, their biological activities attracted much attention.[12] In 2015, Elie *et al*., reported that benzimidazolium salts perform antibacterial effects by the impairment of membrane permeability.[13] In another study, Liu *et al.,* reported that carbazole substituted benzimidazolium salts perform strong cytotoxicity against various cancer cell lines.[14] Additionally, enzyme inhibitory properties of some benzimidazolium salts were reported.[15–18]

In recent years, many research groups focused on the synthesis and anti-cancer properties of hybrid compounds of coumarin with other bio-active heterocyclic moieties such as pyrimidine,[19] chalcone,[20] β-carboline,[21] indole-triazole,[22] artemisinin,[23] thiazole,[24] and isooxazilones.[25]In the meantime, hybrid compounds of benzimidazole and their anticancer properties were reported with various heterocyclic moieties such as triazine,[26] ellipticine,[27] tetrazine,[28] deoxynucleosides,[29] thiazoles,[30] and chrysin.[31]However, according to our literature survey, coumarin-benzimidazole hybrids are very rare. Hwu and co-workers reported the synthesis and anti-viral properties of coumarin-benzimidazole hybrids.[32,33] Paul and co-workers synthesized coumarin-benzimidazole hybrids and showed that these compounds are highly active and selective anticancer agents.[34] Most recently, Holiyachi and co-workers reported the synthesis, antimicrobial, and anticancer properties of coumarin-benzimidazole-sulphonamide hybrids.[35] Based on the above information, herein, we report the synthesis and anticancer properties of coumarin substituted benzimidazolium salts. For this purpose, six 6-substituted-4-chloromethylene coumarin derivatives and sixteen benzimidazolium chlorides were synthesized and characterized. Cytotoxic properties of all compounds were tested against human prostate (PC-3) and ovarian (A2780) cancer cell lines.

2. Experimental

2.1 *Reagents and equipment*

Benzyl chloride, 2,3,4,5,6-pentamethylbenzyl chloride, 3,4,5-trimethoxybenzyl chloride, 4-methylphenol, 4-ethylphenol, 4-ethoxyphenol, 4-*iso*-propylphenol, 4-*tert*-butylphenol, 4-benzylphenol, ethyl-4-chloroacetoacetate and solvents were purchased from Aldrich Chemical Co and Alfa Aesar (Istanbul, Turkey) and used as received. The C, H and N elemental analysis were determined by LECO CHNS-932 elemental analyser. Melting points were determined in open capillary tubes by Electrothermal-9200 melting point apparatus. IR spectra in the range of 4000–400 cm^{-1} were obtained in ATR Sampling Accessory with Perkin Elmer UATR Two Spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded using a Bruker UltraShield 300 operating at 300.13 MHz (^1H), 75.47 MHz (^{13}C) using DMSO-d_6 as a solvent. Chemical shifts are given in ppm relative to tetramethylsilane (TMS). NMR multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, sep = septet, m = multiplet signal. Coupling constants, J, are given in Hz.

2.2 *Synthesis and characterization data of compounds*

2.2a Synthesis of 4-chloromethyl coumarin derivatives and characterization data (1a–f). These compounds were synthesized by the previously described procedure by Frasinyuk[36] with a minor modification. The crude products were re-crystallized from ethanol instead of dioxane. The mixture of 18 mmol (3 g) of ethyl-4-chloroacetoacetate and 18 mmol of corresponding phenol derivative was stirred in 40 mL 70% H_2SO_4 for 24 h at room temperature. Then the mixture was slowly poured into an ice bath and the precipitate was collected by filtration. The crude product was re-crystallized from ethanol. Among the **1a–f**, only **1a** was previously reported by Frasinyuk and co-workers.[22]

4-Chloromethyl-6-methyl-2*H*-chromene-2-one, 1a. White solid, yield: 2.8 g (75%), M.p.: 145–146 °C. Elemental analysis: Calculated for $C_{11}H_9ClO_2$; C, 63.32; H, 4.35; Found: C, 63.16; H, 4.24. IR (cm^{-1}): 3088, 2922, 1726 (-C=O), 1609, 1570, 1494, 1442. ^1H NMR (300 MHz, DMSO-d_6): δ 7.66 (m, 1H, Ar***H***), 7.47 (m, 1H, Ar***H***), 7.33 (m, 1H, Ar***H***), 6.66 (s, 1H, -C***H***=C-), 5.02 (s, 2H, -C***H***$_2$Cl), 2.39 (s, 3H, ArC***H***$_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.7 (-***C***=O), 151.4, 150.5, 133.7, 133.2, 124.9, 116.7, 116.5, 115.3 (-***C***H=C-), 41.2 (-***C***H$_2$Cl), 20.4 (Ar***C***H$_3$).

4-Chloromethyl-6-ethyl-2*H*-chromene-2-one, 1b. Yellow solid, yield: 1.6 g (40%), M.p.: 141–142 °C. Elemental analysis; Calculated for $C_{12}H_{11}ClO_2$; C, 64.73; H, 4.98; Found: C, 64.66; H, 4.91. IR (cm^{-1}): 3090, 2982, 2934, 1726 (-C=O), 1625, 1609, 1570, 1493, 1442. ^1H NMR (300 MHz, DMSO-d_6): δ 7.67 (m, 1H, Ar***H***), 7.51 (m, 1H, Ar***H***), 7.35 (m, 1H, Ar***H***), 6.66 (s, 1H, -C***H***=C-), 5.04 (s, 2H, -C***H***$_2$Cl), 2.69 (q, 2H, ArC***H***$_2$CH$_3$, J = 7.6 Hz), 1.22 (t, 3H,

$ArCH_2CH_3$, J = 7.6 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.7 (-***C***=O), 151.6, 150.6, 140.0, 132.1, 123.8, 116.7, 116.6, 115.3 (-***C***H=C-), 41.2 (-***C***H_2Cl), 27.6 (Ar***C***H_2CH_3), 15.6 ($ArCH_2$***C***H_3).

4-Chloromethyl-6-ethoxy-2*H*-chromene-2-one, 1c. Green solid, yield: 1.8 g (42%), M.p.: 149–150 °C. Elemental analysis; Calculated for $C_{12}H_{11}ClO_3$; C, 60.39, H, 4.65; Found: C, 60.22; H, 4.53. IR (cm^{-1}): 3090, 2982, 2876, 1729 (-C=O), 1624, 1568, 1499, 1477. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.38 (m, 1H, Ar***H***), 7.29 (m, 1H, Ar***H***), 7.24 (m, 1H, Ar***H***), 6.67 (s, 1H, -C***H***=C-), 5.04 (s, 2H, -C***H***$_2$Cl), 4.10 (q, 2H, ArOC***H***$_2CH_3$, J = 6.9 Hz), 1.36 (t, 3H, $ArOCH_2$C***H***$_3$, J = 6.9 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.7 (-***C***=O), 154.7, 150.4, 147.4, 119.7, 117.8, 117.5, 115.7 (-***C***H=C-), 108.8, 63.8 (ArO***C***H_2CH_3), 41.3 (-***C***H_2Cl), 14.5 ($ArOCH_2$***C***H_3).

4-Chloromethyl-6-isopropyl-2*H*-chromene-2-one, 1d. Beige solid, yield: 2.3 g (54%), M.p.: 106–107 °C. Elemental analysis; Calculated for $C_{13}H_{13}ClO_2$; C, 65.97; H, 5.54; Found: C, 65.81; H, 5.44. IR (cm^{-1}): 3089, 3033, 2922, 1726 (-C=O), 1625, 1609, 1570, 1493, 1442. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.69 (m, 1H, Ar***H***), 7.56 (m, 1H, Ar***H***), 7.37 (m, 1H, Ar***H***), 6.67 (s, 1H, -C***H***=C-), 5.07 (s, 2H, -C***H***$_2$Cl), 3.00 (sep, 1H, ArC***H***$(CH_3)_2$, J = 6.8 Hz), 1.25 (d, 6H, $ArCH(CH_3)_2$, J = 6.8 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.7 (-***C***=O), 151.6, 150.7, 144.6, 130.6, 122.6, 116.7, 116.6, 115.2 (-***C***H=C-), 41.3 (-***C***H_2Cl), 33.0 (Ar***C***$H(CH_3)_2$), 23.8 ($ArCH(CH_3)_2$).

4-Chloromethyl-6-*tert*-butyl-2*H*-chromene-2-one, 1e. This compound was ready from our previous study.[17]

4-Chloromethyl-6-benzyl-2*H*-chromene-2-one, 1f. White solid, yield: 1.9 g (37%), M.p.: 116–117 °C, Elemental analysis; Calculated for $C_{17}H_{13}ClO_2$; C, 71.71; H, 4.60; Found: C, 71.56; H, 4.53. IR (cm^{-1}): 3091, 2945, 1717 (-C=O), 1626, 1610, 1572, 1493, 1454. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.79 (m, 1H, Ar***H***), 7.49 (m, 1H, Ar***H***), 7.36 (m, 1H, Ar***H***), 7.33-7.16 (m, 5H, Ar***H***), 6.68 (s, 1H, -C***H***=C-), 5.02 (s, 2H, -C***H***$_2$Cl), 4.04 (s, 2H, ArC***H***$_2$Ar). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.7 (-***C***=O), 151.8, 150.5, 140.8, 137.6, 132.9, 128.6, 128.5, 126.1, 124.9, 116.9, 116.8, 115.4 (-***C***H=C-), 41.2 (-***C***H_2Cl), 40.3 (Ar***C***H_2Ar).

2.2b General procedure for the synthesis of benzimidazolium salts and characterization data (2a–f, 3a–e, 4a–e). The mixture of 2.4 mmol of 1-benzylbenzimidazole derivative and 2.4 mmol of 4-chloromethylne coumarin derivative in 10 mL of DMF was stirred at 80 °C for 24 h. Later, DMF was removed under reduced pressure. The crude product was dissolved in 20 mL of hot ethanol and then ethanol was evaporated under reduced pressure to half of initial volume. Twice of the last volume of diethyl ether was added to mixture. Obtained crystals were collected, washed three times with diethyl ether (3 × 10 mL) and dried under reduced pressure.

1-Benzyl-3-((6-methyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 2a. White solid, yield: 0.78 g (78%), M.p.: 248–249 °C. Elemental analysis; Calculated for $C_{25}H_{21}ClN_2O_2$; C, 72.02; H, 5.08; N, 6.72; Found: C, 71.85; H, 4.92; N, 6.60. IR (cm^{-1}): 3118, 3030, 1702 (-C=O), 1687, 1617, 1576, 1557, 1482, 1452. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.18 (s, 1H, -NC***H***N-), 8.09–8.03 (m, 2H, Ar***H***), 7.76–7.66 (m, 3H, Ar***H***), 7.61–7.53 (m, 3H, Ar***H***), 7.48–7.38 (m, 4H, Ar***H***), 6.23 (s, 2H, -NC***H***$_2$coumarin), 6.03 (s, 1H, -C***H***=C-), 5.84 (s, 2H, -NC***H***$_2$Ph), 2.43 (s, 3H, ArC***H***$_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.5 (-***C***=O), 151.1, 148.5, 143.6 (-N***C***HN-), 134.0, 133.7, 133.5, 131.4, 131.2, 129.0, 128.8, 128.5, 127.1, 127.0, 124.4, 116.63, 116.58, 114.1, 113.9, 112.9 (-***C***H=C-), 50.2 (-N***C***H_2Ph), 46.6 (-N***C***H_2coumarin), 20.4 (Ar***C***H_3).

1-Benzyl-3-((6-ethyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 2b. White solid, yield: 0.37 g (36%), M.p.: 181–183 °C. Elemental analysis; Calculated for $C_{26}H_{23}ClN_2O_2$; C, 72.47; H, 5.38; N, 6.50; Found: C, 72.31; H, 5.30; N, 6.38. IR (cm^{-1}): 3119, 3029, 2969, 1704 (-C=O), 1632, 1613, 1576, 1558, 1492, 1482, 1457. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.18 (s, 1H, -NC***H***N-), 8.11–8.04 (m, 2H, Ar***H***), 7.74–7.67 (m, 3H, Ar***H***), 7.62–7.54 (m, 3H, Ar***H***), 7.47–7.39 (m, 4H, Ar***H***), 6.26 (s, 2H, -NC***H***$_2$coumarin), 6.04 (s, 1H, -C***H***=C-), 5.84 (s, 2H, -NC***H***$_2$Ph), 2.72 (q, 2H, ArC***H***$_2CH_3$, J = 7.6 Hz), 1.22 (t, 3H, $ArCH_2$C***H***$_3$, J = 7.6 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.5 (-***C***=O), 151.3, 148.6, 143.6 (-N***C***HN-), 140.3, 133.6, 132.5, 131.3, 131.1, 129.0, 128.8, 128.5, 127.1, 127.0, 123.3, 116.7, 116.6, 114.1, 113.9, 112.9 (-***C***H=C-), 50.2 (-N***C***H_2Ph), 46.6 (-N***C***H_2coumarin), 27.6 (Ar***C***H_2CH_3), 15.6 ($ArCH_2$***C***H_3).

1-Benzyl-3-((6-ethoxy-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 2c. Yellow solid, yield: 0.33 g (31%), M.p.: 245–247 °C. Elemental analysis; Calculated for $C_{26}H_{23}ClN_2O_3$; C, 69.87; H, 5.19; N, 6.27; Found: C, 69.66; H, 5.11; N, 6.12. IR (cm^{-1}): 2882, 2774, 1729 (-C=O),1613, 1577, 1561, 1497, 1483, 1454. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.17 (s, 1H, -NC***H***N-), 8.11–8.03 (m, 2H, Ar***H***), 7.74–7.65 (m, 2H, Ar***H***), 7.61–7.54 (m, 2H, Ar***H***), 7.49–7.38 (m, 4H, Ar***H***), 7.36–7.29 (m, 2H, Ar***H***), 6.23 (s, 2H, -NC***H***$_2$coumarin), 6.08 (s, 1H, -C***H***=C-), 5.83 (s, 2H, -NC***H***$_2$Ph), 4.10 (q, 2H, ArOC***H***$_2CH_3$, J = 6.9 Hz), 1.34 (t, 3H, $ArOCH_2$C***H***$_3$, J = 6.9 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.5 (-***C***=O), 154.9, 148.3, 147.2, 143.6 (-N***C***HN-), 133.7, 131.4, 131.1, 129.0, 128.8, 128.5, 127.1, 127.0, 120.1, 117.9, 117.3, 114.2, 113.9, 113.5 (-***C***H=C-), 108.3, 63.9 (ArO***C***H_2CH_3), 50.2 (-N***C***H_2Ph), 46.6 (-N***C***H_2coumarin), 14.5 ($ArOCH_2$***C***H_3).

1-Benzyl-3-((6-isopropyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 2d. White solid, yield: 0.55 g (52%), M.p.: 219–221 °C. Elemental analysis; Calculated for $C_{27}H_{25}ClN_2O_2$; C, 72.88; H, 5.66; N, 6.30; Found: C, 72.71; H, 5.60; N, 6.17. IR (cm^{-1}): 3033, 2966, 1720 (-C=O), 1610, 1562, 1491, 1457. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.13 (s, 1H, -NC***H***N-), 8.14–8.04 (m, 2H, Ar***H***),

7.75–7.52 (m, 6H, Ar***H***), 7.48–7.38 (m, 4H, Ar***H***), 6.28 (s, 2H, -NC***H***$_2$coumarin), 6.05 (s, 1H, -C***H***=C-), 5.83 (s, 2H, -NC***H***$_2$Ph), 3.02 (sep, 1H, ArC***H***$(CH_3)_2$, J = 6.9 Hz), 1.23 (d, 2H, ArCH(C***H***$_3)_2$, J = 6.9 Hz). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 159.5 (-***C***=O), 151.3, 148.7, 144.9, 143.6 (-N***C***HN-), 133.6, 131.5, 131.11, 131.06, 129.0, 128.8, 128.5, 127.1, 127.0, 121.8, 116.8, 116.6, 114.1, 114.0, 112.9 (-***C***H=C-), 50.2 (-N***C***H$_2$Ph), 46.6 (-N***C***H$_2$coumarin), 33.0 (Ar***C***H$(CH_3)_2$), 23.7 (ArCH(***C***H$_3)_2$).

1-Benzyl-3-((6-*tert*-butyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 2e. White solid, yield: 0.87 g (79%), M.p.: 220–222 °C. Elemental analysis; Calculated for $C_{28}H_{27}ClN_2O_2$; C, 73.27; H, 5.93; N, 6.10; Found: C, 73.11; H, 5.80; N, 6.02. IR (cm^{-1}): 3032, 2965, 1721 (-C=O), 1612, 1563, 1490, 1458, 1449. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 10.10 (s, 1H, -NC***H***N-), 8.17–8.03 (m, 2H, Ar***H***), 7.82–7.67 (m, 4H, Ar***H***), 7.58–7.37 (m, 6H, Ar***H***), 6.32 (s, 2H, -NC***H***$_2$coumarin), 6.07 (s, 1H, -C***H***=C-), 5.83 (s, 2H, -NC***H***$_2$Ph), 1.30 (s, 9H, ArC(C***H***$_3)_3$). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 159.5 (-***C***=O), 151.1, 148.8, 147.1, 143.6 (-N***C***HN-), 133.7, 131.5, 131.1, 130.2, 129.0, 128.8, 128.4, 127.1, 127.0, 120.5, 116.5, 116.1, 114.2, 114.0, 113.0 (-**C**H=C-), 50.2 (-N***C***H$_2$Ph), 46.7 (-N***C***H$_2$coumarin), 34.5 (Ar***C***$(CH_3)_3$), 30.9 (ArC(***C***H$_3)_3$).

1-Benzyl-3-((6-benzyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 2f. White solid, yield: 0.53 g (45%), M.p.: 156–158 °C. Elemental analysis; Calculated for $C_{31}H_{25}ClN_2O_2$; C, 75.53; H, 5.11; N, 5.68; Found: C, 75.34; H, 5.03; N, 5.56. IR (cm^{-1}): 2925, 1725 (-C=O), 1666, 1613, 1571, 1492, 1455. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 10.13 (s, 1H, -NC***H***N-), 8.12–8.03 (m, 2H, Ar***H***), 7.82 (m, 1H, Ar***H***), 7.75–7.65 (m, 2H, Ar***H***), 7.63–7.53 (m, 3H, Ar***H***), 7.48–7.38 (m, 4H, Ar***H***), 7.32–7.15 (m, 5H, Ar***H***), 6.21 (s, 2H, -NC***H***$_2$coumarin), 6.06 (s, 1H, -C***H***=C-), 5.83 (s, 2H, -NC***H***$_2$Ph), 4.07 (s, 2H, ArC***H***$_2$Ar). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 159.4 (-***C***=O), 151.5, 148.5, 143.6 (-N***C***HN-), 140.7, 137.9, 133.6, 133.3, 131.5, 131.1, 129.0, 128.8, 128.6, 128.51, 128.49, 127.1, 127.0, 126.2, 124.2, 117.0, 116.7, 114.1, 113.9, 113.1 (-***C***H=C-), 50.2 (-N***C***H$_2$Ph), 46.6 (-N***C***H$_2$coumarin), 40.3 (Ar***C***H$_2$Ar).

1-(2,3,4,5,6-Pentamethylbenzyl)-3-((6-methyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 3a. White solid, yield: 0.71 g (61%), M.p.: 217–219 °C. Elemental analysis; Calculated for $C_{30}H_{31}ClN_2O_2$; C, 73.98; H, 6.42; N, 5.75; Found: C, 74.18; H, 6.54; N, 5.67. IR (cm^{-1}): 2902, 1723 (-C=O), 1611, 1561, 1446. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 9.33 (s, 1H, -NC***H***N-), 8.37 (d, 1H, Ar***H***, J = 8.1 Hz), 8.05 (d, 1H, Ar***H***, J = 8.1 Hz), 7.85–7.67 (m, 3H, Ar***H***), 7.57–7.51 (m, 1H, Ar***H***), 7.41–7.36 (m, 1H, Ar***H***), 6.15 (s, 2H, -NC***H***$_2$coumarin), 5.91 (s, 1H, -C***H***=C-), 5.79 (s, 2H, -NC***H***$_2$Ph$(CH_3)_5$), 2.41 (s, 3H, ArC***H***$_3$-coumarin), 2.24 (s, 9H, ArC***H***$_3$ − *o* and *p*), 2.22 (s, 6H, ArC***H***$_3$-*m*). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 159.5 (-***C***=O), 151.0, 149.1, 142.2 (-N***C***HN-), 136.9, 133.94, 133.87, 133.5, 133.0, 131.8, 131.7, 127.2, 126.9, 125.6, 124.2, 116.6, 116.5, 114.2, 113.9, 111.9 (-***C***H=C-), 46.7 (-N***C***H$_2$Ph$(CH_3)_5$), 46.6 (-N***C***H$_2$coumarin), 20.4 (Ar***C***H$_3$-coumarin), 17.0 (Ar***C***H$_3$), 16.7(Ar***C***H$_3$), 16.4 (Ar***C***H$_3$).

1-(2,3,4,5,6-Pentamethylbenzyl)-3-((6-ethyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 3b. Yellow solid, yield: 0.55 g (46%), M.p.: 249–250 °C. Elemental analysis; Calculated for $C_{31}H_{33}ClN_2O_2$; C, 74.31; H, 6.64; N, 5.59; Found: C, 74.47; H, 6.71; N, 5.66. IR (cm^{-1}): 2971, 1714 (-C=O), 1571, 1430. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 9.26 (s, 1H, -NC***H***N-), 8.33 (d, 1H, Ar***H***, J = 8.1 Hz), 8.05 (d, 1H, Ar***H***, J = 8.1 Hz), 7.86–7.66 (m, 3H, Ar***H***), 7.60–7.54 (m, 1H, Ar***H***), 7.43–7.38 (m, 1H, Ar***H***), 6.14 (s, 2H, -NC***H***$_2$coumarin), 5.87 (s, 1H, -C***H***=C-), 5.77 (s, 2H, -NC***H***$_2$Ph$(CH_3)_5$), 2.71 (q, 2H, Ar***C***H$_2$CH$_3$, J = 7.6 Hz), 2.24 (s, 3H, ArC***H***$_3$ − *p*), 2.23 (s, 6H, ArC***H***$_3$ − *o*), 2.22 (s, 6H, ArC***H***$_3$ − *m*), 1.22 (t, 3H, ArCH$_2$C***H***$_3$,J = 7.6 Hz). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 159.5 (-***C***=O), 151.1, 149.2, 142.2 (-N***C***HN-), 140.3, 136.3, 133.8, 133.0, 132.4, 131.8, 131.7, 127.3, 127.0, 125.5, 123.1, 116.7, 116.6, 114.2, 113.9, 111.8 (-***C***H=C-), 46.7 (-N***C***H$_2$Ph$(CH_3)_5$), 46.6 (-N***C***H$_2$coumarin), 27.6 (Ar***C***H$_2$CH$_3$), 16.9 (Ar***C***H$_3$), 16.7 (Ar***C***H$_3$), 16.4 (Ar***C***H$_3$), 15.6 (ArCH$_2$***C***H$_3$).

1-(2,3,4,5,6-Pentamethylbenzyl)-3-((6-ethoxy-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 3c. Yellow solid, yield: 0.52 g (42%), M.p.: 175–177 °C. Elemental analysis; Calculated for; $C_{31}H_{33}ClN_2O_3$; C, 72.01, H, 6.43; N, 5.42; Found: C, 72.24; H, 6.53; N, 5.44. IR (cm^{-1}): 2967, 1724 (-C=O), 1611, 15771, 1448. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 9.33 (s, 1H, -NC***H***N-), 8.34 (d, 1H, Ar***H***, J = 8.1 Hz), 8.05 (d, 1H, Ar***H***, J = 8.1 Hz), 7.85–7.70 (m, 2H, Ar***H***), 7.46–7.40 (m, 1H, Ar***H***), 7.35–7.29 (m, 2H, Ar***H***), 6.18 (s, 2H, -NC***H***$_2$oumarin), 5.94 (s, 1H, -C***H***=C-), 5.79 (s, 2H, -NC***H***$_2$Ph$(CH_3)_5$), 4.12 (q, 2H, ArOC***H***$_2$CH$_3$, J = 6.9 Hz), 2.25 (s, 3H, ArC***H***$_3$ − *p*), 2.24 (s, 6H, ArC***H***$_3$ − *o*), 2.22 (s, 6H, ArC***H***$_3$ − *m*), 1.36 (t, 3H, ArOCH$_2$C***H***$_3$, J = 6.9 Hz). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 159.5 (-***C***=O), 154.9, 149.0, 147.1, 142.2 (-N***C***HN-), 136.3, 133.9, 133.0, 131.9, 131.7, 127.2, 126.9, 125.6, 120.1, 117.9, 117.3, 114.2, 113.9, 112.4 (-***C***H=C-), 108.2, 64.0 (ArO***C***H$_2$CH$_3$), 46.8 (-N***C***H$_2$Ph$(CH_3)_5$), 46.6 (-N***C***H$_2$coumarin), 17.0 (Ar***C***H$_3$), 16.7 (Ar***C***H$_3$), 16.4 (Ar***C***H$_3$), 14.5 (ArOCH$_2$***C***H$_3$).

1-(2,3,4,5,6-Pentamethylbenzyl)-3-((6-isopropyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 3d. White solid, yield: 0.63 g (51%), M.p.: 237–241 °C. Elemental analysis; Calculated for; $C_{32}H_{35}ClN_2O_2$; C, 74.62; H, 6.85; N, 5.44; Found: C, 74.93; H, 7.01; N, 5.31. IR (cm^{-1}): 2957, 1717 (-C=O), 1610, 1564, 1471, 1434. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 9.29 (s, 1H, -NC***H***N-), 8.34 (d, 1H, Ar***H***, J = 8.1 Hz), 8.09 (d, 1H, Ar***H***, J = 7.9 Hz), 7.87–7.57 (m, 4H, Ar***H***), 7.45–7.38 (m, 1H, Ar***H***), 6.20 (s, 2H, -NC***H***$_2$coumarin), 5.86 (s, 1H, -C***H***=C-), 5.79 (s, 2H, -NC***H***$_2$Ph$(CH_3)_5$), 3.02 (sep, 1H, ArC***H***$(CH_3)_2$, J = 6.6 Hz), 2.24 (m, 15H, ArC***H***$_3$), 1.25 (d, 6H, ArCH(C***H***$_3)_2$, J = 6.6 Hz). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 159.5 (-***C***=O), 151.2, 149.5, 144.8, 142.2 (-N***C***HN-), 136.3, 133.8, 132.9, 131.84,

131.79, 131.0, 127.2, 126.9, 125.6, 121.7, 116.7, 116.6, 114.2, 113.9, 111.5 (-***C***H=C-), 46.8 (-N***C***H_2Ph(CH_3)$_5$), 46.6 (-N***C***H_2coumarin), 33.1 (Ar***C***H(CH_3)$_2$), 23.8 (ArCH(***C***H_3)$_2$), 17.0 (Ar***C***H_3), 16.7 (Ar***C***H_3), 16.4 (Ar***C***H_3).

1-(2,3,4,5,6-Pentamethylbenzyl)-3-((6-*tert*-butyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 3e. White solid, yield: 0.95 g (75%), M.p.: 261–262 °C. Elemental analysis; Calculated for $C_{33}H_{37}ClN_2O_2$; C, 74.91; H, 7.05; N, 5.29; Found: C, 75.06; H, 7.13; N, 5.14. IR (cm^{-1}): 2963, 1720 (-C=O), 1611, 1562, 1475, 1429. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.27 (s, 1H, -NC***H***N-), 8.34 (d, 1H, Ar***H***, J = 8.1 Hz), 8.09 (d, 1H, Ar***H***, J= 8.0 Hz), 7.85–7.70 (m, 4H, Ar***H***), 7.45–7.40 (m, 1H, Ar***H***), 6.25 (s, 2H, -NC***H***$_2$ coumarin), 5.85 (s, 1H, -C***H***=C-), 5.79 (s, 2H, -NC***H***$_2$Ph(CH_3)$_5$), 2.24 (s, 3H, ArC***H***$_3$ − *p*), 2.23 (s, 6H, ArC***H***$_3$ − *o*), 2.22 (s, 6H, ArC***H***$_3$ − *m*), 1.33 (s, 9H, ArC(C***H***$_3$)$_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.5 (-***C***=O), 150.9, 149.6, 147.1, 142.2 (-N***C***HN-), 136.3, 133.8, 132.9, 131.82, 131.79, 130.2, 127.2, 126.9, 125.6, 120.4, 116.4, 116.2, 114.2, 114.0, 111.5 (-***C***H=C-), 46.8 (-N***C***H_2Ph(CH_3)$_5$), 46.6 (-N***C***H_2coumarin), 34.5 (Ar***C***(CH_3)$_3$), 31.0 (ArC(***C***H_3)$_3$), 17.0 (Ar***C***H_3), 16.7 (Ar***C***H_3), 16.4 (Ar***C***H_3).

1-(3,4,5-Trimethoxybenzyl)-3-((6-methyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 4a. White solid, yield: 0.81 g (67%), M.p.: 176–177 °C. Elemental analysis; Calculated for $C_{28}H_{27}ClN_2O_5$; C, 66.34; H, 5.37; N, 5.53; Found: C, 66.23; H, 5.26; N, 5.40. IR (cm^{-1}): 2964, 1724 (-C=O), 1603, 1593, 1574, 1560, 1508, 1449. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.26 (s, 1H, -NC***H***N-), 8.26–8.20 (m, 1H, Ar***H***), 8.10–8.04 (m, 1H, Ar***H***), 7.79–7.65 (m, 3H, Ar***H***), 7.60–7.52 (m, 1H, Ar***H***), 7.45–7.37 (m, 1H, Ar***H***), 7.03 (s, 2H, Ar***H***), 6.26 (s, 2H, -NC***H***$_2$coumarin), 5.98 (s, 1H, -C***H***=C-), 5.72 (s, 2H, -NC***H***$_2$Ph(OCH_3)$_3$), 3.78 (s, 6HO, ArOC***H***$_3$ − *m*), 3.65 (s, 3H, ArOC***H***$_3$ − *p*), 2.43 (s, 3H, ArC***H***$_3$). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.4 (-***C***=O), 153.2, 151.1, 148.7, 143.5 (-N***C***HN-), 137.7, 134.0, 133.5, 131.4, 131.2, 128.8, 127.0, 126.9, 124.4, 116.6, 114.3, 113.8, 112.6 (-***C***H=C-), 106.6, 59.9 (ArO***C***H_3), 56.1 (ArO***C***H_3), 50.5 (-N***C***H_2Ph(OCH_3)$_3$), 46.5 (-N***C***H_2coumarin), 20.4 (Ar***C***H_3).

1-(3,4,5-Trimethoxybenzyl)-3-((6-ethyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 4b. Yellow solid, yield: 0.74 g (59%), M.p.: 212–213 °C. Elemental analysis; Calculated for $C_{29}H_{29}ClN_2O_5$; C, 66.85; H, 5.61; N, 5.28; Found: C, 66.70; H, 5.52; N, 5.31. IR (cm^{-1}): 2968, 1724 (-C=O), 1630, 1593, 1575, 1558, 1507, 1479, 1445. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.23 (s, 1H, -NC***H***N-), 8.27–8.20 (m, 1H, Ar***H***), 8.13–8.06 (m, 1H, Ar***H***), 7.78-7.66 (m, 3H, Ar***H***), 7.63–7.55 (m, 1H, Ar***H***), 7.47–7.40 (m, 1H, Ar***H***), 7.03 (s, 2H, Ar***H***), 6.28 (s, 2H, -NC***H***$_2$coumarin), 5.98 (s, 1H, -C***H***=C-), 5.71 (s, 2H, -NC***H***$_2$Ph(OCH_3)$_3$), 3.77 (s, 6HO, ArOC***H***$_3$ − *m*), 3.65 (s, 3H, ArOC***H***$_3$ − *p*), 2.72 (q, 2H, ArC***H***$_2$CH$_3$, J = 7.5 Hz), 1.22 (t, 3H, ArCH$_2$C***H***$_3$, J = 7.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.5 (-***C***=O), 153.2, 151.3, 148.8, 143.5 (-N***C***HN-), 140.3, 137.7, 132.5, 131.4, 131.2, 128.8, 127.0, 126.9, 123.3, 116.7, 116.6, 114.3, 113.8, 112.6 (-***C***H=C-), 106.6, 59.9 (ArO***C***H_3), 56.1 (ArO***C***H_3), 50.5 (-N***C***H_2Ph(OCH_3)$_3$), 46.5 (-N***C***H_2coumarin), 27.6 (Ar***C***H_2CH$_3$), 15.6 (ArCH$_2$***C***H_3).

1-(3,4,5-Trimethoxybenzyl)-3-((6-ethoxy-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 4c. Yellow solid, yield: 0.67 g (52%), M.p.: 130–131 °C. Elemental analysis; Calculated for $C_{29}H_{29}ClN_2O_6$; C, 64.86; H, 5.44; N, 5.22; Found: 64.62; H, 5.30; N, 5.08. IR (cm^{-1}): 2966, 1718 (-C=O), 1595, 1574, 1508, 1468, 1444. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.16 (s, 1H, -NC***H***N-), 8.25–8.19 (m, 1H, Ar***H***), 8.12–8.05 (m, 1H, Ar***H***), 7.79–7.66 (m, 2H, Ar***H***), 7.49–7.42 (m, 1H, Ar***H***), 7.38–7.29 (m, 2H, Ar***H***), 7.00 (s, 2H, Ar***H***), 6.26 (s, 2H, -NC***H***$_2$ coumarin), 6.03 (s, 1H, -C***H***=C-), 5.70 (s, 2H, -NC***H***$_2$Ph(OCH_3)$_3$), 4.11 (q, 2H, ArOC***H***$_2$CH$_3$, J = 7.0 Hz), 3.77 (s, 6HO, ArOC***H***$_3$ − *m*), 3.65 (s, 3H, ArOC***H***$_3$ − *p*), 1.34 (t, 3H, ArOCH$_2$C***H***$_3$, J = 7.0 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.4 (-***C***=O), 154.9, 153.2, 148.5, 147.2, 143.4 (-N***C***HN-), 137.7, 131.4, 131.2, 128.8, 127.1, 126.9, 120.1, 117.9, 117.3, 114.3, 113.8, 113.3 (-***C***H=C-), 108.4, 106.6, 63.9 (ArO***C***H_2CH$_3$), 59.9 (ArO***C***H_3), 56.0 (ArO***C***H_3), 50.5 (-N***C***H_2Ph(OCH_3)$_3$), 46.5 (-N***C***H_2coumarin), 14.5 (ArOCH$_2$***C***H_3).

1-(3,4,5-Trimethoxybenzyl)-3-((6-isopropyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 4d. Yellow solid, yield: 0.91 g (71%), M.p.: 191–193 °C. Elemental analysis, Calculated for; $C_{30}H_{31}ClN_2O_5$; C, 67.35; H, 5.84; N, 5.24; Found: C, 67.18; H, 5.77; N, 5.09. . IR (cm^{-1}): 2968, 1721 (-C=O), 1629, 1593, 1575, 1560, 1508, 1481, 1444. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.19 (s, 1H, -NC***H***N-), 8.26–8.20 (m, 1H, Ar***H***), 8.15–8.10 (m, 1H, Ar***H***), 7.78–7.67 (m, 3H, Ar***H***), 7.66–7.60 (m, 1H, Ar***H***), 7.47–7.41 (m, 1H, Ar***H***), 7.02 (s, 2H, Ar***H***), 6.30 (s, 2H, -NC***H***$_2$coumarin), 5.99 (s, 1H, -C***H***=C-), 5.71 (s, 2H, -NC***H***$_2$Ph(OCH_3)$_3$), 3.76 (s, 6HO, ArOC***H***$_3$-*m*), 3.64 (s, 3H, ArOC***H***$_3$ − *p*), 3.02 (sep, 1H, ArC***H***(CH_3)$_2$, J = 6.9 Hz), 1.22 (d, 6H, ArCH(C***H***$_3$)$_2$, J = 6.9 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ 159.5 (-***C***=O), 153.2, 151.3, 148.9, 144.9, 143.4 (-N***C***HN-), 137.7, 131.4, 131.2, 131.1, 128.8, 127.1, 126.9, 121.8, 116.7, 116.6, 114.3, 113.9, 112.6 (-***C***H=C-), 106.6, 59.9 (ArO***C***H_3), 56.0 (ArO***C***H_3), 50.5 (-N***C***H_2Ph(OCH_3)$_3$), 46.6 (-N***C***H_2coumarin), 33.0 (ArC***H***(CH_3)$_2$), 23.7 (ArCH(***C***H_3)$_2$).

1-(3,4,5-Trimethoxybenzyl)-3-((6-*tert*-butyl-2*H*-chromene-4-yl)methyl)benzimidazolium chloride, 4e. Yellow solid, yield: 1.10 g (84%), M.p.: 230–231 °C. Elemental analysis; Calculated for $C_{31}H_{33}ClN_2O_5$; C, 67.81; H, 6.06; N, 5.10; Found: C, 67.64; H, 6.01; N, 5.02. IR (cm^{-1}): 2966, 1724 (-C=O), 1603, 1593, 1560, 1574, 1508, 1449. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.15 (s, 1H, -NC***H***N-), 8.26–8.18 (m, 1H, Ar***H***), 8.18-8.10 (m, 1H, Ar***H***), 7.81–7.68 (m, 4H, Ar***H***), 7.48–7.41 (m, 1H, Ar***H***), 6.99 (s, 2H, Ar***H***), 6.34 (s, 2H, -NC***H***$_2$ coumarin), 6.03 (s, 1H, -C***H***=C-), 5.70 (s, 2H, -NC***H***$_2$Ph(OCH_3)$_3$), 3.75

(s, 6H, ArOC$\boldsymbol{H}_3$ − *m*), 3.64 (s, 3H, ArOC$\boldsymbol{H}_3$ − *p*), 1.28 (s, 9H, ArC(C$\boldsymbol{H}_3$)$_3$). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 159.5 (-***C***=O), 153.2, 151.1, 148.9, 147.1, 143.4 (-N***C***HN-), 137.7, 131.4, 131.1, 130.2, 128.8, 127.1, 127.0, 120.6, 116.5, 116.2, 114.3, 113.9, 112.9 (-***C***H=C-), 106.5, 59.9 (ArO***C***H$_3$), 56.0 (ArO***C***H$_3$), 50.5 (-N***C***H$_2$Ph(OCH$_3$)$_3$), 46.6 (-N***C***H$_2$coumarin), 34.5 (Ar***C***(CH$_3$)$_3$), 30.9 (ArC(***C***H$_3$)$_3$).

2.3 *Cytotoxicity studies*

Cell cultures. A2780 and PC-3 cell lines were preserved in RPMI-1640 culture medium supplemented with L-glutamine (10% heat-inactivated fetal bovine serum, 100 μ/mL penicillin-streptomycin), with the addition of 10 mM nonessential amino acids for the culture of prostate cancer cells. The cell lines were kept at 37 °C in a 5% CO_2 humidified incubator (Panasonic, Japan).

MTT assay. The synthesized compounds were screened for their antitumor activities against different type cancer cell lines (PC-3 and A2780) by MTT assay. The pale-yellow tetrazolium salt, MTT, was transformed by active mitochondria to form a dark blue formazan that was determined by a microplate reader.[37]

The MTT method provides a simple way to detect living and growing cells without using radioactivity. For all compounds, firstly, 100 μM and 50 mL solutions were prepared by the dissolving 0.005 mmol of the compound in 100 μL of DMSO and (less than 1% DMSO) and diluting the final volume to 50 mL with RPMI-1640. The other concentrations of compounds were prepared by the diluting of this solution. Shortly, the prostate and ovarian cancer cells were seeded into 96-well plates at 15×10^3 cells/well in a final volume of 100 μL and treated with different concentrations of compounds (1, 10 and 100 μM) in RPMI-1640. Then, the cells were incubated at 37 °C for 24 h in a 5% CO_2 humidified incubator. After 24 h, MTT (0.005 g/mL in phosphate buffer saline) was added to the cell culture and incubated for 3 h. The formazan crystals formed during the reaction of active mitochondria with MTT were dissolved in 0.04 N (100 mL) isopropanol and readings were recorded on a microplate reader (BioTek, Synergy HTX, USA) using a 570 nm filter.[38] By having the control wells read, the average of the obtained absorbance values was taken, and this value was accepted as 100% live cell. The absorbance values obtained from the solvent (the group into which only DMSO was added), as well as agent-applied wells, were proportioned to the control absorbance values, in addition to which the percentage (%) viability values were calculated. Each value represented an average of 10 measurements. All cellular results were determined against control cells.[39,40]

Statistical analyses. Quantitative data were presented as mean ± standard deviation (SD). Normal distribution was confirmed by the Kolmogorov-Smirnov test. Quantitative data were analysed using the Kruskal-Wallis H test following the Mann-Whitney U test with Bonferroni adjustment as a post-hoc test.

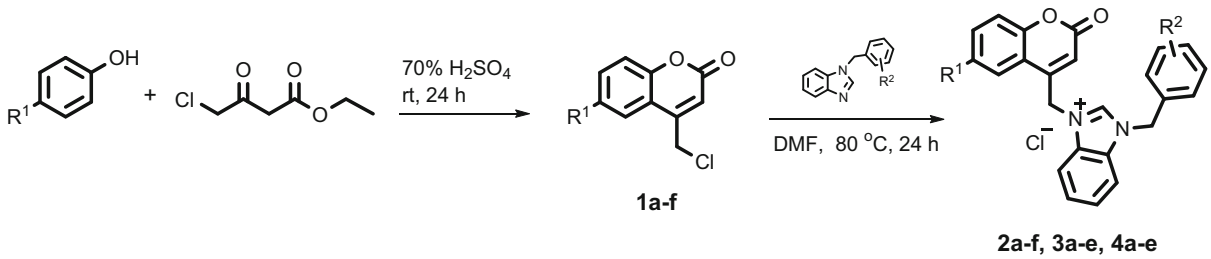
All P values < 0.05 were considered statistically significant. All analyses were done by IBM SPSS Statistics 22.0 for Windows. The $LogIC_{50}$ values were determined by using % cell viability values of compounds by the GraphPad Prism 6 program.

3. Results and Discussion

3.1 *Synthesis and characterization*

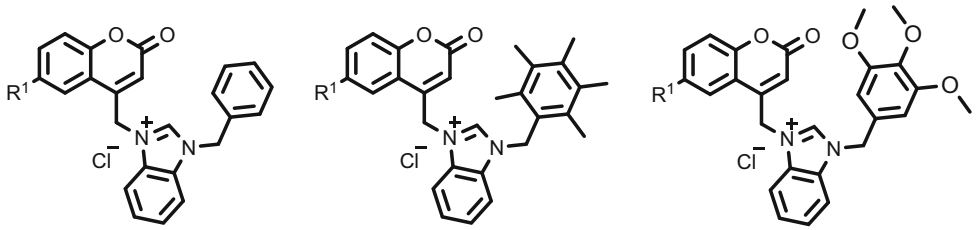
Firstly, 6-substituted-4-chloromethylene coumarin derivatives (**1a–f**) were synthesized by the previously reported procedure.[36] The structures of **1a–f** are given in Scheme 1. Compound **1e** was available from our previous study.[17] Other coumarin derivatives were characterized by ^{1}H and ^{13}C NMR, IR spectroscopic techniques and elemental analyses. In the ^{1}H NMR spectra of compounds, olefinic hydrogens were observed in the range of 6.66–6.68 ppm. Methylene protons were observed as singlets in the range of 5.02–5.07 ppm. In the ^{13}C NMR spectra of compounds, signals of olefinic carbons (-*C*H=C-) were observed in the range of 115.3–115.7 ppm. Signals of carbonyl carbons were observed at 159.7 ppm for all compounds. Other signals in the ^{1}H and ^{13}C NMR spectra of compounds were observed in agreement with the expected integrities and coupling patterns (See Supplementary Information for spectra). In the IR spectra of compounds, sharp peaks of carbonyl group were observed in the range of 1717–1729 cm^{-1}. Elemental analyses results are also supportive for structures and purities of compounds.

After the synthesis of **1a–f**, benzimidazolium salts were synthesized by the reaction of **1a–f** and *N*-benzylbenzimidazole derivatives at 80 °C during 24 hours. Synthetic route and structures of compounds are outlined in Scheme 1. Sixteen benzimidazolium salts (**2a–f**, **3a–e**, **4a–e**) were synthesized and characterized by ^{1}H and ^{13}C NMR (see Supplementary Information for spectra), IR spectroscopic techniques and elemental analyses. All salts are stable against oxygen and moisture of air, and daylight. Unfortunately, the products of the reaction of **1f** with **3** and **4** could not be purified and therefore, these compounds were not included in cytotoxicity assay. In the ^{1}H NMR spectra of benzimidazolium salts, the resonances of acidic -NC*H*N-hydrogens were observed in the range of 10.10–10.18 ppm for **2a–f**, 9.26–9.33 ppm for **3a–e**, and 10.15–10.26 ppm for **4a–e**. The appearance of these signals clearly proves the formation of benzimidazolium chlorides. In the ^{1}H NMR spectra of pentamethylbenzyl substituted benzimidazolium chlorides (**3a–e**), acidic signals



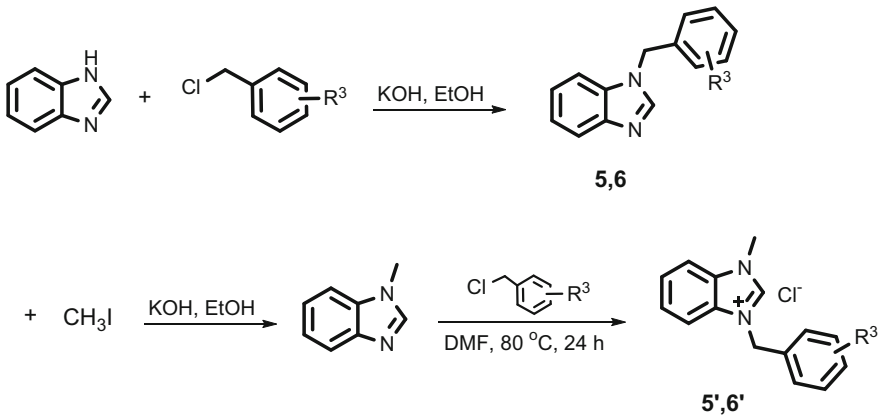
1a-f

2a-f, 3a-e, 4a-e



2a-f 3a-e 4a-e

a, R^1 = -CH_3 ; **b**, R^1 = -CH_2CH_3 ; **c**, R^1 = -OCH_2CH_3 ; **d**, R^1 = -$CH(CH_3)_2$; **e**, R^1 = -$C(CH_3)_3$; **f**, R^1 = -$CH_2C_6H_5$



5,6

5',6'

for **5** and **5'**, R^3 = H ;
for **6** and **6'**, R3 = 2,3,4,5,6-$(CH_3)_5$

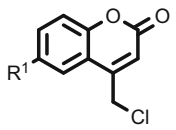
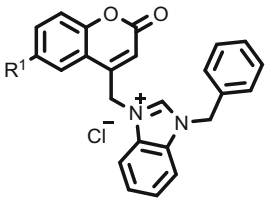
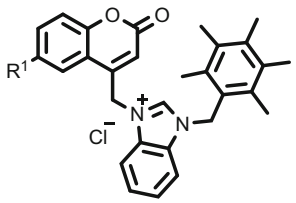
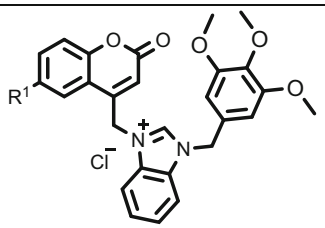
Scheme 1. Synthesis and structures of compounds.

were observed at high field compared to other benzimidazolium chlorides due to strong electron donating properties of methyl groups. The resonances of imino carbons (-N*C*HN-) were observed at 143.6 ppm for **2a–f**, 142.2 ppm for **3a–e** and 143.4 and 143.5 ppm for **4a–e**. In the ^{1}H NMR spectra, the signals of olefinic hydrogens were observed at the high field in the range of 5.85–6.08 ppm, compared with **1a–f**. Conversely, the signals of methylene hydrogens shifted to downfield compared with **1a–f**. Other NMR signals, IR spectra and elemental analyses results are also in agreement with the expected structures.

3.2 *Cytotoxicity studies*

In our previous studies, we had reported the human carbonic anhydrase I and II[16] and human paraoxonase I[17] inhibitory properties, and antimicrobial activities[41] of some coumarin substituted benzimidazolium chlorides. We had shown that these compounds have enzyme inhibitory properties but antimicrobial properties are dependent on their lipophilicity. In this study, we decided to synthesize a series of 6-substituted coumarin derivatives and their benzimidazolium salts in order to investigate their anticancer properties. The cytotoxic

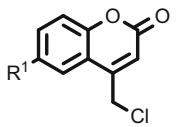
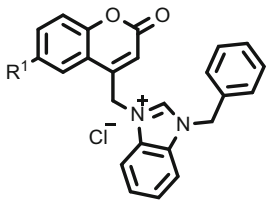
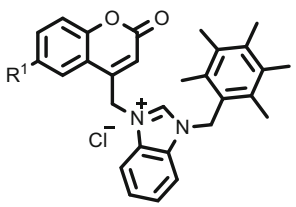
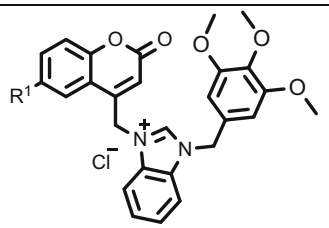
Table 1. Dose dependent cell viability results of PC-3 cells after 24 h treatment of coumarin substituted benzimidazolium salts. Each data point is an average of 10 viability measurements.

Compound	General Structure	R^1	1 µM	10 µM	100 µM
1a		$-CH_3$	95.26 ± 8.47	65.91 ± 7.23*	42.64 ± 10.22*
1b		$-CH_2CH_3$	90.77 ± 8.22	60.93 ± 10.68*	53.99 ± 11.91*
1c		$-OCH_2CH_3$	101.87 ± 12.08	92.37 ± 10.93	66.87 ± 8.29*
1d		$-CH(CH_3)_2$	60.79 ± 6.89*	35.62 ± 5.62*	20.86 ± 4.11*
1e		$-C(CH_3)_3$	53.51 ± 6.21*	30.75 ± 5.56*	30.15 ± 6.90*
1f		$-CH_2C_6H_5$	63.25 ± 7.16*	32.49 ± 10.93*	25.70 ± 5.85*
2a		$-CH_3$	64.62 ± 8.01*	42.72 ± 9.95*	18.26 ± 5.23*
2b		$-CH_2CH_3$	63.28 ± 8.27*	50.22 ± 8.76*	32.97 ± 4.41*
2c		$-OCH_2CH_3$	96.21 ± 12.68	55.16 ± 7.81*	30.91 ± 5.76*
2d		$-CH(CH_3)_2$	54.60 ± 5.23*	52.61 ± 5.26*	32.54 ± 7.29*
2e		$-C(CH_3)_3$	60.42 ± 7.29*	50.72 ± 10.14*	18.50 ± 3.51*
2f		$-CH_2C_6H_5$	94.49 ± 10.23	102.56 ± 18.45	36.64 ± 5.52*
3a		$-CH_3$	95.63 ± 5.36	105.09 ± 12.74	46.06 ± 5.29*
3b		$-CH_2CH_3$	108.47 ± 15.34	72.11 ± 8.81*	64.17 ± 8.91*
3c		$-OCH_2CH_3$	100.99 ± 11.02	84.89 ± 10.99	59.08 ± 8.23*
3d		$-CH(CH_3)_2$	103.26 ± 15.82	65.26 ± 10.87*	62.67 ± 9.35*
3e		$-C(CH_3)_3$	108.05 ± 13.95	61.65 ± 5.17*	46.67 ± 5.33*
4a		$-CH_3$	91.56 ± 11.03	63.93 ± 9.21*	23.67 ± 2.84*
4b		$-CH_2CH_3$	67.94 ± 7.81*	45.72 ± 5.26*	31.99 ± 4.81*
4c		$-OCH_2CH_3$	60.13 ± 9.28*	42.18 ± 6.89*	30.49 ± 5.23*
4d		$-CH(CH_3)_2$	66.36 ± 5.96*	53.29 ± 7.09*	41.27 ± 7.37*
4e		$-C(CH_3)_3$	44.53 ± 5.36*	44.44 ± 6.98*	34.17 ± 8.03*
N-benzylbenzimidazole (**5**)			101.96 ± 14.83	65.39 ± 10.21*	15.51 ± 3.42*
N-(2,3,4,5,6-pentamethyl)benzylbenzimidazole (**6**)			103.31 ± 11.67	106.52 ± 14.77	61.61 ± 9.99*
N-benzyl-*N*-methylbenzimidazolium chloride (**5'**)			101.82 ± 15.99	99.23 ±16.61	30.89 ± 6.69*
N-(2,3,4,5,6-pentamethyl)benzyl-*N*-methylbenzimidazolium chloride (**6'**)			101.65 ± 10.02	100.49 ± 13.94	84.82 ± 13.47
Docetaxel			31.45 ± 7.89*	20.25 ± 3.92*	2.12 ± 0.68*

Control value is 96.73 ± 9.13, *p < 0.05.

effects of all compounds were tested against human prostate (PC-3) and ovarian (A2780) cancer cell lines by MTT assay at three different concentration (1, 10 and 100 µM). The % cell viabilities after 24 h treatment of compound solutions are presented in Tables 1 and 2. Docetaxel was used as a standard drug for comparison. All compounds reported in this study performed lower cytotoxicity than Docetaxel which has some adverse effects in clinical use.[42] In fact, it is difficult to make precise generalizations, but we must point out that all compounds performed significant cytotoxicity against both cancer cells at 100 µM. The compounds **1d**, **1e**, **1f**, **2a**, **2b**, **2d**, **2e**, **4b**, **4c**, **4d** and **4e** performed significant cytotoxicity against PC-3 cells at 1 µM and **4e** was found out as the most active among all compounds. Pentamethylbenzyl substituted salts, **3** performed lower activities than other compounds against PC-3 cell lines. The compounds,

Table 2. Dose dependent cell viability results of A2780 cells after 24 h treatment of coumarin substituted benzimidazolium salts. Each data point is an average of 10 viability measurements.

Compound	General Structure	R^1	1 µM	10 µM	100 µM
1a		$-CH_3$	106.41 ± 9.76	85.70 ± 20.81	21.55 ± 5.06*
1b		$-CH_2CH_3$	98.87 ± 16.31	82.13 ± 8.29	60.23 ± 8.29*
1c		$-OCH_2CH_3$	95.10 ± 9.41	55.41 ± 8.96*	34.23 ± 9.06*
1d		$-CH(CH_3)_2$	82.49 ± 18.21	50.47 ± 5.98*	31.08 ± 6.21*
1e		$-C(CH_3)_3$	95.21 ± 9.13	69.88 ± 8.14*	30.22 ± 5.21*
1f		$-CH_2C_6H_5$	72.57 ± 4.26*	60.17 ± 9.41*	25.29 ± 8.28*
2a		$-CH_3$	91.60 ± 8.22	57.72 ± 6.28*	31.94 ± 6.11*
2b		$-CH_2CH_3$	60.26 ± 6.06*	58.26 ± 7.29*	48.24 ± 3.14*
2c		$-OCH_2CH_3$	92.33 ± 7.06	58.07 ± 10.02*	45.75 ± 3.94*
2d		$-CH(CH_3)_2$	66.28 ± 6.35*	60.14 ± 8.91*	35.78 ± 5.67*
2e		$-C(CH_3)_3$	90.14 ± 12.69	58.16 ± 19.85*	36.43 ± 7.96*
2f		$-CH_2C_6H_5$	92.26 ± 10.33	63.64 ± 9.95*	43.23 ± 9.09*
3a		$-CH_3$	101.39 ± 10.13	104.83 ± 14.75	54.51 ± 7.26*
3b		$-CH_2CH_3$	102.04 ± 15.25	79.98 ± 10.26*	60.13 ± 10.21*
3c		$-OCH_2CH_3$	106.63 ± 8.14	75.28 ± 10.22*	50.67 ± 7.61*
3d		$-CH(CH_3)_2$	82.41 ± 7.77	39.41 ± 7.13*	36.89 ± 4.09*
3e		$-C(CH_3)_3$	103.05 ± 14.25	55.98 ± 12.81*	43.04 ± 8.12*
4a		$-CH_3$	108.13 ± 14.44	58.14 ± 17.63*	27.26 ± 5.99*
4b		$-CH_2CH_3$	86.83 ± 8.99	90.46 ± 15.62	46.48 ± 3.38*
4c		$-OCH_2CH_3$	50.73 ± 8.36*	49.64 ± 8.39*	25.32 ± 5.02*
4d		$-CH(CH_3)_2$	101.20 ± 18.14	100.25 ± 19.88	53.53 ± 8.96*
4e		$-C(CH_3)_3$	95.09 ± 16.92	65.55 ± 13.44*	54.51 ± 9.23*
N-benzylbenzimidazole (**5**)			95.80 ± 10.13	60.15 ± 7.41*	23.66 ± 2.34*
N-(2,3,4,5,6-pentamethyl)benzylbenzimidazole (**6**)			108.24 ± 8.42	96.79 ± 11.34	66.52 ± 12.43*
N-benzyl-*N*-methylbenzimidazolium chloride (**5'**)			93.73 ± 14.23	92.44 ± 15.21	43.41 ± 5.34*
N-(2,3,4,5,6-pentamethyl)benzyl-*N*-methylbenzimidazolium chloride (**6'**)			99.85 ± 12.42	92.28 ± 10.86	61.16 ± 7.12*
Docetaxel			28.65 ± 6.19	16.79 ± 4.14	0.68 ± 0.04

Control value is 91.34 ± 11.24, *$p < 0.05$.

1f, **2b**, **2d**, **4c** performed significant cytotoxicity against A2780 cells at 1 µM and **4c** was found out as the most active among all compounds. The simple benzyl substituted salts, **2** performed stronger cytotoxicity than other compounds against A2780 cell lines. Additionally, methyl containing derivatives (**5'** and **6'**) of **5** and **6** were synthesized according to literature[43] in order to compare with coumarin substituted salts (Scheme 1). The cell viabilities of PC-3 and A2780 after treatment of **5**, **5'**, **6**, and **6'** were also given in Tables 1 and 2. When the results compared for PC-3, **5**, **5'**, **6**, and **6'** performed comparable activities only at 100 µM. On the other hand, compound **5** performed stronger activity than all coumarin substituted salts at 100 µM against A2780. **5'**, **6**, and **5'** performed weaker cytotoxicity than coumarin substituted salts at all concentrations. After the completion of 24 h tests, we investigated cell viabilities of both cell lines after the treatment of selected

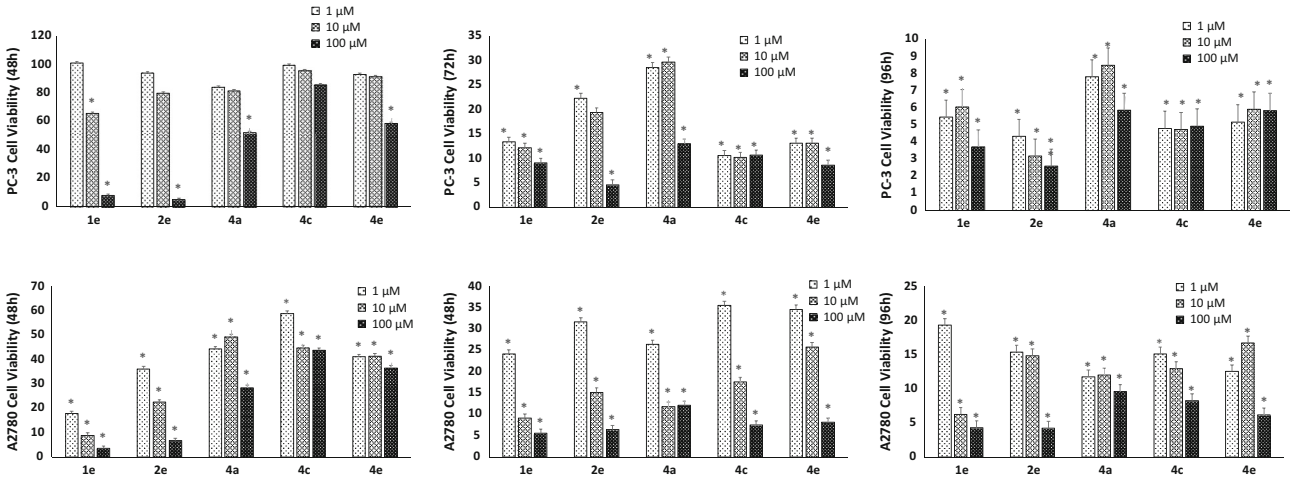


Figure 1. Cell viabilities of PC-3 (top) and A2780 (bottom) after 48 h (left), 72 h (middle), and 96 h (right) treatment of selected compounds (**1e**, **2e**, **4a**, **4c**, **4e**).

compounds **1e**, **2e**, **4a**, **4c**, **4e** during 48, 72, and 96 hours. The results were given in Figure 1. As seen from Figure 1, the cell viabilities significantly decreased after 72 and 96 hours of treatment of selected compounds.

The mechanisms of action of imidazolium or benzimidazolium salts have been investigated by some research groups. In 2009, Minematsu and co-workers reported the cellular uptake of ^{14}C-labelled 1-(2-methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1*H*-naphtho[2,3-*d*]imidazolium bromide (YM155 monobromide) into PC-3 cell lines.[44] The authors showed that YM155 suppress the Survivin which acts as anti-apoptotic protein in tumor cells. In a similar study, Lambrecht and co-workers showed that ^{131}I-labeled imidazolium bromide salt performs significant uptake efficiency in MCF-7 and PC-3 cell lines.[45] In 2015, Wright and co-workers showed that naphthalene-substituted lipophilic imidazolium salts induce PARP-1 cleavage and reduction in procaspase-3 so that causes the apoptotic pathway in NCI-H460 (human lung) cancer cells.[46] Yang and co-workers synthesized carbazole[14] and tetrahydrobenzodifuran[47] imidazolium and benzimidazolium salts and showed that these salts induce cell cycle arrest and apoptosis in SMMC-7721 cancer cell lines. All mechanistic studies clearly show that imidazolium and benzimidazolium salts can enter into the cancer cells. In this study, it is possible that the synthesized benzimidazolium salts performed their anticancer activity with different ways than impairment of membrane integrity. Additionally, fluorescence properties of coumarin derivatives are well known and we think that this is an advantage in the future studies for intracellular imaging.

4. Conclusions

In summary, we synthesized six 6-substituted-4-chloromethylene coumarin derivatives and their sixteen benzimidazolium chlorides. All compounds were characterized by ^{1}H and ^{13}C NMR, IR spectroscopic techniques and elemental analyses. Cytotoxic properties of all compounds were tested against PC-3 and A2780 cancer cells and all compounds performed significant activities at different concentrations. Although these results are preliminary, some compounds performed promising anticancer effects at low concentrations and in future we are planning to carry out mechanistic studies for reported compounds.

Supplementary Information (SI)

^{1}H and ^{13}C NMR spectra of all compounds can be found at www.ias.ac.in/chemsci.

Acknowledgements

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