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Research Article

**SYNTHESIS, CHARACTERISATION OF COUMARIN
DERIVATIVES AND THEIR ANTI CANCER ACTIVITIES****Karupakula Pravalika*¹, Dr. K. Hariprasad²**Department Of Chemistry ,Princeton College Of Pharmacy, Pocharam, Hyderabad, Telangana
500088.**Abstract:**

Coumarin and its derivatives are a prominent class of benzopyrone compounds known for diverse pharmacological properties, including anticancer, antimicrobial, and antioxidant activities. The present study focuses on the synthesis, characterization, and evaluation of anticancer activity of novel coumarin derivatives. The synthesis was carried out through Pechmann condensation and subsequent functional modifications at various positions of the coumarin nucleus to enhance biological efficacy. The synthesized compounds were characterized by FT-IR, ¹H-NMR, ¹³C-NMR, and Mass spectroscopic techniques to confirm their structural integrity and purity. The anticancer potential of the synthesized derivatives was assessed using MTT assay against selected human cancer cell lines, such as MCF-7 (breast cancer), HeLa (cervical cancer), and A549 (lung cancer). Results revealed that several derivatives exhibited significant cytotoxicity, which may be attributed to the presence of electron-donating or withdrawing substituents influencing the coumarin core. The findings suggest that structural modification of coumarin scaffolds can lead to promising lead molecules for future anticancer drug development.

Keywords: Coumarin derivatives, synthesis, characterization, anticancer activity, MTT assay, structure–activity relationship.

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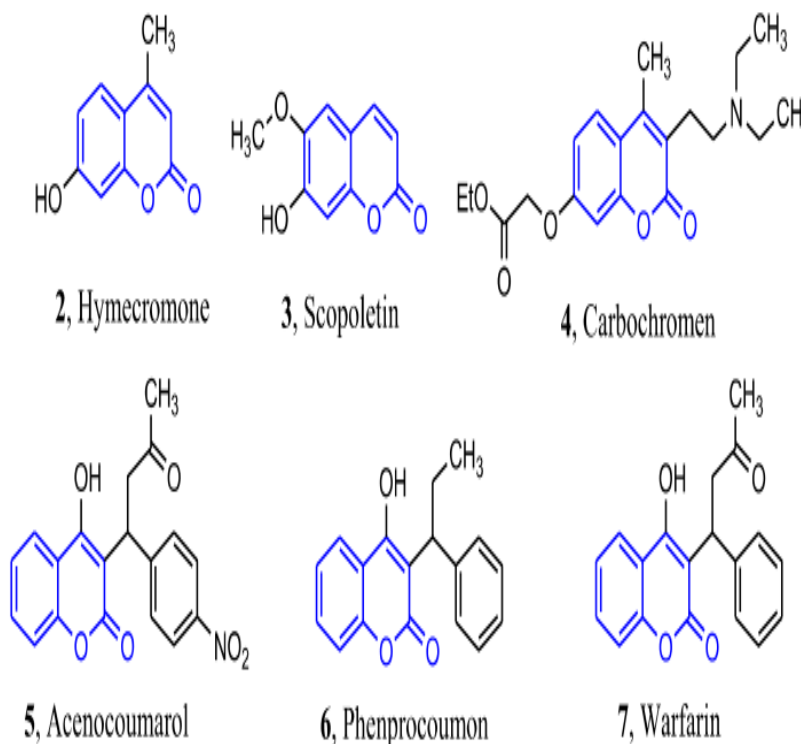


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INTRODUCTION:

According to a projection by the World Health Organization (WHO), more than 13 million cancer-related deaths will occur in 2030 [1,2]. This makes cancer one of the clearest causes of death globally. One study found that 20% of the population will get cancer in their lifetime [3]. Unchecked growth of cells without division is a hallmark of most malignancies [4,5]. This is because basic chemicals and other proteins that regulate cell division and extension become dysregulated. Despite numerous attempts to treat cancer and significant progress from diagnosis to therapy, some cancer patients either do not respond to treatment or experience a recurrence after an initial response. Chemotherapy is still an option to consider when dealing with cancer [6]. Cure resistance to different anticancer medicines is one of the most important fundamental problems in chemotherapy. There was a focus on chemotherapeutic master doses and drug-induced toxicities in the fight against mild resistance [7, 8]. It is essential to disclose untapped anticancer agents that show promise in research and have a strong track record of effective treatment [9]. The bicyclic heterocycle

(2H-1-benzopyran-2-one or 2H-chromen-2-one) in the core of coumarin (1, 2H-chromen-2-one or 2H-1) may be seen in Figure 1. All sorts of natural and synthetic compounds known as coumarins belong to the flavonoid group of plant assistant metabolites. These compounds have been tested in pharmacological studies for their anti-inflammatory, antioxidant, antinociceptive, hepatoprotective, antithrombotic, antiviral, antimicrobial, antituberculosis, anti-carcinogenic, upper, There are a variety of naturally occurring and synthetic medications that include the coumarin system that are clinically well-known masters. The choleric and antispasmodic effects were administered by hymecromone (4-methylumbelliferone, 2) in this instance [23]. Scopoletin, a compound with antioxidant, hepatoprotective, anti-inflammatory, and antifungal effects, is derived from a small number of plant species [24,25]. Carbochem is an effective medicine for cardiovascular disease (4) [26]. As anticoagulants and vitamin K antagonists, the 4-hydroxycoumarin derivatives acenocoumarol (5), phenprocoumon (6), warfarin (7), difenacoum (8), and brodifacoum [27] are useful.

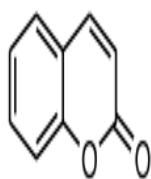


The following pharmacologically active administrators have been developed and are used extensively in clinical practice: armillarisin A (10), novobiocin (11), geiparvarin (12), auraptene (13), and ensaculin (14), all of which treat dementia [28-

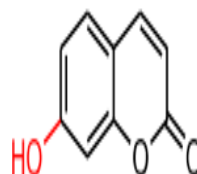
31]. Coumarins continue to be one of the most versatile classes of chemicals for anticancer medication arrangement and disclosure, despite the fact that they have a number of negative effects when investigated for bioactive ingredients. Coumarins

have been the subject of much research into their potential use in cancer cell therapy. The cytotoxic and cytostatic effects of coumarin (1) and 7-hydroxycoumarin on renal cell cancer were shown in vitro [32]. Also, xenograft models and in vitro experiments showed that both chemicals were cytotoxic to several human cancer cell lines, including gastric carcinoma, colon carcinoma, a cell line derived from hepatoma, and a lymphoblastic cell line [33–37]. As a particular anti-proliferative drug, 6-nitro-7-hydroxycoumarin inhibits p38, thrust activated protein kinase (SAPK), and the p21WAF1/CIP1 cyclin subordinate kinase, according to in vitro investigations [38]. In order to prevent the spread of leukemic cells, scopoletin (3,6-methoxy-7-hydroxycoumarin) and esculetin (17,6,7-dihydroxycoumarin) trigger cell death [39,40].

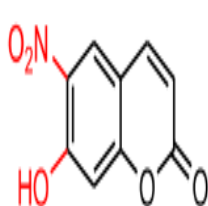
Warfarin (7), an anticoagulant, inhibits the spread of Mtlm3 tumors in rats, but it has no effect on the tumors' fundamental growth [41,42]. Coumarins have anticancer effects at obvious cancer stages by many mechanisms, such as regulating DNA-associated proteins like topoisomerase, modifying the estrogen receptor (ER), or stopping the cell cycle [43–51]. There has been a lot of work on the arrangement and combination of coumarin auxiliaries with significant advancements anticancer development because of the possible uses of coumarins in cancer chemotherapy. We have done our best to ensure that most future reports within this review's zone will use the same graph. Cancer therapies based on coumarin have been categorized according to the kind and position of the crucial pharmacophoric substituent on the coumarin core structure.



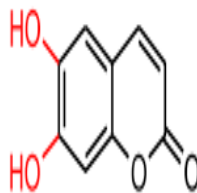
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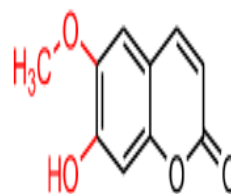
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17, Esculetin



3, Scopoletin

MATERIAL AND METHODS:

1. Arrange the Reagents and Conditions: (a) Piperidine, EtOH, and reflux; (b) NaOH (2 N), residual temperature, and at that moment HCl (2 N); (c) CDI, THF, and reflux; (ii) TFA, residual temperature. Nicolet 740 FT-IR Defiant and Perkin Elmer illustrate 283B are used to record infrared spectra, with values reported in cm⁻¹. The Bruker UX-NMR instruments varianGemini-200, varianunity-200, and advance-400 MHz capture proton nuclear attractive resonance spectra. The results are presented on the d scale and are based on experiments conducted in chloroform-d(1:1) or

DMSO-d₆, with tetramethylsilane (MeSi) as the internal standard. The ESI mass spectra were acquired using the ESI+ software, a smaller scale mass Quattro LC with a capillary voltage of 3.98 kV, and an ESI mode positive molecule trap locator. All byproducts are diluted with water and ethyl acetoacetate before being concentrated at a lower weight on a Buchi-R-3000 rotating screen operated at a temperature below 50°C. The fine print refers to the detached yields of materials that have been evaluated for homogeneity using techniques like thin-layer chromatography and nuclear magnetic resonance spectroscopy. All the factors were seen using TLC

procedures with the right dissolvable system for progress. The VARFIO EL, se Elementor underwent a necessary evaluation. Conventional syringe-septum methods are used to conduct clamminess-sensitive responses. It is possible to refine sodium benzophenone ketyl into dry ether and dry toluene, and to orchestrate the production of dry methanol using potassium hydroxide. There are 254 glass plates with a thickness of 0.5mm. Using iodine vapor or ultraviolet light, the spots on TLC plates are shown. The Melter Fp-51 device recorded the softening centers without correction. The reagents and solvents used in the exploratory strategy were supplied by Merck & Co., Inc. (Cerros Organics, Thermofisher, Belgium) and Darmatadt, Germany, and used them without purifying assistance. We acquired Entinostat and vorinostat from EuroAsia Chemicals (www.euroasiarnd.com). To screen the development of all responses, thin-layer chromatography was performed using 0.25 mm Silica gel plates (60 GF-254-Merck & Co, Darmatadt, Germany). To see the results, iodine vapour and ultraviolet light were employed. The electrothermal softening point device (Stafford, UK) was used to measure the uncorrected softening centers. An infrared spectrum was captured using a spectrometer manufactured by Perkin Elmer (KBr disks, Massachusetts, USA) called the Illustrate 1420. Bruker FT-300 MHz (Karlsruhe, Germany) ¹H NMR and ¹³C NMR spectra were selected at 300 MHz and 75 MHz, respectively, using DMSO-d₆, TMS. Separately, the chemical shifts (δ) and coupling constants (J) are conveyed in ppm and Hz, respectively. A turn item is denoted as s, d, t, q, sextet, or m, depending on its number of pieces. An electrospray ionization (ESI) interface was used to acquire mass spectra from a 6410 Agilent LC-MS triple quadrupole mass spectrometer (LC-MS, Santa Clara, USA). Standard tests were conducted using a Cos-Tec appear EAS 4010 instrument from Cernusco, Italy, and the results are within ±0.4% of the predicted values. The following cell lines were obtained from the Pasteur Institute of Iran: HCT116, A2780, MCF7, A549, HL60, PC3, and Huvec. A standard procedure for the condensation of ethyl 2-oxo-2H-chromene-3-carboxylates (3a-d) was followed: 2.11 mmol of diethyl malonate and 2 mL of piperidine were added to a solution of the appropriate salicylaldehyde intermediates (1a-d) (10 mmol) in 20 mL of ethanol. The resulting mixture was refluxed for 14–15 hours until the beginning materials disappeared, as observed by TLC. Following cooling, the vile substance was passed through a filter, rinsed with cold ethanol, and then recrystallized from the ethanol. Mixing 2-oxo-2H-chromene-3-carboxylic acids (4a-d): a typical

approach The esters (3a-v) were dissolved in 20 mL of watery sodium hydroxide (2 N) and then mixed at room temperature for 5 to 7 hours. The process was further advanced with hydrochloric acid (2 N) after cooling until a white precipitate formed. The white substance was filtered, rinsed with water, and then dried in order to eliminate acids. The conventional method for combining 4-((2-oxo-2H-chromene-3-carboxamido) methyl) benzoic destructive subordinates (6a-d) involved adding 5-mmol of comparing 2-oxo-2H-chromene-3-carboxylic acids (3a-d) to a solution of 1, 1'-carbonyldimidazole (CDI, 25 mmol) in dry tetrahydrofuran (THF, 10 mL), and then mixing the mixture for 2 hours at room temperature. Next, 5-(aminomethyl) benzoic destructive (5, 25 mmol) and trifluoroacetic destructive (TFA, 1.2 mL) were added and combined for another 10 hours at room temperature. Drying over anhydrous Na₂SO₄ and concentration under decreased weight followed the dissolution of the mixture in empty THF, followed by removal with EtOAc. The development filtering process made use of preparative inclination layer chromatography.

4-((2-oxo-2H-chromene-3-carboxamido) methyl) benzoic destructive (6a) White solid; yield:78%; mp: 261–263 °C; R_f = 0.56 (petroleum ether: ethyl acidic corrosive determination = 3:1); IR (KBr): $\hat{\nu}_{\text{max}}$ (cm⁻¹) 3312 (NH), 1709 1602 (CO); ¹H NMR (300 MHz, DMSO-d₆) δ 4.63 (d, 2H, J = 6.0 Hz, CH₂), 7.44–7.54 (m, 4H, Ar-H), 7.77 (t, 1H, J = 6.0 Hz, Ar-H), 7.92 (d, 2H, J = 9.0 Hz, Ar-H), 7.99 (d, 1H, J = 9.0 Hz, Ar-H), 8.90 (s, 1H, CH), 9.23 (t, 1H, J = 6.0 Hz, NH); ¹³C NMR (75 MHz, DMSO-d₆) δ 43.04, 116.62, 118.94, 119.57, 125.62, 127.78, 129.88, 130.06, 130.75, 134.61, 144.56, 148.08, 154.40, 160.73, 161.96, 167.60; Butt-centric. calcd. for C₁₈H₁₃NO₅: C, 66.87; H, 4.05; N, 4.33; found: C 66.80, H 4.09, N 4.24. 4-((8-methoxy-2-oxo-2H-chromene-3-carboxamido) methyl) benzoic destructive (6b) White solid; yield: 73%; mp: 238–240 °C; R_f = 0.49 (petroleum ether: ethyl acidic corrosive induction = 3:1); IR (KBr): $\hat{\nu}_{\text{max}}$ (cm⁻¹) 3347 (NH), 1712 & 1606 (CO); ¹H NMR (300 MHz, DMSO-d₆) δ 3.95 (s, 1H, OCH₃), 4.62 (d, 2H, J = 6.0 Hz, CH₂), 7.35–7.54 (m, 5H, Ar-H), 7.91 (d, 1H, J = 8.1 Hz, Ar-H), 8.85 (s, 1H, CH), 9.24 (t, 1H, J = 5.7 Hz, NH); ¹³C NMR (75 MHz, DMSO-d₆) δ 43.05, 56.65, 116.53, 119.46, 119.57, 121.62, 125.54, 127.81, 129.36, 137.81, 143.69, 144.56, 146.73, 148.30, 160.44, 161.83, 167.60; Butt-centric. calcd. for C₁₉H₁₅NO₆: The compound in question is a pale yellow solid with a yield of 79%, melting point ranging from 246 to 248 °C, and an R_f value of 0.43 (based on a petroleum ether: ethyl acidic corrosive determination of 3:1).

Infrared spectroscopy (IR) reveals a $\hat{\nu}_{\text{max}}$ (cm^{-1}) of 3335 (NH), 1707 (CO), and 1653 (CO). ^1H NMR (300 MHz, DMSO- d_6) $\hat{\nu}$ 1.42 (t, 3H, J = 6.9Hz, CH₃), 4.20 (q, 2H, J = 6.9Hz, OCH₂), 4.62 (d, 2H, J = 6.0Hz, CH₂), 7.34 (d, 1H, J = 7.5Hz, Ar-H), 7.42–7.48 (m, 3H, Ar-H), 7.52 (dd, 1H, J_1 = 7.5Hz, J_2 = 1.2Hz, Ar-H), 7.92 (d, 2H, J = 8.1Hz, Ar-H), 8.86 (s, 1H, CH), 9.23 (t, 1H, J = 6.0Hz, NH); ^{13}C NMR (75 MHz, DMSO- d_6) $\hat{\nu}$ 15.04, 43.03, 65.02, 117.43, 119.56, 119.59, 121.62, 125.56, 127.82, 129.89, 137.82, 143.79, 144.63, 145.98, 148.37, 160.53, 161.96, 167.66;

Butt-centric. calcd. for C₂₀H₁₇NO₆: C, 65.39; H, 4.66; N, 3.81; found: C 65.30, H 4.73, N 3.75. 4-((7-methoxy-2-oxo-2H-chromene-3-carboxamido)methyl) benzoic destructive (6d) Irradiation (KBr): $\hat{\nu}_{\text{max}}$ (cm^{-1}) 3346 (NH), 1701, 1659, and 1614 (CO); light brown color; yield: 89%; melting point: 242 to 244 $^{\circ}\text{C}$; Rf = 0.46 (petroleum ether: ethyl acidic corrosive induction = 3:1); ^1H NMR (300 MHz, DMSO- d_6) $\hat{\nu}$ 3.89 (s, 3H, OCH₃), 4.62 (d, 2H, J = 6.0Hz, CH₂), 7.02 (dd, 1H, J_1 = 8.7Hz, J_2 = 2.4Hz, Ar-H), 7.09 (d, 1H, J = 2.1Hz, Ar-H), 7.45 (d, 2H, J = 8.1Hz, Ar-H), 7.90 (m, 3H, Ar-H), 8.83 (s, 1H, CH), 9.17 (t, 1H, J = 6.0Hz, NH), 12.91 (brs, 1H, Goodness); ^{13}C NMR (75MHz, DMSO- d_6) $\hat{\nu}$ 42.98, 56.67, 100.69, 112.54, 114.05, 115.16, 127.77, 129.84, 129.90, 131.99, 144.77, 148.47, 156.64, 161.24, 162.20, 164.90, 167.65; Butt-centric. calcd. for C₁₉H₁₅NO₆: C, 64.59; H, 4.28; N, 3.96; found: C 64.48, H 4.34, N 3.89.

benzyl-N-(4-((2-aminophenyl) carbamoyl) 8a-Chromene-3-carboxamide, 2-oxo-2H- White solid; yield: 90%; mp: 207–209 $^{\circ}\text{C}$; Rf = 0.76 (petroleum ether: ethyl acidic corrosive induction = 3:2); IR (KBr): $\hat{\nu}_{\text{max}}$ (cm^{-1}) 3404 and 3322 (NH and NH₂), 1720, 1653 and 1609 (CO); ^1H NMR (300 MHz, DMSO- d_6) $\hat{\nu}$ 4.64 (d, 2H, J = 6.0 Hz, CH₂), 4.90 (s, 2H, NH₂, D₂O exchangeable), 6.62 (t, 1H, J = 6.6 Hz, Ar-H), 6.79 (d, 1H, J = 7.5 Hz, Ar-H), 6.93 (t, 1H, J = 7.5 Hz, Ar-H), 7.18 (d, 1H, J = 6.0 Hz, Ar-H), 7.48–7.55 (m, 4H, Ar-H), 7.75 (t, 1H, J = 6.0 Hz, Ar-H), 7.98 (m, 2H, Ar-H), 8.90 (s, 1H, CH), 9.22 (t, 1H, J = 6.0 Hz, NH, D₂O exchangeable), 9.64 (s, 1H, NH, D₂O exchangeable); ^{13}C NMR (75 MHz, DMSO- d_6) The given values are: 43.03, 116.63, 116.79, 118.95, 119.58, 123.70, 125.63, 126.93, 127.05, 127.63, 128.36, 129.93, 130.75, 133.75, 134.62, 143.00, 148.05, 154.39, 160.78, 161.91, 165.53.

In the butt-centric calculation for C₂₄H₁₉N₃O₄, the values were calculated as C 69.72, H 4.63, and N 10.16. The results, according to the LCMS(ESI, m/z): C 69.78, H 4.69, and N 10.06.

N-(4-((2-aminophenyl) carbamoyl) benzyl)-8-ethoxy-2-oxo-2H-chromene-3-carboxamide (8c) was determined by

chemical shift calculations using LCMS(ESI, m/z): [M+1]⁺ 444.0, (2 M + 1)⁺ 887.3. The ^1H NMR spectrum was recorded using DMSO- d_6 at 300 MHz. 4.63 (s, 2H, J = 5.4 Hz, CH₂), 4.92 (s, 2H, NH₂), 1.43 (t, 3H, J = 6.9 Hz, CH₃), 4.23 (q, 2H, J = 6.9 Hz, OCH₂), 6.61 (t, 1H, J = 6.9 Hz, Ar-H), 7.78 (d, 1H, J = 7-methoxy-2-oxo-2H-chromene-3-carboxamide is N-(4-((2-aminophenyl)carbamoyl)benzyl) (8d). The ^1H NMR spectrum was recorded using DMSO- d_6 at 300 MHz. The structures are as follows: 3.91 for s, 3H in OCH₃, 4.62 for d, 2H in CH₂, 4.94 for brs, 2H in NH₂, 6.61 for t, 1H in Ar-H, J = 7.2Hz, 6.78 for d, 1H in Ar-H, J = 7.8Hz, 7.05 for m, 3H in Ar-H, 7.48 for d,

Biological Activity

The MTT Test was used to assess the anticancer potential of the synthetic compounds in relation to the MDAMB (breast cancer) cell line. The proliferation and viability of the cells were determined using the 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) level [60]. The transformation of the light yellow tetrazolium salt (MTT) into a dark blue, insoluble formazan by metabolically active cells allows for its quantitative measurement after dissolving in DMSO. The amount of sensitive cells is directly proportional to the dissolvable formazan absorbance. Each well of the 96-well plate was seeded with 200 μL of medium and 1×10^4 cells. Before the creation of the exploratory compounds, the 96-well microtiter plates were brooded for 24 hours. The cells were exposed to either the vehicle (0.4% DMSO) or compounds (1, 10, and 25 M) for 48 hours. The medicines are now hydrolysed in DMSO. The experiment was carried out for 60 minutes at 37 $^{\circ}\text{C}$ and finished with the addition of 10% MTT solution. The plates were air-dried after sucking the supernatant, and 100 mL of DMSO was used to break up the MTT-formazan diamonds. A TECAN multimode peruser was used to measure the optical thickness (O.D.) at 560 nm. Each treatment well in the 96-well plate had its advancement rate computed using test wells compared to control wells [61]. The limitation of cell advancement was calculated using measurement response twists that relate to a plot of the rate of surviving cells versus the steady concentration. The anti-proliferative growth of the cancer cells was evaluated using the IC₅₀ value, which is the concentration of the substance that reduced absorbance by 50% relative to the control.

RESULTS AND DISCUSSION:

Compounds 8a and 8c exhibited robust action against MDAMB cell lines, with IC₅₀ values of around 9.68 and 15.2 $\mu\text{g}/\text{ml}$, respectively. In contrast,

compounds 8b and 8d shown development in down and out prevention.

Table 5:IC₅₀ (μM) for the synthesized compounds and (STD) on the cells MDAMB (breast cancer) determined by MTT assay:

Compound code	MDAMB (breast cancer)
8a	15.2 ± 0.39
8b	22.39 ± 0.47
8c	9.68 ± 0.78
8d	32.57 ± 0.53
STD	8.45 ± 0.49

STD= Fluorouracil

Data represented as mean ± standard deviation

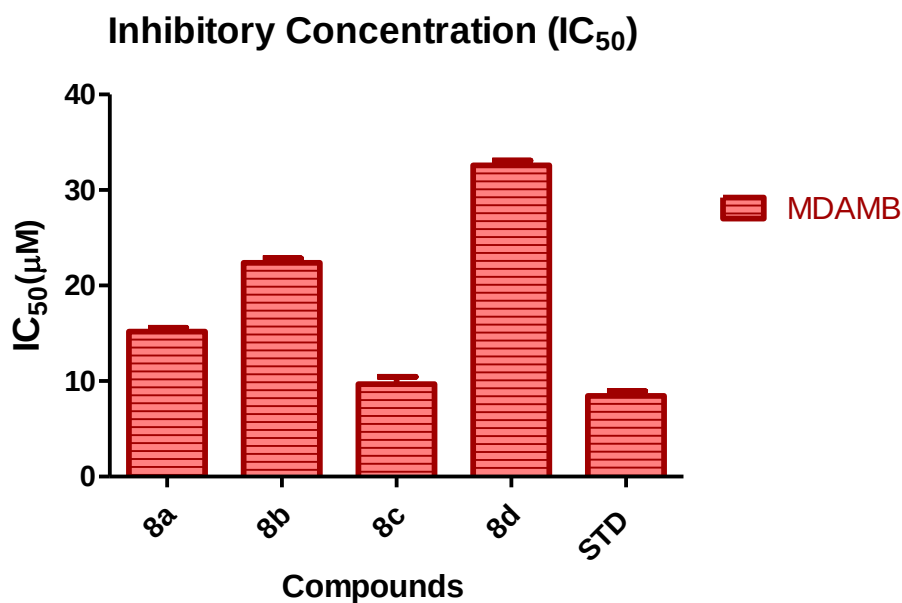
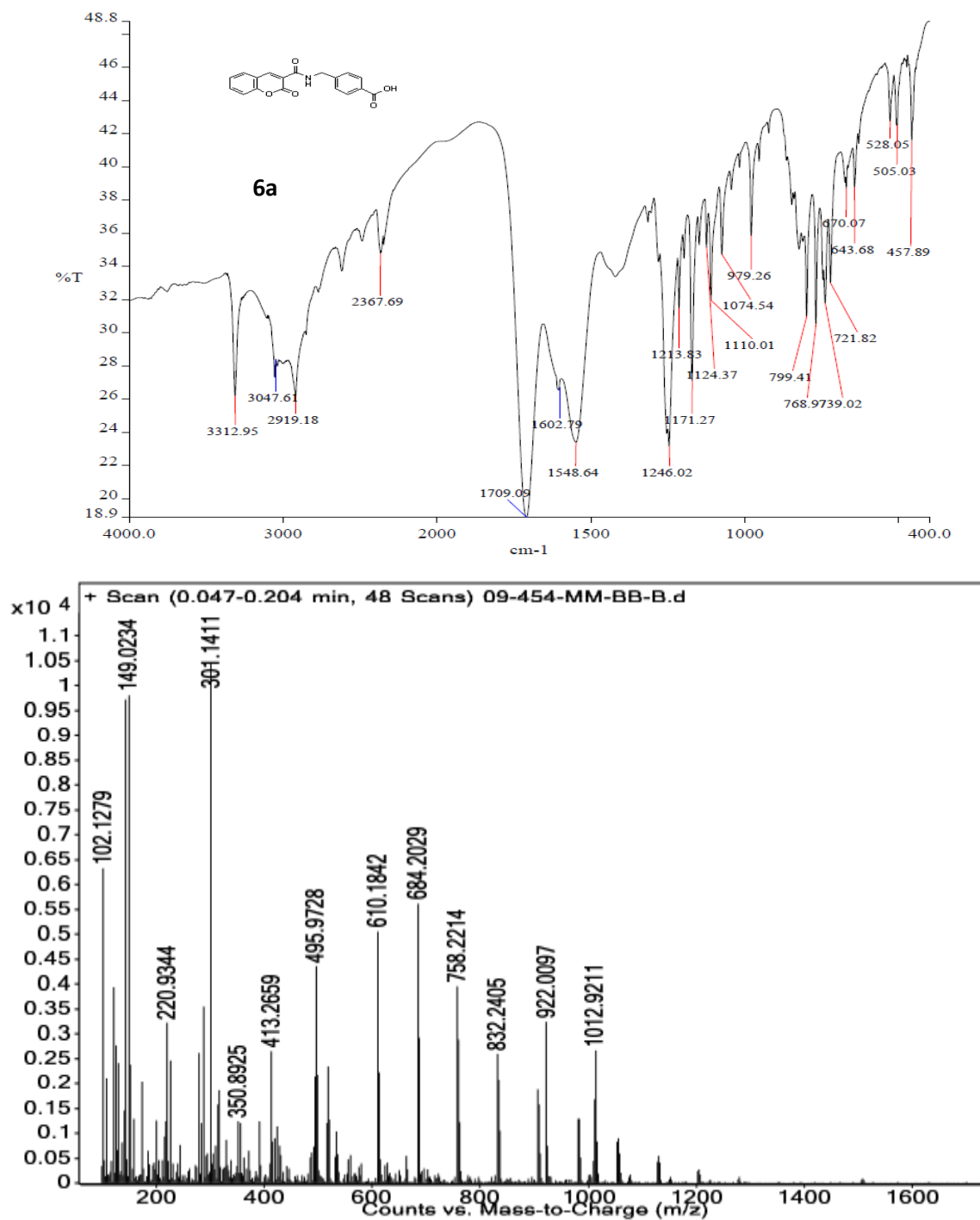
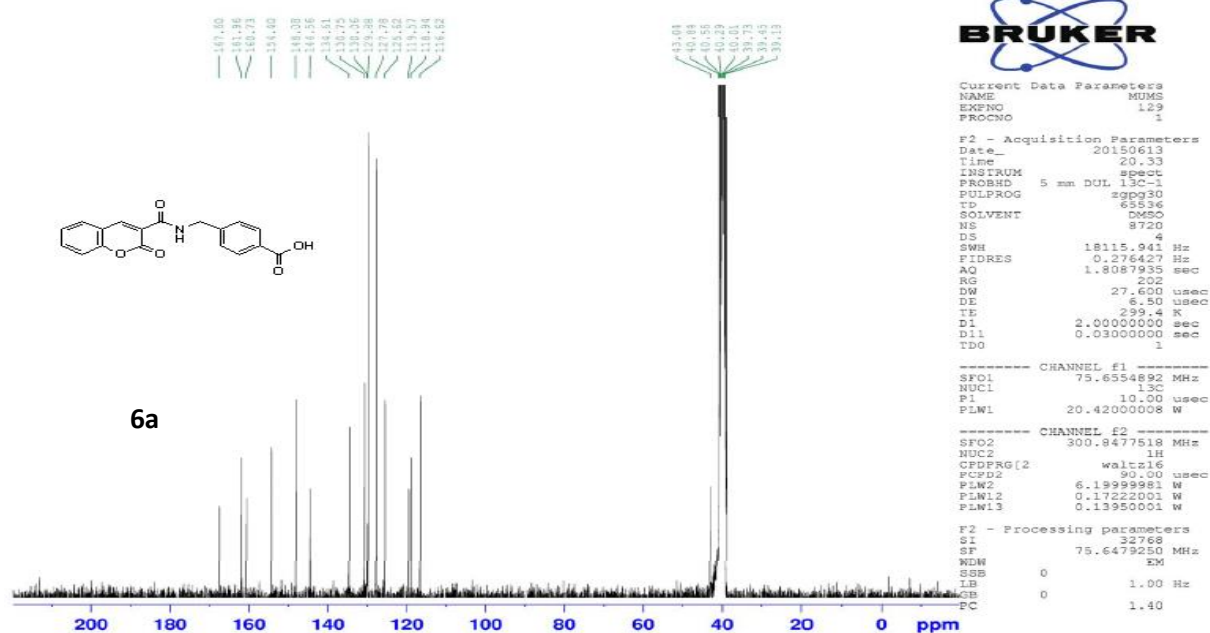


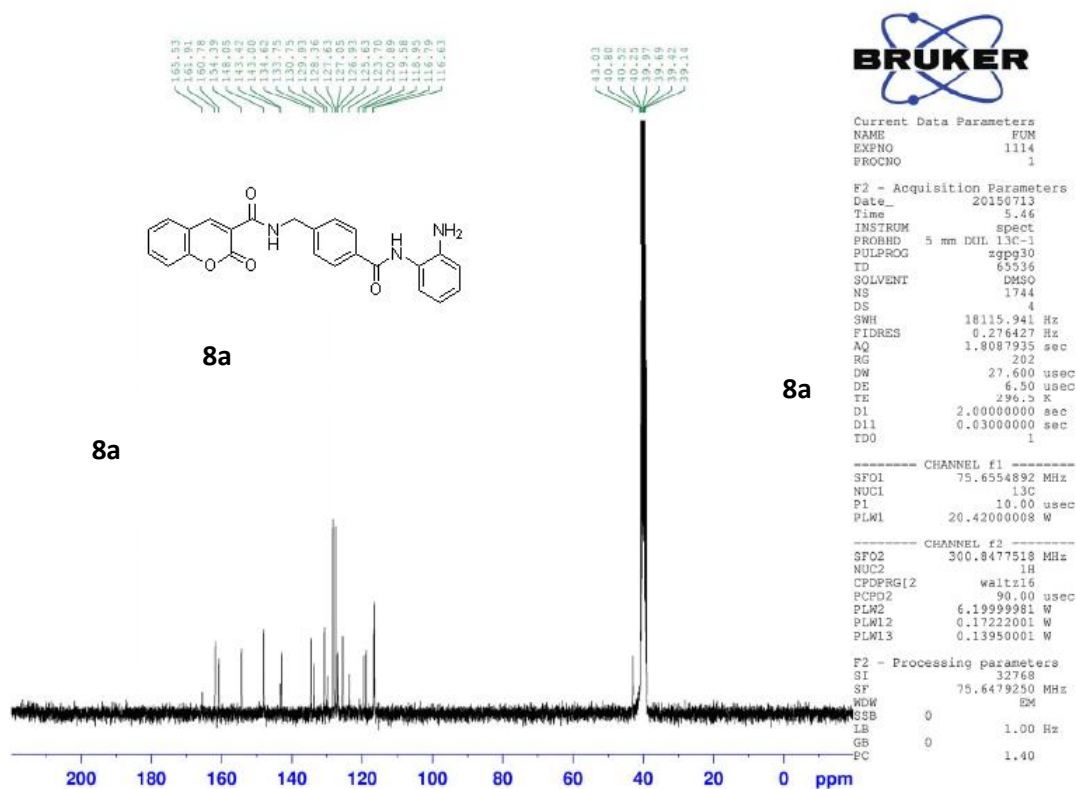
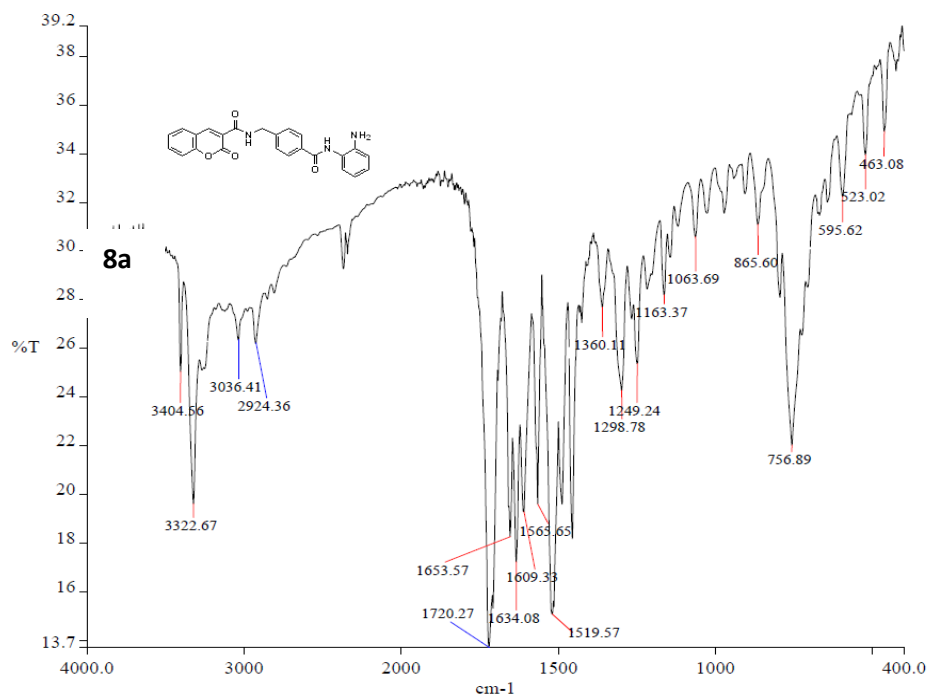
Chart 1:

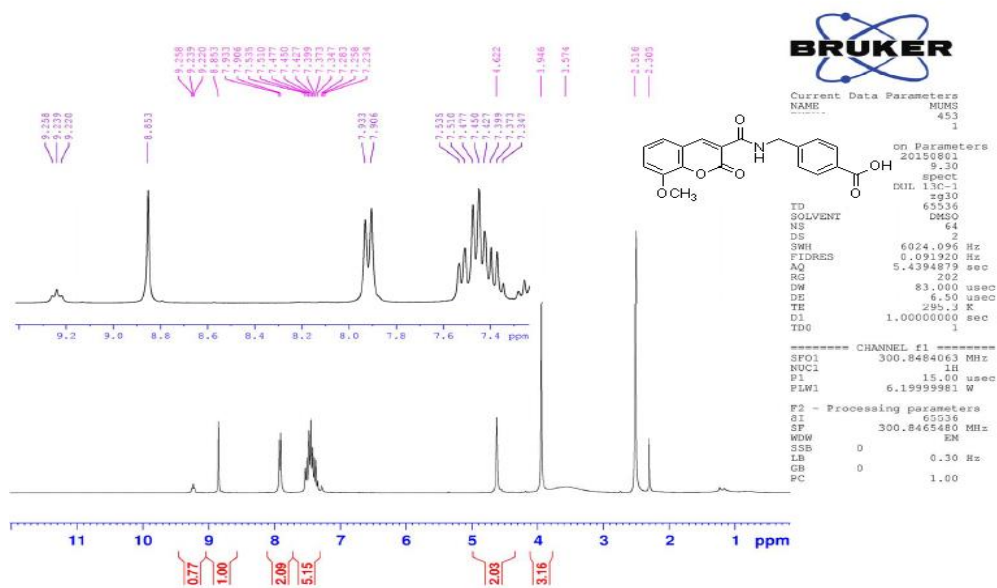
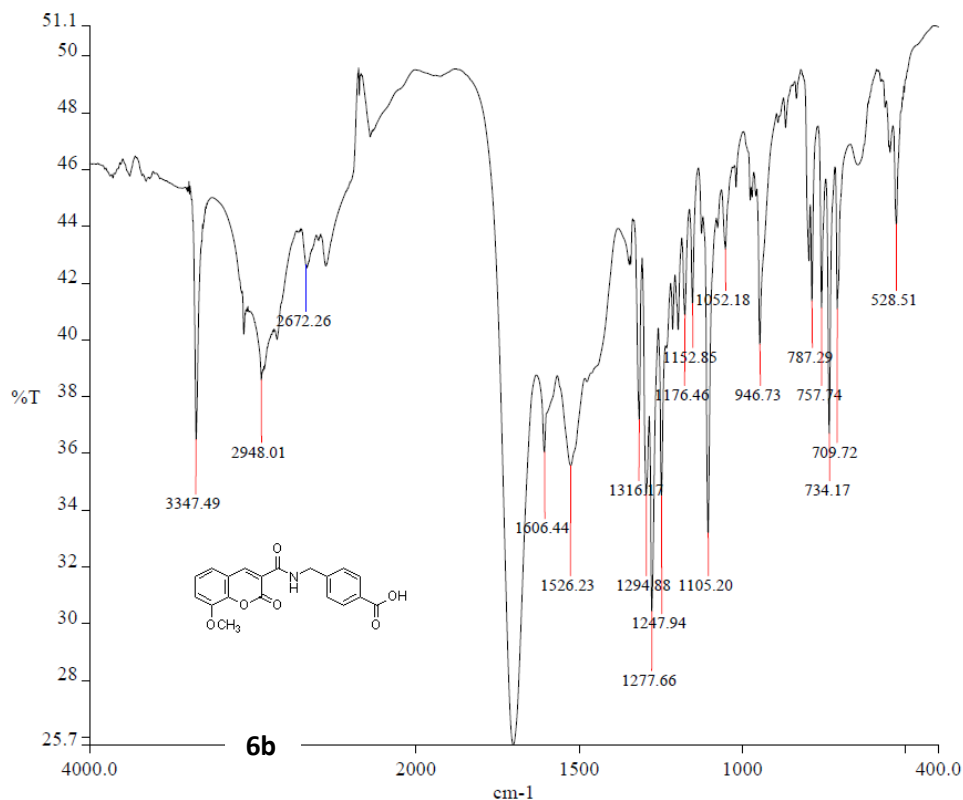
Evaluation of the produced compounds' and STD's IC₅₀ values on MDAMB cells In summary

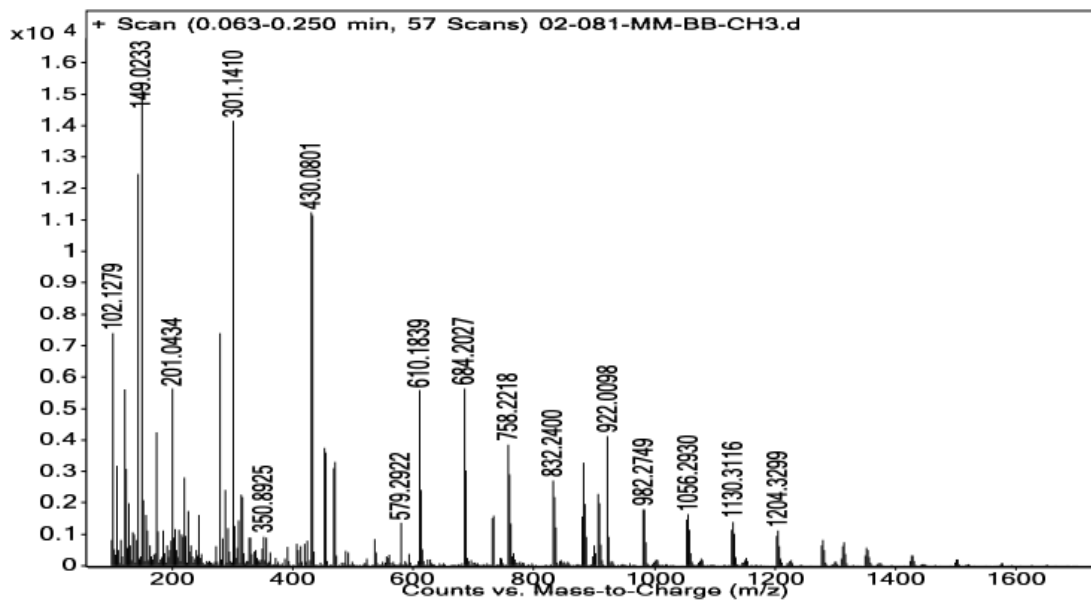
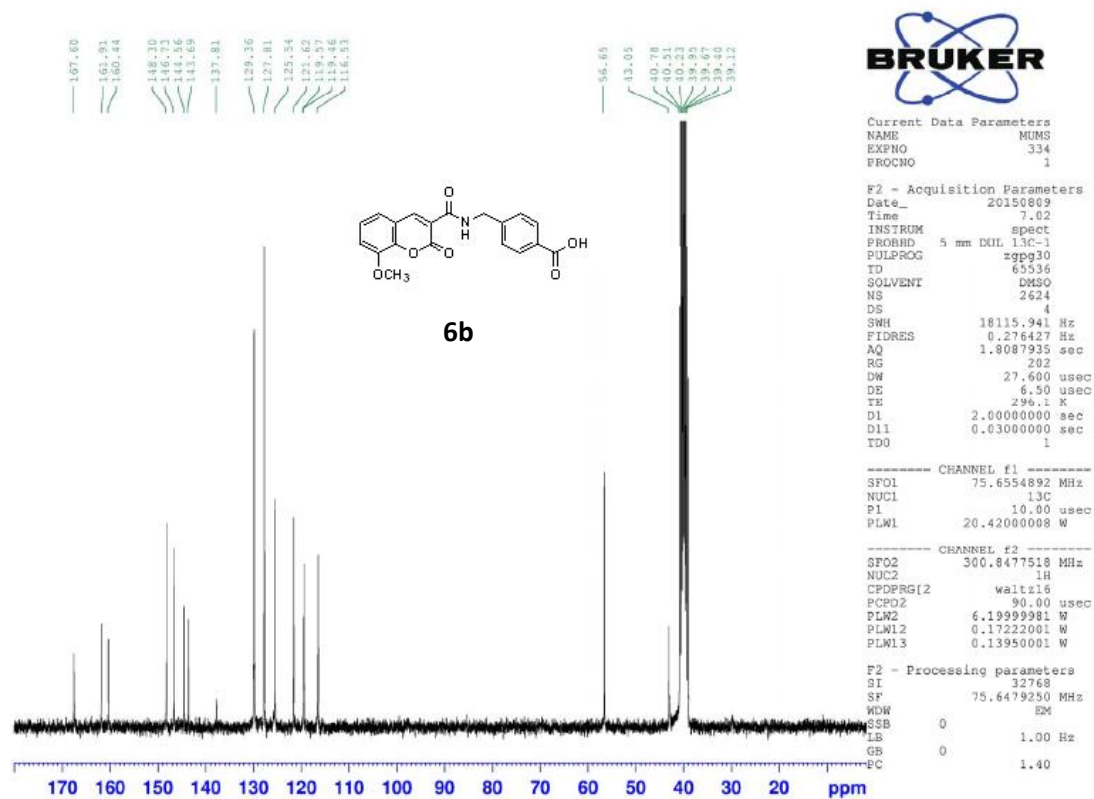
Therefore, coumarin 8a and 8c are great places to start when looking to empower research into the development of effective cytotoxic administrations.

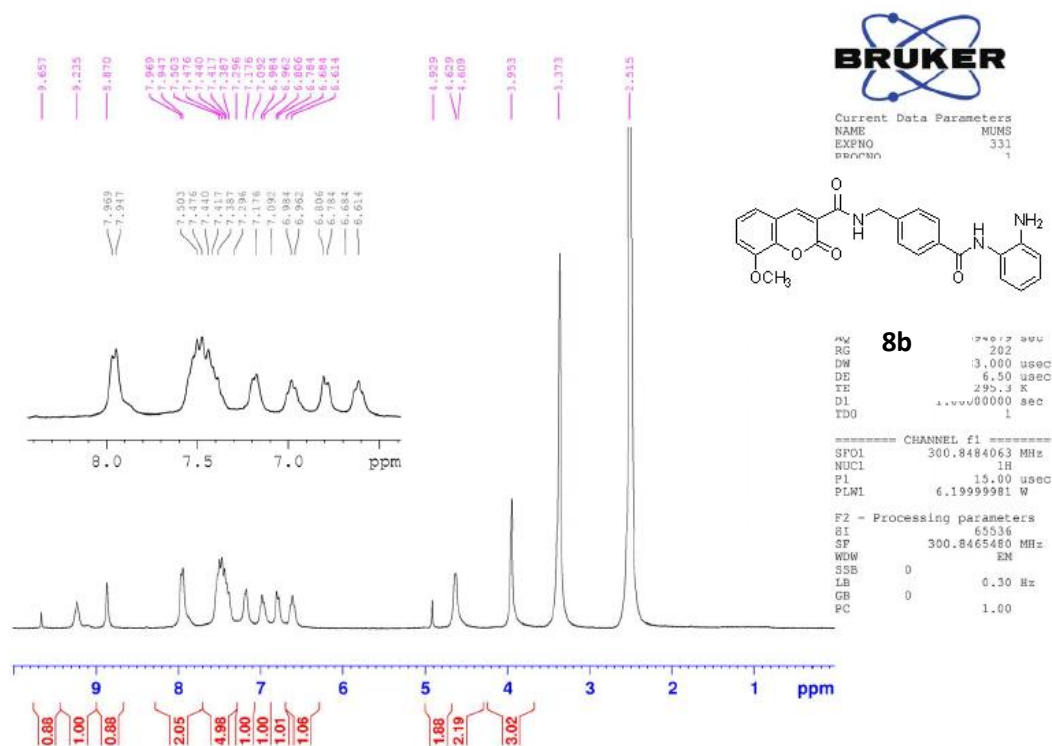












CONCLUSION:

The present study successfully achieved the synthesis, characterization, and biological evaluation of novel coumarin derivatives. The synthesized compounds were confirmed by spectroscopic analyses such as FT-IR, ^1H -NMR, ^{13}C -NMR, and Mass spectrometry, ensuring structural integrity and purity. The in vitro anticancer screening revealed that several derivatives exhibited promising cytotoxic activity against selected human cancer cell lines, indicating the strong potential of coumarin as a core scaffold for anticancer drug design. Structure–activity relationship (SAR) analysis suggested that substituent variations on the coumarin ring significantly influence biological activity, guiding future optimization strategies. Overall, this work highlights coumarin derivatives as potential lead molecules for the development of safer and more effective anticancer agents, warranting further pharmacological and molecular docking studies to explore their mechanism of action and therapeutic potential.

REFERENCES:

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. *Global Cancer Statistics. CA Cancer J Clin.* 2011;61(2):69–90.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. *GLOBOCAN 2008 v1.2: Cancer Incidence and Mortality Worldwide.* Lyon, France: International Agency for Research on Cancer; 2010.
3. Kim S-K. *Handbook of Marine-Based Anticancer Drugs.* Springer Worldwide Distributors, Switzerland; 2015.
4. Mareel M, Leroy A. *Physiological Reviews.* 2003;83:337–376.
5. Wesche J, Haglund K, Haugsten EM. *Biochem J.* 2011;437:199–213.
6. Give SK. *Cell Mol Life Sci.* 2009;66:1163–1177.
7. Solyanik G. *Exp Oncol.* 2011;32:181–185.
8. Vijayaraghavalu S, et al. *Mol Pharm.* 2012;9:2730–2742.
9. Riedl SD, Zweglick Lohner K. *Chem Phys Lipids.* 2011;164:766–781.
10. Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, Nicolaidis DN. *Curr Pharm Des.* 2004;10:3813–3833.