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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of a series of novel imidazolidinone analogues of 6-aminoflavone as anticancer and anti-inflammatory agents

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Abstract The flavone moiety is a potential pharmacophore known for its diverse range of pharmacological activities. Aminoflavones have recently been the subject of considerable attention as lead molecules in several cancer research projects. Imidazolidinone heterocycles represent another biologically active scaffold with known cytotoxic properties. In an attempt to provide synergistic cytotoxic activity, these two moieties have been combined, and the resulting novel analogues evaluated for their anticancer and anti-inflammatory activities. The results revealed that the cytotoxicities of these compounds were fivefold greater than those of aminoflavone. DNA histograms obtained from cell cycle analysis in the presence of these compounds were apoptotic in their nature. Furthermore, the in vivo screening of these compounds using Ehrlich's ascites tumour model showed an increase in life span, whereas an in vivo anti-inflammatory study resulted in the enhancement of the anti-inflammatory potential. The results therefore supported the hypothesis that there is a relationship between inflammation and cancer.

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Keywords 6-Aminoflavone · Imidazolidinone · Cytotoxicity · Ehrlich's ascites · Anti-inflammatory · Carrageenan

Introduction

Flavonoids are a group of natural substances with a broad range of biological activities that can be found abundantly in many food items. Research in the field of flavonoids has increased steadily since the discovery of the French paradox. The flavonoid heterocycle is an established pharmacophore containing a chromone ring system. Compounds of this structural class typically possess a variety of different biological activities at non-toxic concentrations (Ren *et al.*, 2003). These molecules can be useful in the prevention of cancer because of their strong antioxidant potential (Stefani *et al.*, 1999; Fotsis *et al.*, 1997) and have established themselves as promising anticancer agents (Ren *et al.*, 2003). Recent studies report an interesting relationship between the chromone structures in flavonoids and their potential anticancer activity (Nijveldt *et al.*, 2001). According to the reports of Scambia *et al.* (1994) and Ferte *et al.* (1999), flavonoids possess multidrug resistance (MDR) modulatory activity. Quercetin and some flavonoids containing *N*-benzyl piperazine side chains in particular have been proven to potentiate Doxorubicin cytotoxicity in MDR cells.

Aminoflavones are a class of flavonoids that contains amino groups attached to the flavone nucleus. They do not occur naturally and must therefore be synthesized. Although reports into the syntheses and biological activities of aminoflavones are scarce, the available literature suggests that compounds containing the aminoflavone moiety have good potential as anticancer agents. Compounds of this particular structural class are potent inhibitors of protein tyrosine

kinase (Cushman *et al.*, 1994). Aminoflavones have also been reported as a new class of antitumour agents in breast cancer (Akama *et al.*, 1997). Reports suggest that aminoflavone is uniquely able to induce its own metabolic activation by CYP1A1/1A2 induction to directly form a cytotoxic DNA damaging species in tumour cells (Kuffel *et al.*, 2002). The basis for this selective toxicity of aminoflavone has been reported to be the result of aryl hydrocarbon receptor activation, leading to a 100-fold induction of CYP1A1 mRNA and a corresponding increase in ethoxyresorufin-O-deethylase activity (Loaiza-Perez *et al.*, 2004). Aminoflavone has also been reported to induce the formation of DNA protein cross links, DNA single strand breaks, and Histone H_2AX phosphorylation (Meng *et al.*, 2005). In a separate report, non-malignant cells (MCF10A) were shown to be resistant to the cytotoxic effects of aminoflavone (McLean *et al.*, 2008).

4-Imidazolidinones represent another heterocyclic structural class with established cytotoxic properties against a variety of different cell lines (Al-Madi *et al.*, 2001; Jung *et al.*, 1998). The inclusion of some aryl functionality at the 5-position of 4-imidazolidinones has been reported to be essential to their anticancer activity, and accordingly, 5-substituted 2-cyanoimino-4-imidazolidinones have been shown to exhibit cytotoxic properties at nano-molar concentrations (Chern *et al.*, 2004; Khodair *et al.*, 1998). The importance of the imidazolidinone motif to the cytotoxicity of 4-phenyl 1-arylsulphonyl imidazolidinones has also been reported (Kim and Jung, 2002; Jung *et al.*, 2001).

Based on the results outlined above, we became interested in the preparation of imidazolidinone analogues of 6-aminoflavone and the assumption that the combination of these two pharmacophores may result in compounds with synergistic anticancer activity and reduced levels of toxicity. The general structure of the proposed analogues is shown in Fig. 1.

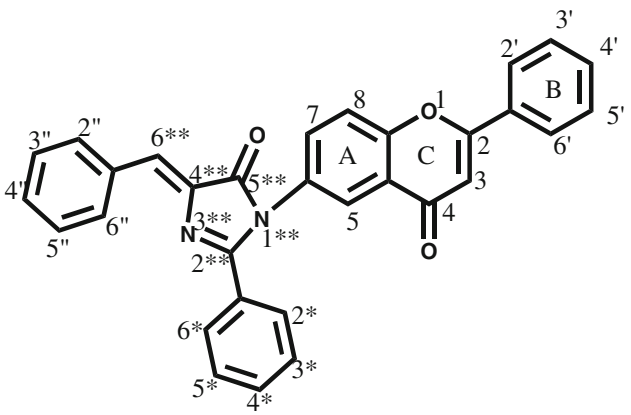
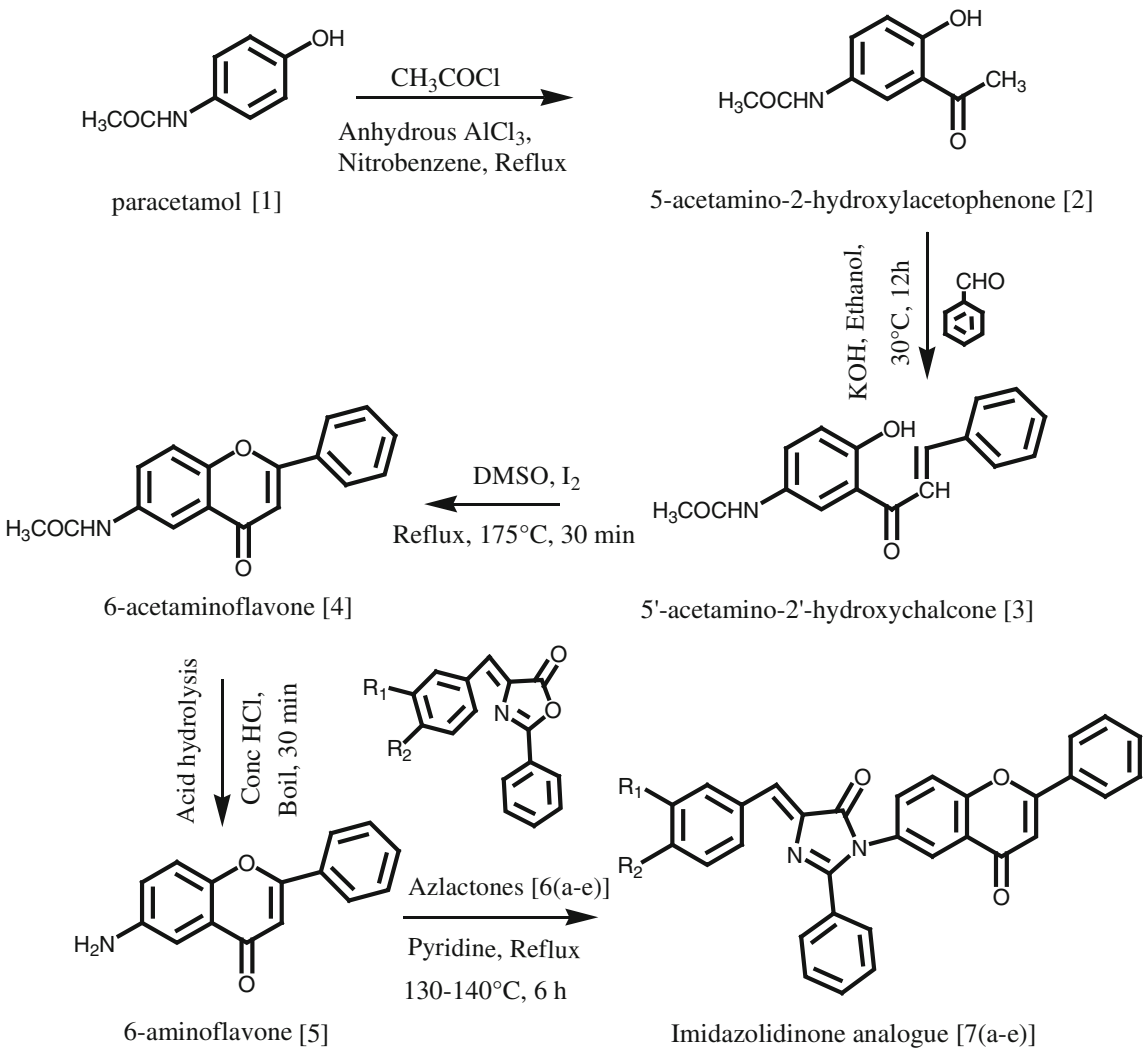


Fig. 1 Structure of the proposed imidazolidinone analogue of flavone

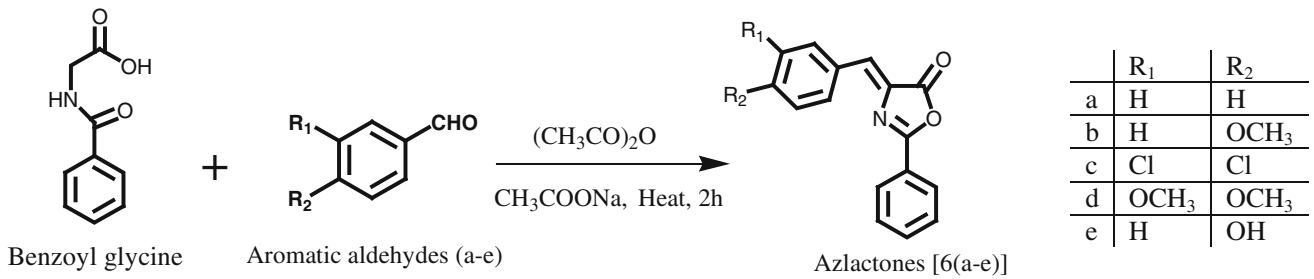
Results and discussion

The 6-aminoflavone (**5**) required for the synthesis of the imidazolidinone analogues was synthesized from paracetamol, as shown in Scheme 1. Acetylation of paracetamol followed by the base-mediated condensation of the product with benzaldehyde yielded the corresponding chalcone **3**. The formation of the chalcone with the acetamido group intact was confirmed by the appearance of the corresponding molecular ion peak at *m/z* 281 in the mass spectrum of the compound. The presence of a ^{1}H NMR signal at δ 2.26 was also consistent with the three hydrogen atoms of the $COCH_3$ group in the product, whereas the singlet at δ 10.02 was consistent with the NH proton of the $NHCOCH_3$ group. Cyclisation of the chalcone in DMSO with a catalytic amount of iodine yielded 6-acetamidoflavone (Nitin and Soni, 2005), which was hydrolysed to give 6-aminoflavone in good yield (92 %). The formation of 6-aminoflavone was confirmed by the presence of the molecular ion at *m/z* 237 in the mass spectrum of the product, and by the presence of a ^{1}H NMR signal at δ 5.5 corresponding to the two amino protons. The 6-aminoflavone was refluxed with a series of different azlactones in pyridine for 6–8 h to give the proposed imidazolidinone analogues **7a–e** in yields in the range of 48–55 %. The formation of the products was confirmed by the disappearance of the primary amino-proton signals of the aminoflavones at δ 5.5 in the ^{1}H NMR spectra of the products. Product formation was also confirmed by mass spectral and ^{13}C NMR spectral analyses. The azlactones used in the syntheses described above were prepared by the Erlenmeyer azlactone synthesis (Brian *et al.*, 1989), as shown in Scheme 2. The five azlactones **6a–e** used in the current study were synthesized from the corresponding benzaldehydes, including benzaldehyde, 4-methoxybenzaldehyde, 3,4-dichlorobenzaldehyde, 3,4-dimethoxybenzaldehyde and 4-hydroxybenzaldehyde, respectively.

All of the newly synthesized imidazolidinone analogues and the original aminoflavone were screened for their in vitro cytotoxicity against HeLa (human epithelial cervical cancer), and MDA MB 435 (human breast cancer) cell lines using the methyl tetrazolium (MTT) and sulphorhodamine B (SRB) assays, respectively. Two different assay techniques were used for the cytotoxicity screening, based on the fact that the aminoflavone is coloured yellow and could interfere with the colour formed in the assay. The occurrence of a matching result across the two assays was expected to confirm the validity of the results because the principles involved in the two techniques were different. The results of the preliminary cytotoxicity screen revealed that all of the newly synthesized imidazolidinone analogues registered higher levels of cytotoxicity than 6-aminoflavone in both the cell lines. The most active



Scheme 1 Synthesis of imidazolidinone analogues of 6-aminoflavone



	R_1	R_2
a	H	H
b	H	OCH_3
c	Cl	Cl
d	OCH_3	OCH_3
e	H	OH

Scheme 2 Synthesis of azlactones **6a–e**

compound in the screen (Compound **7d**) was about five times more active than the parent 6-aminoflavone. The results are presented in Table 1 and Fig. 2. The data obtained showed that the conversion of the aminoflavone to its imidazolidinone analogue effectively enhanced the cytotoxic potential of the original compound. Of the imidazolidinones synthesized and tested for their cytotoxic activity, compounds containing hydroxy and methoxy substituents on the imidazolidinone segment were more cytotoxic than those containing chloro substituents or no substitution at all. Furthermore, the presence of two methoxy groups on the benzene ring had a considerable impact on the cytotoxicity, as can be seen in the case of compound **7d**. The increase in the activity of this particular

Table 1 Results of the in vitro cytotoxicity studies

Compound	IC_{50} value[a] (μg/mL)			
	MTT assay		SRB assay	
	HeLa cell line	MDA MB cell line	HeLa cell line	MDA MB cell line
5	55.75 ± 2.37	59.48 ± 1.11	63.61 ± 1.38	78.37 ± 0.89
7a	20.12 ± 1.54	24.29 ± 2.62	20.89 ± 1.86	26.37 ± 0.55
7b	14.24 ± 2.75	18.50 ± 1.03	17.62 ± 0.79	21.74 ± 0.86
7c	15.23 ± 0.89	16.89 ± 0.60	15.98 ± 1.39	20.15 ± 0.19
7d	9.87 ± 3.13	10.79 ± 2.23	11.08 ± 2.57	11.95 ± 0.50
7e	16.40 ± 0.56	18.03 ± 0.69	18.90 ± 2.60	21.36 ± 1.16
Dox	0.69 ± 0.12	0.77 ± 0.19	0.81 ± 0.12	0.93 ± 0.19

Dox Doxorubicin standard

[a] Values are the average of three determinations ± SDEV

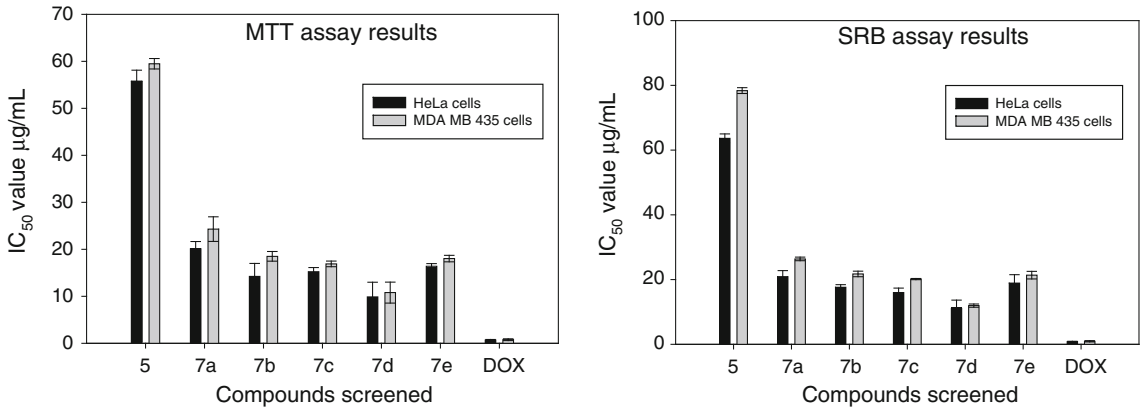
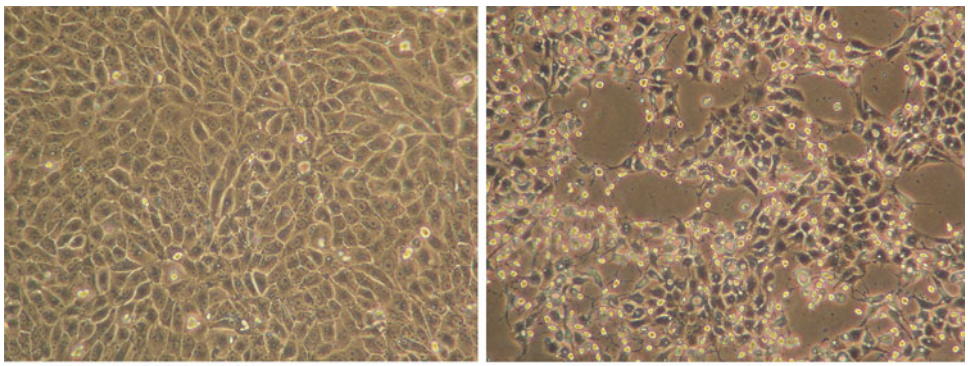


Fig. 2 Comparison of results of the in vitro cytotoxicity studies performed for compounds **7a–e** using MTT and SRB assays

Fig. 3 Microscopic pictures of HeLa cells taken after 24 h of treatment during the MTT assay

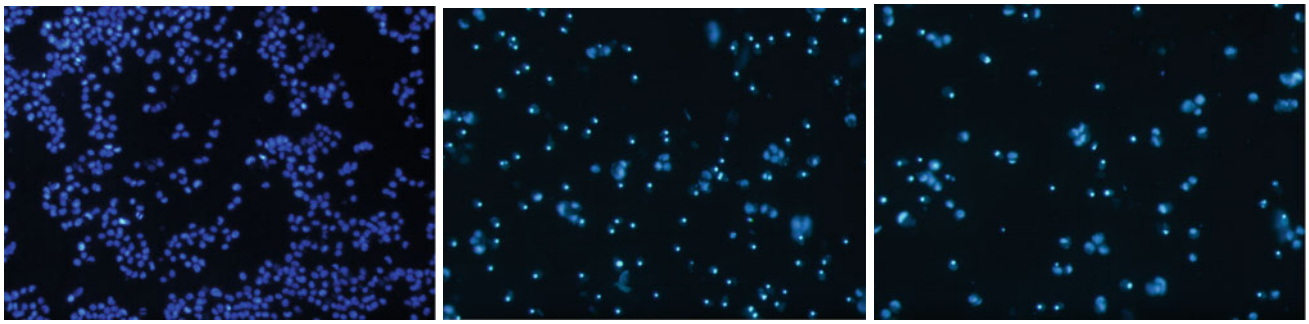


compound may be attributed to the presence of two electron-donating methoxy groups. The presence of a single hydroxyl, methoxy or halogen group did not provide substantial increases in the level of cytotoxic activity. During the cytotoxicity assay, microscopic pictures were also taken 24 h after the initiation of the treatment with the compounds. These pictures revealed characteristic morphological changes such as cell rounding and detachment of cells from the surface of the well, which confirmed the cytotoxicities of the compounds being tested (Fig. 3).

The most active compound (**7d**) in the in vitro cytotoxicity screen was selected to further study the possibility of any apoptotic potential. Apoptotic potential was studied at a concentration equal to the IC_{50} value of the compound. Accordingly, HeLa cells treated with compound **7d** were stained with Hoechst 33342 stain, and the appearance of chromatin condensation and the fragmentation of the nuclei were monitored according to the procedure described by Hu *et al.* (2009). Morphological changes in the nuclei of the apoptotic cells were visualised by fluorescence microscopy.

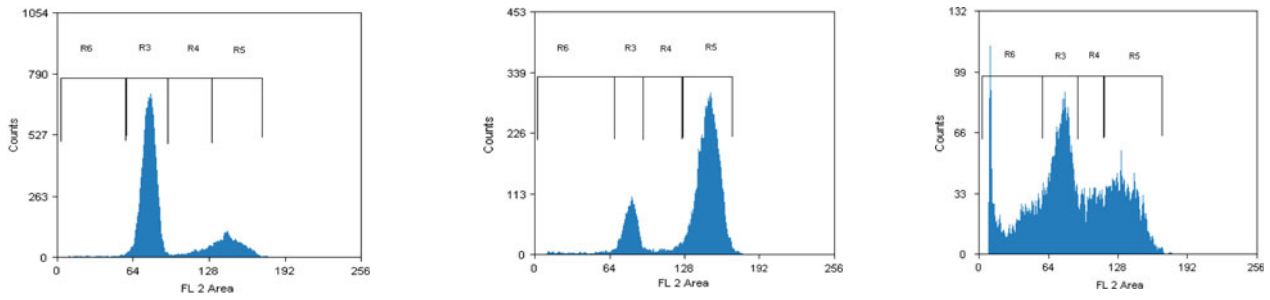
The results of the Hoechst stain analysis revealed that the control cells possessed regular and round-shaped nuclei, whereas the cells treated with compound **7d** and Doxorubicin (standard drug) showed condensation and fragmentation of their nuclei characteristic of apoptotic cells. As can be seen from the results obtained with the Hoechst staining technique (Fig. 4), the cells showed DNA fragmentation characterised by the occurrence of a condensed nucleus (highly fluorescent), which is characteristic of apoptosis. DNA fragmentation is considered to be the key biochemical event in apoptosis. One method for identifying DNA fragmentation involves analysis of the DNA content by flow cytometry (Telford *et al.*, 1991, 1992). Fluorescence-activated cell sorting (FACS) analyses were performed on compound **7d** to study the effect of the synthesized compound on cell cycle progression. Figure 5 shows the results of the FACS analysis following 48 h of treatment as DNA histograms. Compound **7d** provided a DNA histogram characteristic of apoptosis, showing an increased percentage of DNA in the $SubG_0$ phase. The presence of the $SubG_0$ phase DNA content is a single parameter indicator of apoptotic cells. Compound **7d** also provided an increase in the percentage DNA content from 18.65 (untreated control) to 28.73 % in the G_2/M phase, which indicated that the compound **7d** had also caused G_2/M phase arrest.

Following the confirmation of its cytotoxicity and apoptotic potential, compound **7d** was further studied for its in vivo anticancer activity. The safe dose was determined to be 100 mg/kg/day as per the results of the acute toxicity study (Ghosh, 1984). In the in vivo studies, parameters such as mean survival time, % increase in life span (%ILS) and % increase in body weight were measured and compared to those of the standard drug Cisplatin. The mean survival time and percentage increase in life span were studied using Ehrlich's Ascites Carcinoma (EAC)-induced mice with two doses of 100 and 50 mg/kg/day. The results for the mean survival time and the % ILS of EAC-induced mice are presented in Table 2. The results were analysed for statistical significance as per Dunnett's multiple comparison test for one-way analysis of variance. All of the results were found to be significant with $p < 0.01$ when compared with the control. A regular rapid increase in ascites tumour volume was also noted in the tumour-bearing mice. In the case of treated mice, as well as in the case of the positive control, the tumour volume was found to be lower. Treatment with compound **7d** led to an



Untreated HeLa cells | HeLa cells treated with doxorubicin | HeLa cells treated with compound **7d**

Fig. 4 Results of the Hoechst stain analysis (fluorescent microscopic pictures)



Untreated (control) Hela cells | Hela cells treated with Doxorubicin | Hela cells treated with compound **7d**

HeLa Control (G_2: R_1 & R_2)					
	Total	R_3	R_4	R_5	R_6
Phase		G_0/G_1	S	G_2/M	$SubG_0$
% Hist	100	74.27	6.34	18.65	1.15

Doxorubicin (G_2: R_1 & R_2)					
	Total	R_3	R_4	R_5	R_6
Phase		G_0/G_1	S	G_2/M	$SubG_0$
% Hist	100	16.62	4.01	76.76	2.22

Compound 7d (G_2: R_1 & R_2)					
	Total	R_3	R_4	R_5	R_6
Phase		G_0/G_1	S	G_2/M	$SubG_0$
% Hist	100	36.8	14.1	28.37	21.63

Fig. 5 Results of the cell cycle analysis (DNA content histograms)

Table 2 Mean survival time and percentage increase in life span of EAC-induced mice during the study

Treatment code	Dose (mg/kg/day)	Mean life span		Median life span	
		Mean survival time (days)	Mean (%ILS)	Median survival time (days)	Median (%ILS)
Negative control	–	17.33 ± 0.21	–	17	–
Cisplatin	3.5	34 ± 0.63*	96.19	34	100
7d	100	23 ± 0.36*	32.72	23	35.29
	50	22.33 ± 0.33*	28.87	22.5	32.35

$N = 6$ animals in each group, Days of treatment = 9, values are expressed as mean ± SEM

* $p < 0.01$ versus control as per Dunnet multiple comparisons test for one-way analysis of variance.

Table 3 Percentage increase in body weight of EAC-induced mice during the study

Treatment code	Average % increase in body weight in grams ± SEM				
	Day 3	Day 6	Day 9	Day 12	Day 15
Negative control (untreated)	4.56 ± 0.52	16.31 ± 0.48	34.31 ± 1.06	44.42 ± 2.82	58.81 ± 2.67
Cisplatin (3.5 mg/kg/day)	3.12 ± 1.2	4.69 ± 1.34*	6.97 ± 1.060*	9.0 ± 0.99*	10.54 ± 0.90*
7d (100 mg/kg/day)	3.58 ± 0.90	9.39 ± 1.32***	13.63 ± 1.29*	17.47 ± 1.2*	19.99 ± 1.29*

$N = 6$ animals in each group, Days of treatment = 9, values are expressed as mean ± SEM

* $p < 0.001$, *** $p < 0.05$ versus control as per Tukey multiple comparisons test for one-way analysis of variance

increase in the survival time of the tumour-bearing mice and also led to a reduction in the ascites fluid volume. The average % increase in body weight was calculated for the EAC-induced liquid tumour-bearing mice at a dose level of 100 mg/kg/day. The intra-peritoneal inoculation of EAC cells in the mice produced a significant increase in their body weight. The increase in tumour weight was attributed to the accumulation of peritoneal fluid, which in turn resulted in an abnormal enlargement of the peritoneal cavity. The untreated mice showed an average increase of 58.8 % in body weight on the 15th day of the treatment programme. In the case of the positive control (Cisplatin treated mice), there was only a 10.54 % increase in body weight on the 15th day of the treatment programme, and the amount of ascitic fluid was also much lower. The % increase in body weight on the 15th day for the mice treated with compound **7d** was 19.99 %, demonstrating a significant antitumour activity (Table 3).

The in vivo anti-inflammatory activities of compounds **7a–e** were studied in Wistar rats according to the method described by Winter *et al.* (1962) involving a Carrageenan-induced rat paw oedema model. A dose of 100 mg/kg/day was selected based on the acute toxicity study. The results revealed an increase in paw volume following Carrageenan administration in the control (0.46 mL) and ibuprofen-treated groups (0.15 mL), which corresponded well with the findings of a previous report (Khaksari *et al.*, 2004). All of the imidazolidinone analogues registered anti-inflammatory activity with a reduction in the paw volume from 0.46 mL (untreated control) to as low as 0.23 mL (Table 4). Compound **7d** was found to be the most active of the compounds tested, suggesting that the presence of

Table 4 Effect of compounds **7a–e** on Carrageenan-induced rat paw oedema

Compound code	Dose (mg/kg, p.o)	Mean rat paw oedema volume after 3 h (ml ± SEM*) ($N = 6$)	Percentage inhibition ± SEM
Control	CMC 2 %	0.46 ± 0.01	–
5	100	0.35 ± 0.01*	24.18 ± 2.8
7a	100	0.29 ± 0.02*	38.25 ± 6.1
7b	100	0.25 ± 0.02*	45.11 ± 3.6
7c	100	0.29 ± 0.01*	36.45 ± 3.1
7d	100	0.23 ± 0.008*	49.81 ± 1.9
7e	100	0.24 ± 0.01*	47.64 ± 2.5
Ibuprofen	50	0.15 ± 0.01*	67.14 ± 2.5

One-way ANOVA followed by post hoc Scheffe's test

* $p < 0.05$ versus control group

the methoxy group may have an influence on the anti-inflammatory activity.

Materials and methods

Chemicals required for the synthesis such as paracetamol, benzaldehyde, 3,4-dichlorobenzaldehyde, 3,4-dimethoxybenzaldehyde, 4-methoxybenzaldehyde and 4-hydroxybenzaldehyde were purchased from Sigma Aldrich Chemical Company, USA. The melting points of the synthesized compounds were determined using digital melting point apparatus (V Scientific India). Thin layer chromatography (TLC) studies were performed on silica gel G coated aluminium plates supplied by Merck, Germany with a solvent system composition of Hexane: Acetone (6:4). FTIR spectra were recorded using 8310 FTIR instrument, Shimadzu, Japan. NMR spectral study was performed with the help of AMX 400, Bruker Spinwin, Germany. EI MS data were obtained using GCMS QP5050, Shimadzu, Japan and ESI–MS data were recorded using Enquire 3000 plus, Bruker Daltonics, Germany. UV λ_{max} values were determined using UV Visible Spectrophotometer, Model UV-2400PC, Shimadzu, Japan. Various chemicals used for biological activity studies such as MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide], SRB, Hoechst 33342 stain, Propidium iodide dye, Carrageenan etc. were obtained from Sigma Aldrich Chemical company, USA. Doxorubicin hydrochloride which was used as the standard for in vitro anticancer study was procured from Sigma Aldrich Chemical Company, USA. Cisplatin the positive control used in the in vivo anticancer study was obtained as a gift sample from Dabur India ltd. Ibuprofen, the standard drug for anti-inflammatory activity, was purchased from Sd fine chemicals, India. The cell lines used such as HeLa (human epithelial cervix cancer) and MDA MB 435 (human breast cancer) were the ones that were maintained at the Department of MRDG, Indian Institute of Science, Bangalore. The anti-inflammatory measurements were carried out on a plethysmometer. Hoechst staining of the cells was viewed on a fluorescent microscope (Olympus, Japan). The cell cycle analysis was carried out on a Fluorescence Assisted Cell Sorting (FACS) machine, MoFlo equipped with 488 nm solid state laser, and data were analysed using Summit software (Beckman Coulter).

Synthesis of imidazolidinone analogues **7a–e**

To an equimolar mixture of **5** and azlactone **6**, was added pyridine (quantity sufficient to dissolve), and the mixture was refluxed at 130–140 °C for 6 h. The completion of the reaction was monitored by TLC. The reaction mixture was then cooled and poured on to 50 ml ice cold water containing 5 N HCl, with stirring until the pH was neutral. The precipitated product was collected by filtration, washed with water to remove pyridine and dried. The dried product was recrystallised from ethanol yielding imidazolidinone analogue in pure form. A series of such analogues **7a–e** were prepared using corresponding oxazolones **6a–e**. The newly synthesized 6-imidazolidinone analogues were characterised by their physical properties and spectral characteristics. Various experimental and spectral data such as melting point, R_f value, % yield, UV, IR, MS, ^{1}H NMR and ^{13}C NMR etc. were recorded.

*4-Benzylidene-1-(4-oxo-2-phenyl-4H-chromen-6-yl)-2-phenyl-1H-imidazol-5-one (**7a**)*

Yield: 55 %; m.p.: 244 °C; R_f value: 0.388; UV λ_{max} (nm): 294; IR (KBr) cm^{-1}: 1685 (C=O str. imidazolidinone), 1649 (C=O str. flavone), 1600 (C=N str. Imidazolidinone). EI–MS *m/z* (% abundance): 468 M^+ (10), 324 (4), 237 (48), 247 (13), 135 (30), 116 (5). ^{1}H NMR DMSO-d_6, 400 MHz, δ 7.22 (s, 1H, Ar–CH=C), 8.48 (s, 1H, H-5), 7.32 (d, 1H, H-8) $J = 12$ Hz, 7.8 (d, 1H, H-7) $J = 12$ Hz, 7.35–7.70 (m, 15H, aromatic protons of the three phenyl rings); ^{13}C NMR DMSO-d_6, 100 MHz, δ 164.52 (C-2), 106.48 (C-3), 176.99 (C-4), 118.81 (C-5), 131.78 (C-6), 134.16 (C-7), 114.46 (C-8), 151.78 (C-9), 123.41 (C-10), 131.20 (C-1′), 128.55 (C-2′, C-6′), 127.91 (C-3′, C-5′), 133.39 (C-4′), 162.39 (C-2**), 129.52 (C-4**), 166.07 (C-5**), 106.48 (C-6**), 136.78 (C-1″), 126.66 (C-2″, C-6″), 128.36 (C-5″), 128.55 (C-3″), 130.76 (C-4″), 127.91 (C-1*), 126.32 (C-2*, C-6*), 129.10 (C-5*, C-3*), 130.76 (C-4*).

*4-(4-Methoxybenzylidene)-1-(4-oxo-2-phenyl-4H-chromen-6-yl)-2-phenyl-1H-imidazol-5-one (**7b**)*

Yield: 53 %; m.p.: 267 °C; R_f value: 0.327; UV λ_{max} (nm): 310; IR (KBr) cm^{-1}: 1680 (C=O str. imidazolidinone), 1639 (C=O str. flavone), 1604 (C=N str. imidazolidinone). EI–MS *m/z* (% abundance): 498 M^+ (2), 237 (12), 279 (5), 146 (5), 135 (20), 105 (100), 77 (41). ^{1}H NMR DMSO-d_6, 400 MHz, δ 3.55 (s, 3H, OCH_3) 7.22 (s, 1H, Ar–CH=C), 8.48 (s, 1H, H-5), 7.79 (d, 1H, H-8) $J = 12$ Hz, 8.18 (d, 1H, H-7) $J = 12$ Hz, 8.08 (dd, 4H, H-2″, 3″, 5″, 6″), $J = 9.4$ Hz, $J = 9.38$ Hz, 7.58 (m, 8H, 2-phenyl of imidazolidinone (5H), 3′, 4′, 5′), 6.97 (d, 2H, H-2′, 6′) $J = 10.16$ Hz; ^{13}C NMR DMSO-d_6, 100 MHz, δ 162.58 (C-2), 103.58 (C-3), 179.79 (C-4), 120.99 (C-5), 129.45 (C-6), 130.62 (C-7), 117.24 (C-8), 153.12 (C-9), 124.97 (C-10), 130.40 (C-1′), 125.59 (C-2′, C-6′), 128.21 (C-3′, C-5′), 129.98 (C-4′), 167.39 (C-2**), 131.80 (C-4**), 170.28 (C-5**), 108.58 (C-6**), 127.98 (C-1″), 126.16 (C-2″, C-6″), 128.38 (C-5″), 128.91 (C-3″), 130.73 (C-4″), 127.54 (C-1*), 127.39 (C-2*, C-6*), 116.20 (C-5*, C-3*), 129.96 (C-4*), 56.26 (C–OCH_3).

*4-(3,4-Dichlorobenzylidene)-1-(4-oxo-2-phenyl-4H-chromen-6-yl)-2-phenyl-1H-imidazol-5-one (**7c**)*

Yield: 58 %; m.p.: 268 °C; R_f value: 0.367; UV λ_{max} (nm): 292; IR (KBr) cm^{-1}: 1685 (C=O str. imidazolidinone), 1649 (C=O str. flavone), 1600 (C=N str. imidazolidinone); ESI–MS *m/z* 555.4 (M + H_2O); ^{1}H NMR DMSO-d_6, 400 MHz, δ 7.02 (s, 1H, H-3), 7.16 (s, 1H, Ar–CH=C), 8.46 (s, 1H, H-5), 7.8 (d, 1H, H-8) J = 9.04 Hz, 8.18 (d, 1H, H-7) J = 8.84 Hz, 8.01 (d, 2H, H-2′, 2*) J = 7.4 Hz, 8.1 (d, 2H, H-6′, 6*) J = 6.84 Hz, 7.89 (s, 1H, H-2″) 7.67 (d, 1H, H-6″) J = 8.4 Hz, 7.58 (m, 7H, 3′, 4′, 5′, 5″, 3*, 4*, 5*); ^{13}C NMR DMSO-d_6, 100 MHz, δ 163.86 (C-2), 105.39 (C-3), 183.02 (C-4), 120.18 (C-5), 129.14 (C-6), 131.37 (C-7), 117.86 (C-8), 152.62 (C-9), 124.16 (C-10), 130.34 (C-1′), 126.48 (C-2′, C-6′), 128.69 (C-3′, C-5′), 128.01 (C-4′), 164.87 (C-2**), 130.57 (C-4**), 171.73 (C-5**), 108.89 (C-6**), 134.81 (C-1″), 125.66 (C-2″), 127.76 (C-6″), 133.69 (C-5″), 130.57 (C-3″), 132.67 (C-4″), 128.01 (C-1*), 126.28 (C-2*, C-6*), 128.85 (C-5*, C-3*), 130.61 (C-4*).

*4-(3,4-Dimethoxybenzylidene)-1-(4-oxo-2-phenyl-4H-chromen-6-yl)-2-phenyl-1H-imidazol-5-one (**7d**)*

Yield: 48 %; m.p.: 240 °C; R_f value: 0.306. UV λ_{max} (nm): 296; IR (KBr) cm^{-1}: 1681 (C=O str. imidazolidinone), 1631 (C=O str. flavone), 1604 (C=N str. imidazolidinone); ESI–MS *m/z* 547.1 (M + H_2O); ^{1}H NMR DMSO-d_6, 400 MHz, δ 3.62 (d, 6H, H–OCH_3) J = 12.6 Hz, 6.98 (s, 1H, H-3), 7.01 (s, 1H, Ar–CH=C), 8.448 (s, 1H, H-5), 7.8 (d, 1H, H-8) J = 9.08 Hz, 8.2 (d, 1H, H-7) J = 7.4 Hz, 8.1 (d, 4H, H-2′, 2*, 6′, 6*) J = 5.92 Hz, 7.23 (d, 1H, H-2″) J = 8.2 Hz, 7.3 (d, 2H, H-5″, 6″) J = 13.48 Hz, 7.51–7.6 (m, 6H, 3′, 4′, 5′, 3*, 4*, 5*); ^{13}C NMR DMSO-d_6, 100 MHz, δ 164.62 (C-2), 104.92 (C-3), 182.26 (C-4), 120.89 (C-5), 127.48 (C-6), 128.72 (C-7), 118.18 (C-8), 152.89 (C-9), 124.63 (C-10), 130.43 (C-1′), 125.98 (C-2′, C-6′), 128.97 (C-3′, C-5′), 127.89 (C-4′), 163.79 (C-2**), 130.38 (C-4**), 170.36 (C-5**), 108.93 (C-6**), 128.18 (C-1″), 120.61 (C-2″), 110.67 (C-6″), 149.94 (C-5″), 119.73 (C-3″), 151.71 (C-4″), 128.58 (C-1*), 126.21 (C-2*, C-6*), 128.93 (C-5*, C-3*), 130.14 (C-4*), 55.89 (C–OCH_3 at C-4″ and C-5″).

*4-(4-Hydroxybenzylidene)-1-(4-oxo-2-phenyl-4H-chromen-6-yl)-2-phenyl-1H-imidazol-5-one (**7e**)*

Yield: 51 %; m.p.: 262 °C; R_f value: 0.347; UV λ_{max} (nm): 296; IR (KBr) cm^{-1}: 3244 (br. Ar–OH str), 1674 (C=O str. imidazolidinone), 1626 (C=O str. flavone), 1601 (C=N str. imidazolidinone); ESI–MS *m/z* 503.1 (M + H_2O); ^{1}H NMR DMSO-d_6, 400 MHz, δ 7.01 (s, 1H, H-3), 7.2 (s, 1H, Ar–CH=C), 8.45 (s, 1H, H-5), 7.8 (d, 1H, H-8) J = 5.48 Hz, 8.19 (d, 1H, H-7) J = 7.4 Hz, 8.08 (dd, 4H, H-2″, 3″, 5″, 6″) J = 7.32 Hz, J = 7.8 Hz, 7.69 (d, 2H, H-6′, 6*) J = 8.64 Hz, 7.5–7.6 (m, 6H, 3′, 4′, 5′, 3*, 4*, 5*), 7.16 (d, 2H, H-2′, 2*) J = 8.6 Hz, 9.78 (s, 1H, H–Ar–OH); ^{13}C NMR DMSO-d_6, 100 MHz, δ 163.87 (C-2), 104.85 (C-3), 183.31 (C-4), 120.20 (C-5), 127.45 (C-6), 128.27 (C-7), 117.94 (C-8), 152.88 (C-9), 124.14 (C-10), 130.33 (C-1′), 126.35 (C-2′, C-6′), 128.71 (C-3′, C-5′), 128.00 (C-4′), 165.92 (C-2**), 130.83 (C-4**), 170.38 (C-5**), 109.18 (C-6**), 127.80 (C-1″), 125.68 (C-2″, C-6″), 114.89 (C-5″, C-3″), 158.37 (C-4″), 128.41 (C-1*), 126.94 (C-2*, C-6*), 128.88 (C-5*, C-3*), 130.62 (C-4*).

Biological activity

The synthesized analogues **7a–e** were evaluated for their in vitro cytotoxic potential by the MTT assay (Denizot and Lang, 1986) and SRB assay (Skehan *et al.*, 1990). The most active compound from the above cytotoxicity screen was studied further by Hoechst staining analysis and flow cytometric analysis (Duvall and Wyllie, 1986; Hu *et al.*, 2009) to establish the apoptotic potential. After confirming the apoptotic potential of compound **7d**, it was further studied to evaluate the in vivo anticancer activity. Ehrlich tumour is a rapidly growing carcinoma with very aggressive behaviour and is able to grow in almost all mice strains (Chen and Watkins, 1970). The in vivo study was performed using Ehrlich's ascites tumour model on Albino mice, and the activity was compared to that of Cisplatin. The study was carried out as per the procedure reported by earlier workers (Devi *et al.*, 1998). The results obtained for in vivo anticancer studies were analysed statistically and the level of significance determined by one-way ANOVA followed by Dunnet multiple comparisons test in the case of mean survival time and by Tukey test in the case of percentage increase in body weight. An in vivo anti-inflammatory screen by the method of Winter *et al.* (1962) also was performed. Statistical analysis was carried out using one-way ANOVA followed by Post hoc Scheffe's test, and $p < 0.05$ was considered significant in the case of anti-inflammatory evaluation. The animal care and handling was carried out in accordance with the guidelines issued by the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal University, Manipal, and the study was approved by the committee (Clearance certificate no.—IAEC/KMC/02/2006–2007).

Conclusion

Five novel imidazolidinone analogues of flavone have been synthesized in good yields in an attempt to explore the

possibilities of higher anticancer potential. All of the novel compounds were completely characterised by ultraviolet (UV), infrared (IR), NMR and mass spectral methods. Preliminary in vitro cytotoxicity studies carried out using MTT and SRB assays on two different cell lines revealed that the conversion to the imidazolidinone analogues enhanced the cytotoxic potential of aminoflavone. Of the analogues tested, compound **7d** [4-(3,4-dimethoxybenzylidene)-1-(4-oxo-2-phenyl-4H-chromen-6-yl)-2-phenyl-1H-imidazol-5-one] showed promising levels of activity with an IC_{50} value of 9.87 μg/mL. It is noteworthy that the most active compound possessed two methoxy groups in its structure. The study also revealed the apoptotic nature of the compound **7d**. Hoechst staining experiment and cell cycle analysis carried out using compound **7d** showed that the cell death that occurred was characteristic of apoptosis. In vivo anticancer studies performed on EAC-induced mice also resulted in promising levels of activity. The synthesized compounds **7a–e** were also evaluated for their anti-inflammatory activities and registered significant levels of activity. These results effectively support the observed anticancer potential of the compounds. Based on these results, we have concluded that SAR studies and anticancer screening against a panel of cancer cell lines need to be performed to confirm and build upon the findings of this study.

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