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Synthesis and Evaluation of New Coumarins as Antitumor and Antioxidant Applicants

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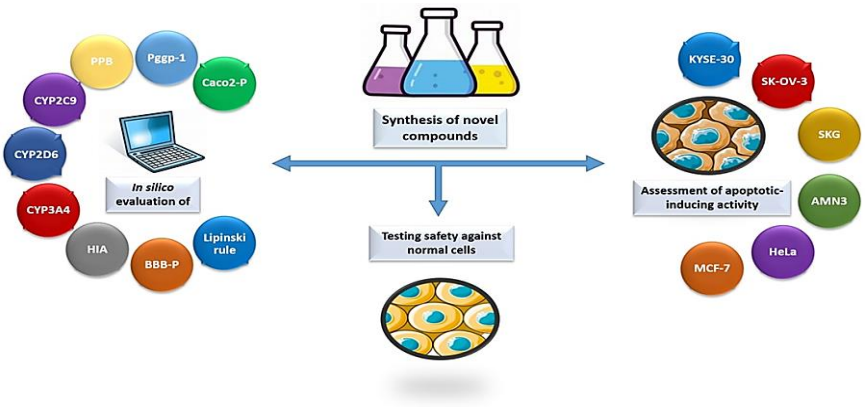
ABSTRACT

This work involves the synthesis of eight novel fused coumarin compounds, which were confirmed by various spectrophotometers and then, assessed for their apoptotic-inducing and free radical-quenching activities. The pharmacokinetic parameters were evaluated *in silico* using pre-ADMET, a free online program. The apoptotic-inducing activity was tested against six tumorigenic cell lines. Also, their safety against normal cells was examined. The free radical-quenching activity was assessed by checking these compounds' ability to eliminate DDPT and hydroxyl moieties. Pharmacokinetic investigations showed that the synthesized fused coumarin compounds have excellent penetration across the GIT mucosa and most of them have poor penetration across the blood-brain barrier. These findings suggest good oral bioavailability along with low neurological toxicity profiles. The evaluation of the apoptotic-inducing activity revealed that all of the compounds have weaker activity as compared to the reference. Among these compounds, SA4 was the most potent one. Nevertheless, all of these new compounds had an excellent safety profile against normal cells. On the other hand, the assessment of the free radical-quenching activity of these synthesized compounds also indicated that all of them were less active than the reference. In this field, SA0 was the strongest free radical-quenching compound. From these realizations, along with the apparent safety and good pharmacokinetic characteristics in accordance with the in silico study, compounds SA4 and SA0 are considered the most promising agents. The authors hope that these new fused coumarin compounds can be utilized in the coming years for the production of new powerful drugs with apoptotic-inducing and free radical-quenching potentials which can help in the battle against many diseases.

GRAPHICAL ABSTRACT



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Introduction

The concept of oxidative stress has been adopted since the eighties of the last century as a leading cause for many diseases that are considered a nightmare for humanity. This kind of stress is caused by either the increased activity of harmful free radical species or the ineffectiveness of the body's defensive mechanisms as a result of a decrease or even lack of powerful antioxidant capability. Many life-threatening disorders, such as malignancy, atherosclerosis, vascular diseases, type 2 diabetes, coronary artery diseases, and many other diseases, are linked to these damaging free radicals. Among these, carcinomas are believed to be the world's most critical health threats, as the rate of their occurrence has risen dramatically in practically every corner of the planet. Despite the discovery and development of numerous experimental anticancer medications from synthetic as well as natural sources, efficient management of various types of cancer is difficult to establish. As a consequence, there is an urgent need to synthesise novel therapeutic agents and investigate their cytotoxic behaviour in order to uncover a viable cure for this perplexing medical condition [1,2].

Coumarins constitute an interesting group of compounds. Researchers have been focused for decades on studying their crucial biological features and preparing analogues for therapeutic applications. Coumarins are a class of heterocyclic compounds with a benzopyrone structure. These molecules offer a number of appealing properties which make them an important part of drug research and innovation. In addition to their multifarious bioactivities, they have a simple structure, low molecular weight, good bioavailability, excellent safety profile, and high solubility in many solvent systems. Their skeletons have been employed as a precursor in the preparation of biologically active heterocyclic compounds with anti-inflammatory, anti-microbial, anti-tumour, painkilling, anti-oxidant, hypoglycaemic, anti-coagulant, and many other activities [3–10].

Fused coumarin compounds are considered promising compounds in the future for the production of modern drugs with favourable biological activities. Fused coumarin compounds are coumarins with bonding phenyl groups to either 3,4-, 5,6-, 6,7-, or 7,8-positions which result in a plethora of appealing bioactivities. They are a prospective family of new compounds due to the make-up of their structure with an expanded π-electron arrangement. F, G, and H, subfamilies of fused coumarin compounds and their related agents contain both an electron-acceptor and an electron donor that are conjugated electronically through the compound's backbone. They are of particular importance owing to their charge-transfer nature intramolecularly. This resulted in great attention to their use as scaffolds for new drug developmental approaches [11–20].

In this work, a number of novel fused coumarin compounds have been synthesised and then, tested for their apoptotic-inducing as well as free radical-quenching activities. Begin by synthesising **SA0** from 6-amino-7-chloronaphthalen-2-ol. **SA0** is then used to create **SA1,** which then produces a series of derivatives by reaction with different phenolic derivatives. These products were checked for possible apoptotic-inducing activity against six tumorigenic cell lines. In addition, their toxicity against normal cells was assessed. The free radical-quenching potential was examined for these compounds against DPPH and hydroxyl harmful radicals. The pre-ADMET online programme was used to obtain *in silico* pharmacokinetic data for them.

Material and Methods

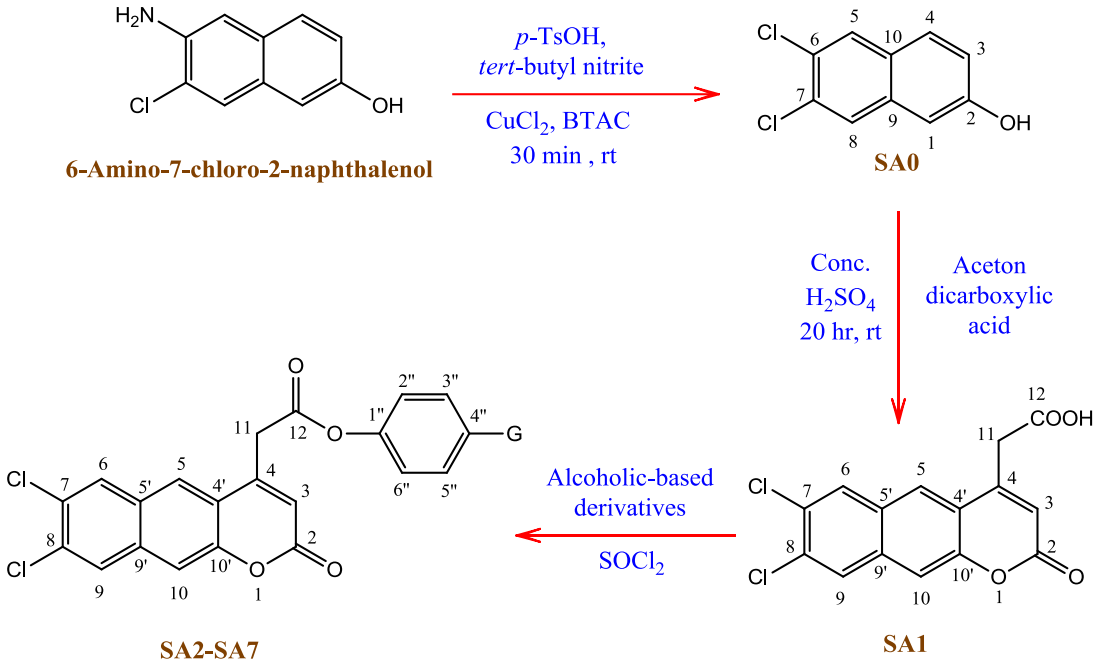
Instruments and Chemicals

Sigma-Aldrich provided the tumorigenic-cell lines used in this study, which were experimental in nature. Chemicals, reagents, and solvents were sourced from reputable international suppliers and used without further purifying. The melting points (Mp) of the synthesised composites were determined using the USP-dependent capillary technique on an electrothermal CIA 9300 apparatus. To ensure the purity of the produced agents and the fulfilment of reactions, thin-layer chromatography (TLC) is being utilised, employing typical silica gel aluminium-based plates and a combination of chloroform ($CHCl_3$) and propanone (4:1) as an eluting solution.

Synthetic composite UV scanning was done by UV-1600PC UV-Vis. Bruker α-ATR-FTIR was used for FTIR scanning. Testing of ^{1}H-and ^{13}C-NMR spectra were carried out by the Bruker Avance DRX-300 MHz spectrophotometer.

Synthetic Protocol

Scheme 1 indicates the steps for the synthesis of **SA0** and its based compounds starting from 6-amino-7-chloro-2-naphthol.



SA2: G= OCH_3; **SA3**: G= CH_3; **SA4**: G= F;
SA5: G= Cl; **SA6**: G= Br; **SA7**: G= I

Scheme 1: Chemical synthesis of SA0 and its based compounds

Synthesis of SA0

At room temperature (RT), a combination of *p*-toluenesulphonic acid (6.00 mmol, 1.03 g), 6-amino-7-chloro-2-naphthol (5.00 mmol, 0.96 g), triethylbenzylammonium chloride (6.00 mmol, 1.37 g), copper dichloride (0.22 mmol, 0.03 g), and 1,1-dimethylethyl nitrite (6.00 mmol, 0.71 mL) were mixed and milled for 30 minutes using a mortar and pestle. Water and ether were used to rinse the mortar separately, using 20 mL of each three times. The prepared crude was recrystallized from aqueous ethyl alcohol after vaporizing the organic phase [21,22].

6,7-Dichloro-2-naphthol (SA0): White crystals; Yield= 52% (0.55 g); Mp=132-134°C; R_f = 0.16; λ_{max} (EtOH)= 267 nm; IR ν_{max} (cm^{-1}): 3300 (broader band, naphtholic O-H), 3076 (m, aryl C-H), 2957 (w, alkyl C-H), 1561 (s, aryl C=C), and 915 (s, C-Cl); ^{1}H-NMR (300 MHz, ppm, DMSO-d_6): δ= 8.15 (1H, d, H-4, *J*=9Hz), 7.72 (1H, s, H-5), 7.62 (1H, s, H-8), 7.55 (1H, s, H-1), 7.22 (1H, d, H-3, *J*=9Hz), and 5.56 (1H, s, OH); ^{13}C-NMR (75 MHz, ppm, DMSO-d_6): δ= 158.1 (C, C-2), 135.5 (C, C-9), 132.5 (C, C-7), 131.4 (CH, C-4), 129.8 (C, C-10), 128.7 (C, C-6), 128.3 (CH, C-5), 127.3 (CH, C-8), 120.1 (CH, C-3), and 111.4 (CH, C-1).

Synthesis of SA1

In a conical flask, 25 mL of concentrated sulphuric acid (H_2SO_4) were cooled using an ice bath. When the temperature dropped below 10°C, 2.75 g of **SA0** (13.22 mmol) and 3.5 mL of acetone dicarboxylic acid (15.00 mmol) were mixed, placed in a separatory funnel, and added drop by drop to the chilled H_2SO_4 with stirring. During the addition, the mixture temperature should be kept below 10°C. After completing the addition, the obtained mixture remained at RT with continuous stirring for 20 hours. Then, it was poured into a beaker containing water and crushed ice and mixed. The formed precipitate was filtered using a filter paper, washed with cold water, and allowed to dry at RT, affording **SA1** compound [23,24].

11-(7,8-Dichloro-2-oxo-2*H*-benzo[*g*]chromen-4-yl)acetic acid (SA1): Pale yellowish powder;

Yield= 48% (0.78 g); Mp=154-156°C; R_f = 0.11; λ_{max} (EtOH)= 411nm; IR ν_{max} (cm^{-1}): 3062 (m, *cis* C-H), 3015 (broader band, carboxylic acid O-H), 2891 (w, alkyl C-H), 1734 (s, cyclic C=O ester), 1692 (s, dimeric carboxylic acid C=O), 1590 (s, *cis* C=C), 1548 (m, aryl C=C), and 941 (s, aryl C-Cl); ^{1}H-NMR (300 MHz, ppm, DMSO-d_6): δ= 11.09 (1H, s, H-12); 7.92 (1H, s, H-5), 7.72 (1H, s, H-6), 7.60 (1H, s, H-9), 7.12 (1H, s, H-10), 6.35 (1H, s, H-3), and 3.12 (2H, s, H-11); ^{13}C-NMR (75 MHz, ppm, DMSO-d_6): δ= 173.1 (C, C-12), 162.2 (C, C-2), 153.0 (C, C-4), 151.8 (C, C-10'), 132.6 (C, C-9'), 130.1 (C, C-8), 129.0 (CH, C-6), 128.1 (C, C-5'), 127.5 (C, C-4'), 126.0 (C, C-7), 125.5 (CH, C-9), 125.1 (CH, C-5), 115.8 (CH, C-3), 113.4 (CH, C-10), and 30.9 (CH_2, C-11).

Synthesis of the Fused Coumarin SA2-SA7 Compounds

A two-nick round-bottomed flask containing a mixture of 25 mL of refreshed sulfinyl chloride and 1.60 g of **SA1** (5.00 mmol) was placed in a salt-ice bath. A stopper containing blue litmus test paper was used to confine the side-nick, while a condenser was attached to the centre. Then, the mixture was stirred gently under anhydrous conditions for 30 minutes, followed by stirring for an additional 30 minutes at RT. After that, the obtained mixture was refluxed for 3 hours. A litmus test paper, which was replaced every 30 minutes, was used to detect the reaction's progress. The excess of sulfinyl chloride was distilled out when the colour of the litmus paper remained blue. The **SA1** acyl compound remained in the concave of the flask as a white solid substance [25,26].

Under water-free conditions, a solution of 4.80 mmol of phenolic derivative with 1 mL of pyridine in 50 mL of anhydrous ethoxyethane was poured into the same flask and stirred for 30 minutes at RT. The mixture refluxing is continued until the colour of the litmus paper remains blue. After that, 50 mL of water was added to the mixture. The organic layer was then isolated, dried, and vaporised. A 1:2 mixture of propane and methylene dichloride was used for the recrystallization to obtain the **SA1** compound [27,28]. The spectrophotometrically collected data from ^{1}H- and ^{13}C-NMR are listed and discussed in the Results and Discussion section.

4''-Methoxyphenyl-11-(7,8-dichloro-2-oxo-2*H*-benzo[*g*]chromen-4-yl)acetate (**SA2**)**:** Off-white powder; Yield= 78% (1.08 g); Mp= 146-148°C; R_f = 0.32; λ_{max} (EtOH)= 345 nm; IR ν_{max} (cm^{-1}): 3096 (m, *cis* C-H), 2917 (w, methoxy C-H), 2821 (w, alkyl C-H), 1731 (s, cyclic C=O ester), 1710 (s, acyclic C=O ester), 1665 (s, *cis* C=C), 1595 (s, aryl C=C), 1216 and 1144 (s, aryl-alkyl ether C-O-C), and 985 (s, aryl C-Cl).

4''-Tolyl-11-(7,8-dichloro-2-oxo-2*H*-benzo[*g*]chromen-4-yl)acetate (**SA3**)**:** Pale yellowish powder; Yield=72% (1.11 g); Mp= 138-140°C; R_f = 0.30; λ_{max} (EtOH)= 398 nm; IR ν_{max} (cm^{-1}): 3090 (m, *cis* C-H), 2877 and 2818 (w, alkyl C-H), 1733 (s, cyclic C=O ester), 1713 (s, acyclic C=O ester), 1668 (s, *cis* C=C), 1597 (s, aryl C=C), and 985 (s, aryl C-Cl).

***4*''-Fluorophenyl-11-(7,8-dichloro-2-oxo-2*H*-benzo[*g*]chromen-4-yl)acetate** (**SA4**)**:** White powder; Yield= 42% (1.13 g); Mp= 144-148°C; R_f = 0.21; λ_{max} (EtOH)= 316 nm; IR ν_{max} (cm^{-1}): 3070 (m, *cis* C-H), 2820 (w, alkyl C-H), 1733 (s, cyclic C=O ester), 1711 (s, acyclic C=O ester), 1666 (s, *cis* C=C), 1597 (s, aryl C=C), 1077 (s, aryl C-F), and 986 (s, aryl C-Cl).

4''-Chlorophenyl-11-(7,8-dichloro-2-oxo-2*H*-benzo[*g*]chromen-4-yl)acetate (**SA5**)**:** Off-white powder; Yield= 43% (1.03 g); Mp= 133-135°C; R_f = 0.24; λ_{max} (EtOH)= 374 nm; IR ν_{max} (cm^{-1}): 3068 (m, *cis* C-H), 2820 (w, alkyl C-H), 1730 (s, cyclic C=O ester), 1710 (s, acyclic C=O ester), 1667 (s, *cis* C=C), 1595 (s, aryl C=C), and 985 (s, aryl C-Cl).

4''-Bromophenyl-11-(7,8-dichloro-2-oxo-2*H*-benzo[*g*]chromen-4-yl)acetate (**SA6**)**:** Pale yellowish powder; Yield= 42% (1.1 g); Mp= 123-125°C; R_f = 0.28; λ_{max} (EtOH)= 409 nm; IR ν_{max} (cm^{-1}): 3066 (m, *cis* C-H), 2819 (w, alkyl C-H), 1732 (s, cyclic C=O ester), 1709 (s, acyclic C=O ester), 1664 (s, *cis* C=C), 1593 (s, aryl C=C), 986 (s, aryl C-Cl), and 900 (s, C-Br).

4''-Iodophenyl-11-(7,8-dichloro-2-oxo-2*H*-benzo[*g*]chromen-4-yl)acetate (**SA7**)**:** Gray-like powder; Yield= 43% (1.3 g); Mp= 112-114 °C; R_f = 0.29; λ_{max} (EtOH)= 326 nm; IR ν_{max} (cm^{-1}): 3064 (m, *cis* C-H), 2823 (w, alkyl C-H), 1733 (s, cyclic C=O ester), 1711 (s, acyclic C=O ester), 1661 (s, *cis*

C=C), 1592 (s, aryl C=C), 986 (s, aryl C-Cl), and 800 (s, aryl C-I).

Theoretical Pharmacokinetic Factors

Using the web application pre-ADMET (https://preadmet.qsarhub.com/adme/), the pharmacokinetic characteristics of the synthesised fused coumarin compounds **SA0**-**SA7** were analysed *in silico*. This analysis involved their absorption, distribution, metabolism, and excretion [29,30].

In-vitro Biological Potentials

Apoptotic-Inducing Activity

The cell viability assay, 3-[4, 5-dimethylthiazol-2-yl]-2,5diphenyl tetrazolium bromide, or MTT, was performed to assess the potential apoptotic-inducing activity of the fused coumarin compounds. The synthesised fused coumarin compounds, along with their control substance, were solubilized in DMSO to obtain seven different concentration levels (400.00, 200.00, 100.00, 50.00, 25.00, 12.50, and 6.25 µg/ml). Then, they were tested against six tumorigenic cell lines: KYSE-30, SK-OV-3, SKG, AMN3, HeLa, and MCF-7 [31,32].

The tested tumorous cell lines were used to obtain 10,000 cells per well by dividing them into a 96-well plate, and each well was treated independently with varying doses of the synthesised fused coumarin compounds after 24 hours. Then, the vitality of the cells was determined after 72 hours of incubation, starting with medium removal followed by the addition of 28µl MTT solution (3.27mM) and cell incubation for 90 minutes at 37°C. The absorption spectrum of the treated well (As) along with the control well (Ac) was measured using a microplate reader set at 492 nm. This experiment was carried out in triplicate for every synthesised compound examined [33–36]. The below mathematical equation was used to obtain the growth inhibition percentage:

$$\textbf{\% Growth inhibition} = \textbf{(Ac-As)/Ac} \times \textbf{100}$$

Free Radical-Quenching Activity

The ability of the synthesised compounds to eliminate DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radicals and hydroxyl moieties as well as donate an electron in redox reactions was measured using vitamin C (L-ascorbic acid, L-AA) as a reference. Using ethanol as a solvent system, a series of seven concentration solutions were produced from the compound under investigation (1 mg/ml), which were: 400.00, 200.00, 100.00, 50.00, 25.00, 12.50, and 6.25 micrograms per millilitre. Several diluted concentrations of L-AA with ethanol were prepared including 200, 100, 50, 25, 12.5, and 6.25 micrograms per millilitre. The L% (liquidating percentage) estimates of the given concentrations were calculated for each compound using this equation:

$$\boldsymbol{L}(\%) = \boldsymbol{Abs}_{\textbf{control}} - \boldsymbol{Abs}_{\textbf{sample}} \div \boldsymbol{Abs}_{\textbf{control}} \times \mathbf{100}$$

The absorptions of the examined and reference samples at a specific wavelength were denoted by the symbols "Abs_{sample}" and "$Abs_{control}$", respectively.

The concentration of the tested compound which can neutralise half of the free radicals or reduce half of the oxidised iron particles is known as the liquidating activity of the compound (LC_{50}). This measurement was created by using a non-linear regression to depict the relationship between L percent value and its associated logarithmic concentration [37–39].

Liquidating Assay of DPPH-Free Radical

A mixture of 1.5 ml of the tested compound with 0.5 ml of an ethanolic DPPH solution at a particular concentration (0.1 mM) was prepared. The mixed solution was overlaid with aluminium platelets to hide it from sunlight. Then, the coated mixture was kept for 30 minutes at 25°C. At 517 nm, the mixture's ability to eliminate the violet colour of the DPPH was measured colorimetrically. To make the reference solution, 1.5 mL of ethanol were combined with 0.5 mL of ethanolic DPPH [40,41].

Liquidating Assay of Hydroxyl-Free Radical

A 1.5 mL solution of the tested compound at the assigned concentration was mixed with 2.4 ml of 0.2 M potassium-phosphate buffer (pH 7.8). The combination was then treated with 150 µl of 0.17 M perhydrol, 60 µl of 0.001 M $FeCl_3$, and 90 µl of 0.001 M pyridino[3,2-*h*]quinoline. The subsequent solution was kept at 25°C for 5 minutes until being spectrophotometrically trialled at 560 nm. All of the aforesaid components

were included in the reference solution, except that employed buffer type was used instead of the tested compound [42,43].

Results and Discussion

Synthetic Pathway

The schematic chemical synthesis for **SA0**-**SA7** compounds was depicted in Scheme 1. Firstly, **SA0** was synthesised by reacting 6-amino-7-phenolchloro-2-naphthol, triethylbenzylammonium chloride, copper dichloride, 1,1-dimethylethyl nitrite, and *p*-toluenesulphonic acid together by an aromatic nucleophilic substitution reaction. Calcium chloride anhydrous was used for drying the organic face, followed by the sample's recrystallization from aqueous ethyl alcohol [44,45].

Concerning **SA1**, which is the precursor of **SA2**–**SA7** compounds, the synthesis method involves the condensation of **SA0** compound and acetone dicarboxylic acid with the aid of concentrated H_2SO_4 via a Pechmann type condensation reaction. This reaction is considered the most widely used one for the synthesis of coumarin-related compounds. With the aid of a condensing agent, the starting materials utilized in this reaction are simple and include β-carbonyl group-containing ester and phenol. The nature of the resulted product and its yield depend on the reactant's reactivity and type. The last step, the synthesis of **SA2**–**SA7** compounds included converting the carboxylic acid moiety of the **SA1** compound into an acid chloride-derived product by reacting with $SOCl_2$. The reaction of the produced intermediate with phenolic derivatives leads to the formation of the final compounds. Each one had a different group substituted at the *para*-position of the benzene ring. These groups are methoxy for **SA2**, methyl for **SA3**, fluoride for **SA4**, chloride for **SA5**, bromide for **SA6**, and iodide for **SA7** [46,47]. Only a few studies exist in the literature aiding the use of halophenol as a starting material in this type of reaction because the nucleophilicity of this form of phenol is poor due to the deactivation effect of the halogen attached to it. In this work, the yields of the synthesized **SA2**–**SA7** compounds were improved by precise monitoring of the reaction conditions [48,49].

Theoretical Pharmacokinetic Parameters

As drug discovery and development are very complex and diverse processes, a number of *in silico* evaluations have been created to offer data on the pharmacokinetic characteristics of the compounds under investigation [48,50]. The examination of the parameters listed in Table-1 revealed a number of interesting points, including that these novel fused coumarin compounds have high HIA percentages ranging from 97.69% to 100.00%, indicating a high theoretical oral bioavailability. They have moderate Caco2 cell permeability with P-glycoprotein (Pggp-1) inhibiting capability. These parameters can indicate good intestinal absorption for these compounds [51]. On the other hand, the inhibitory capacity of these fused coumarin compounds against the CYP2C9 enzyme could suggest good anti-inflammatory activity as this enzyme produces eicosatrienoic acid epoxide, which is an inflammatory signalling molecule, from arachidonic acid metabolism [52,53]. While the inhibition of CYP3A4 by these compounds (except **SA1**) can result in a decrease in the metabolism of some toxins, including the parent drug, that leads to its accumulation and an increased risk of toxicity. Moreover, this action can affect the metabolism of other drugs taken simultaneously with these compounds, leading to drug-drug interaction [54]. Additionally, the produced fused coumarin compounds have a very high plasma protein binding capacity which can result in a decrease in the volume of distribution and a reduction in the half-life of these compounds [55]. Finally, the poor penetration across the blood-brain barrier (except for **SA0**) might mean that these compounds will have low toxicity as a result of a lack of neurological side effects. This limited number of adverse effects is critical in determining CNS toxicity [56].

Table 1: Theoretical pharmacokinetic parameters for the synthesised SA0-SA7 compounds

Compound symbol	Lipinski rule	BBB-P	HIA	CYP3A4	CYP2D6	CYP2C9	PPB	Pggp-1	Caco2-P
SA0	Yes; 0 violation	6.61	100.00	Inhibitor	Non	Inhibitor	98.99	Inhibitor	38.41
SA1	Yes; 0 violation	0.06	97.75	Non	Non	Inhibitor	95.68	Inhibitor	15.19
SA2	Yes; 1 violation: MLOGP>4.15	0.11	97.69	Inhibitor	Non	Inhibitor	96.18	Inhibitor	36.42
SA3	Yes; 1 violation: MLOGP>4.15	0.33	97.87	Inhibitor	Non	Inhibitor	97.51	Inhibitor	36.40
SA4	Yes; 1 violation: MLOGP>4.15	0.14	97.82	Inhibitor	Non	Inhibitor	100.00	Inhibitor	36.26
SA5	Yes; 1 violation: MLOGP>4.15	0.21	98.04	Inhibitor	Non	Inhibitor	100.00	Inhibitor	38.99
SA6	Yes; 1 violation: MLOGP>4.15	0.23	98.15	Inhibitor	Non	Inhibitor	100.00	Inhibitor	36.91
SA7	No; 2 violations: MW>500, MLOGP>4.15	0.23	98.31	Inhibitor	Non	Inhibitor	100.0	Inhibitor	35.56

Chemical Backbones of the Synthetic Fused Coumarin SA2-SA7 Compounds

In addition to the physical properties and IR data analysis listed in the experimental section, the chemical backbones of the fused coumarin **SA2-SA7** compounds were established by investigating their NMR outcomes. The findings revealed that these fused coumarins share a core structure, as displayed in Figure 1.

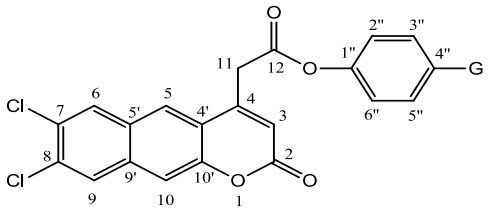


Figure 1: The shared core structure of the fused coumarins SA2-SA7 compounds

The gathered NMR scores and their interpretation regarding this central structure are illustrated below. The ^{1}H-NMR (300 MHz, ppm, DMSO-d_6) chemical shifts included 7.92 (1H, s, H-5), 7.72 (1H, s, H-6), 7.60 (1H, s, H-9), 7.12 (1H, s, H-10), 6.35 (1H, s, H-3), and 3.12 (2H, s, H-11). While the ^{13}C-NMR (75 MHz, ppm, DMSO-d_6) chemical shifts involved 169.5 (C, C-12), 162.2 (C, C-2), 153.0 (C ,C-4), 151.8 (C, C-10'), 132.6 (C, C-9'), 130.1 (C, C-8), 129.0 (CH, C-6), 128.1 (C, C-5'), 127.5 (C, C-4'), 126.4 (CH, C-9), 125.1 (CH, C-5), 124.0 (C, C-7), 115.8 (CH, C-3), 113.4 (CH, C-10), and 28.3 (CH_2, C-11).

The differences in the NMR spectra of the fused coumarins **SA2-SA7** compounds involving those related to their ^{1}H- and ^{13}C-NMR are reported in Tables 2 and 3, respectively.

Table 2: The variation in the 1H-NMR spectra of the fused coumarins SA2-SA7 compounds compared to their core structure

Compound symbol	H-2'',6'' (2H, d, *J*= 6Hz, ppm)	H-3'',5'' (2H, d, *J*= 6Hz, ppm)	Variable functional group at position 4''
SA2	6.74	7.01	4.12 ppm (3H, s, OCH_3)
SA3	7.02	7.25	2.75 ppm (3H, s, CH_3)
SA4	7.26	7.04	----------
SA5	7.35	7.53	----------
SA6	6.95	7.77	----------
SA7	6.83	7.85	----------

Table 3: The variation in the 13C-NMR spectra of the fused coumarins SA2-SA7 compounds compared to their core structure

Compound symbol	C-1'' (C, ppm)	C-2'' and 6'' (CH, ppm)	C-3'' and 5'' (CH, ppm)	C-4'' (C, ppm)	Variable functional group at position 4''
SA2	144.6	112.3	120.1	156.4	51.1 ppm (CH_3, OCH_3)
SA3	149.3	119.0	122.0	134.2	24.1 ppm (CH_3, CH_3)
SA4	147.9	120.7	108.5	158.7	----------
SA5	150.4	120.5	122.9	132.0	----------
SA6	151.3	121.3	123.6	118.5	----------
SA7	151.2	120.7	129.6	93.0	----------

Assessment of Apoptotic-Inducing Activity

An MTT-assisted cell viability test was used to evaluate the synthesised compounds for apoptotic-inducing activity. In this study, DMSO was used as a negative criterion and 5-fluorouracil (5-FU) as a basic apoptotic-inducing agent. Six tumorigenic cell lines were employed in this research: KYSE-30 (94072011, squamous cell carcinoma abstracted from Asian esophageal), SK-OV-3 (91091004, Caucasian ovary adenocarcinoma), SKG (C27676, human papillomavirus-related cervical squamous cell carcinoma), AMN3 (CVCL-M395, murine mammary adenocarcinoma), HeLa (93021013, epithelioid cervix carcinoma), and MCF-7 (86012803, caucasian breast adenocarcinoma). Table 4 revealed the obtained data from this apoptotic-inducing activity test.

Table 4: The apoptotic-inducing activity of **SA0-SA7**

Symbol	IC50 (µM) ± SD (n=3)						
	KYSE-30	**SK-OV-3**	**SKG**	**AMN3**	**HeLa**	**MCF-7**	**RWPE-1**
5-FU	32.01±1.01	22.86 ±1.01	22.08 ±1.05	25.18 ±1.11	13.84 ±1.12	12.63 ±1.01	34.86±1.03
SA0	89.34±0.97	75.98 ±1.14	52.63 ±1.16	53.21 ±1.12	57.39 ±1.08	56.46 ±1.19	124.38±1.12
SA1	103.2±1.04	94.73 ±1.03	61.56 ±1.01	93.11 ±1.02	76.34 ±1.03	93.61 ±1.02	89.01±1.02
SA2	74.22±0.96	68.12 ±1.01	46.41 ±1.02	41.23 ±0.95	29.13 ±0.97	36.67 ±0.99	62.47±1.05
SA3	78.92±1.12	70.46 ±0.97	40.17 ±1.07	41.39 ±0.98	38.22 ±1.01	39.91 ±1.06	57.24±1.07
SA4	48.16±1.14	35.78 ±1.01	33.12 ±1.06	29.03 ±0.97	19.62 ±0.95	23.56 ±1.07	131.67±0.95
SA5	55.08±1.12	59.02 ±0.99	34.09 ±1.20	37.91 ±1.12	19.83 ±1.08	26.94 ±1.20	97.04±0.98
SA6	96.17±1.02	85.14 ±1.15	68.34 ±1.12	76.29 ±1.21	48.42 ±1.12	77.93 ±1.09	75.14±0.95
SA7	96.85±0.94	84.65 ±1.12	83.02 ±1.02	64.44 ±1.21	78.06 ±0.99	73.12 ±1.12	69.96±1.08

The order of the of the synthesised fused coumarin compounds' apoptotic-inducing activity as compared to 5-FU is illustrated in Table 5.

Table 5: The order of apoptotic-inducing activity **SA0-SA7**

Order of activity	KYSE-30	SK-OV-3	SKG	AMN3	HeLa	MCF-7
1	**SA4**	**SA4**	**SA4**	**SA4**	**SA4**	**SA4**
2	**SA5**	**SA5**	**SA5**	**SA5**	**SA5**	**SA5**
3	**SA2**	**SA2**	**SA3**	**SA2**	**SA2**	**SA2**
4	**SA3**	**SA3**	**SA2**	**SA3**	**SA3**	**SA3**
5	**SA0**	**SA0**	**SA0**	**SA0**	**SA6**	**SA0**
6	**SA6**	**SA7**	**SA1**	**SA7**	**SA0**	**SA7**
7	**SA7**	**SA6**	**SA6**	**SA6**	**SA1**	**SA6**
8	**SA1**	**SA1**	**SA7**	**SA1**	**SA7**	**SA1**

From these two tables, a number of observations were made concerning the tumorigenic cell lines. Firstly, the fluorinated and chlorinated compounds (SA4 and SA5) have the most potent apoptotic-inducing activity against all tested cell lines. This may be attributed to the strong electron withdrawing capacity of fluoride and chloride moieties which makes the resultant compound more active. Furthermore, numerous studies have found a strong link between apoptotic-inducing activity and fluoride substitution. Secondly, the methoxylated and methylated compounds (SA2 and SA3) have very close and overlapping activity against the tested cell lines. This may be attributed to the similarity between the substitutions in these two compounds. Finally, the apoptotic-inducing activity of all tested fused coumarin compounds was weaker than that of 5-FU, as the reference [57].

The toxicity of these compounds was assessed against RWPE-1 (human normal prostate epithelial cells). This assessment showed that the entire novel fused coumarin compounds were safer than 5-FU against normal cells.

Assessment of Free Radical-Quenching Activity

Research on free radical-quenching activity has gotten a lot of attention recently because of its possible involvement in the prophylaxis and control of numerous illnesses which influence human health including malignancy, Alzheimer's, diabetes mellitus, hypertension, coronary artery disease, and many other diseases. The discovery of novel free radical-quenching agents has attracted the public's attention. Table 6 illustrates the free-radical quenching activity of the synthesised fused coumarin compounds.

Table 6: The free radical-quenching activity of **SA0-SA7**

Symbol of the compound	Assay and results			
L-AA	**Liquidating assay of DPPH-free radical LC_{50}±SD**	45.84±1.04	**Liquidating assay of hydroxyl-free radical LC_{50}±SD**	50.79±1.01
SA0		56.12±1.25		59.41±1.12
SA1		90.06±1.12		77.52±1.00
SA2		62.45±1.20		64.26±0.98
SA3		63.67±1.03		68.03±1.11
SA4		87.23±0.95		85.29±1.18
SA5		84.35±1.08		73.81±1.02
SA6		94.46±1.02		92.47±1.09
SA7		97.14±1.17		92.89±0.99

From this table, a number of issues were observed. Firstly, SA0 had the strongest free radical-quenching activity as compared to L-AA, the standard. This might be attributed to the presence of a hydroxyl group that attached directly to the coumarin nucleus, which had an electron-donating ability that made the compound more active in quenching free radicals. Secondly, SA7 had the weakest activity among this group. This might be due to the size of the iodide moiety in this

compound, which is considered large compared to other halogens, resulting in the weaker activity of the resultant compound. Lastly, the order of free radical-quenching activity of these fused coumarin compounds is: SA0 > SA2 > SA3 > SA5 > SA1 > SA6 > SA7 [58].

Conclusion

This work demonstrated the synthesis of eight novel fused coumarin compounds from 6-amino-7-phenolchloro-2-naphthol as a starting material. From the pharmacokinetic parameters gathered from the web application pre-ADMET, these compounds were indicated to have good oral bioavailability which makes them promising orally administered drugs in the future. The apoptotic-inducing and free radical-quenching activities of the synthesised fused coumarin compounds revealed a number of significant findings. Firstly, all of the synthesised compounds have a weaker apoptotic-inducing activity than 5-FU, the standard. Among them, **SA4** had the most potent and the closest activity to 5-FU. In addition, all of these compounds were less toxic than the standard against non-tumorigenic cells. Secondly, the free radical-quenching activity of the fused coumarin compounds was weaker than that of L-AA, with **SA0** having the most powerful activity as compared to the others. From these findings, along with their good oral absorption profiles and low penetration across the blood-brain barrier, **SA4** and **SA0** could provide a valuable platform for the scanning of new apoptotic-inducing and free radical-quenching drugs in the coming days.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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