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Original research paper

Synthesis of coumarin heterocyclic derivatives with antioxidant activity and *in vitro* cytotoxic activity against tumour cells

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The aim of the present work was to synthesise coumarinyl heterocycles and to elucidate the potential role of these compounds as antioxidants and cytotoxic agents against Dalton's lymphoma ascites tumour cells (DLA) and Ehrlich ascites carcinoma cells (EAC). The synthesis of coumarin derivatives containing pyrazole, pyrazolone, thiazolidin-4-one, 5-carboxymethyl-4-thiazolidinone and 3-acetyl-1,3,4-oxadiazole ring is reported. 4-Methylcoumarinyl-7-oxyacetic acid hydrazide (**1**) reacted with arylazopropanes or hydrazono-3-oxobutyrate derivatives to form pyrazole (**3a-c**) and pyrazolone derivatives (**5a-c**). Heterocyclisation of Schiff's bases of **1** with thioglycolic acid, thiomalic acid or acetic anhydride afforded novel heterocyclic derivatives 4-thiazolidinones (**7a-c**), 5-carboxymethyl-4-thiazolidinones (**8a-c**) and oxadiazoles (**9a-c**), respectively. Some of the compounds showed promising antioxidant activity *in vitro* and cytotoxic activity against DLA cells and EAC cells.

Keywords: pyrazole, pyrazolone, thiazolidin-4-one, oxadiazole, antioxidant activity

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Coumarin derivatives possess a wide spectrum of biological activities (1–3). Also, it is well documented that pyrazoles, pyrazolin-5-ones, 4-thiazolidinones and 1,3,4-oxadiazoles display pronounced antioxidant (4–6) and antineoplastic activity (7–10). In view of the considerable importance of the coumarins and heterocycles mentioned above, the present work is aimed at the design and synthesis of new heterocyclic compounds bearing coumarin moiety. Moreover, the study includes testing of target compounds for their cytotoxic activity against Dalton's lymphoma ascites (DLA) and Ehrlich ascites carcinoma (EAC) cells.

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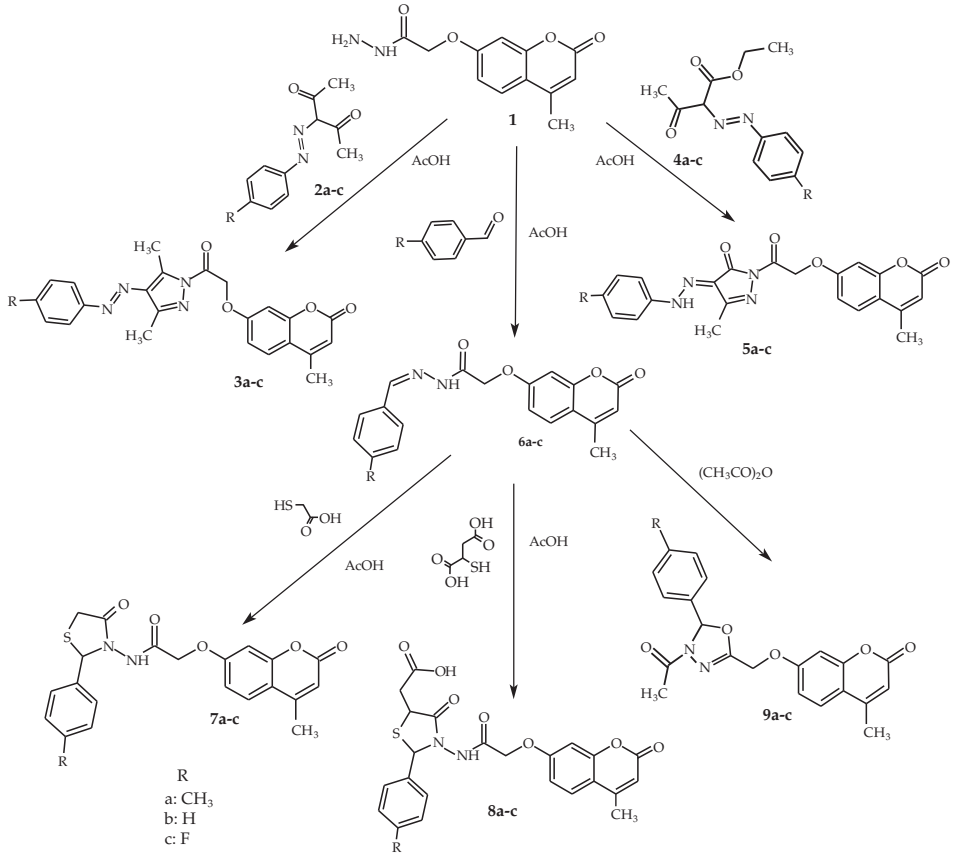
EXPERIMENTAL

Melting points were determined in open capillaries and were uncorrected. IR spectra (KBr discs) were recorded on a JASCO FT/IR-410 spectrophotometer (Japan). ^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer (Switzerland). Mass spectra were recorded on LC-MS/MS (API-4000), MDS SCIEX (Canada). Microanalysis was done on a Perkin-Elmer model 2400 CHN analyser (USA). The purity of all compounds was established by single spot on the TLC plates (Merck, Germany). Iodine vapour was used as developing agent. The solvent system used was toluene: methanol (3:7).

The starting material 4-methylcoumarinyl-7-oxyacetic acid hydrazide (**1**) was prepared according to a literature procedure (11). 3-(Arylazo)-2,4-pentanediones (**2a-c**) and ethyl-2-(substitutedphenyl) hydrazono-3-oxobutyrates (**4a-c**) were prepared according to reported procedures (12–14). Synthetic pathway of newly synthesised compoundsies presented in Scheme 1 and their physicochemical and spectral data are given in Tables I and II.



Scheme 1

Table I. Physico-chemical data of synthesised compounds

Compd.	Yield (%)	M.p. (°C)	Mol. formula (M_r)	Elemental analysis Calcd./found (%)		
				C	H	N
3a	56	246–248	$C_{24}H_{22}N_4O_4$ (430.46)	66.97 66.94	5.15 5.13	13.02 13.16
3b	54	282–284	$C_{23}H_{20}N_4O_4$ (416.43)	66.34 65.95	4.84 4.87	13.45 13.34
3c	72	192–194	$C_{23}H_{19}FN_4O_4$ (434.42)	63.59 63.54	4.41 4.50	12.90 12.79
5a	52	223–225	$C_{23}H_{20}N_4O_5$ (432.44)	63.88 63.65	4.66 4.58	12.96 12.78
5b	45	210–212	$C_{22}H_{18}N_4O_5$ (418.40)	63.15 63.38	4.34 4.28	13.39 13.28
5c	60	276–278	$C_{22}H_{17}FN_4O_5$ (436.39)	60.55 60.45	3.93 3.91	12.84 12.79
6a	62	224–226	$C_{20}H_{18}N_2O_4$ (350.37)	68.56 68.38	5.18 5.22	8.00 7.85
6b	68	222–224	$C_{19}H_{16}N_2O_4$ (336.34)	67.85 67.64	4.79 4.92	8.33 8.56
6c	83	238–240	$C_{19}H_{15}FN_2O_4$ (354.33)	64.40 64.54	4.27 4.37	7.91 7.68
7a	64	154–155	$C_{22}H_{20}N_2O_5S$ (424.47)	62.25 62.19	4.75 4.76	6.60 6.76
7b	57	219–220	$C_{21}H_{18}N_2O_5S$ (410.44)	61.45 61.51	4.42 4.33	6.83 6.78
7c	72	228–230	$C_{21}H_{17}FN_2O_5S$ (428.43)	58.87 59.10	4.00 3.95	6.54 6.61
8a	64	196–197	$C_{24}H_{22}N_2O_7S$ (482.51)	59.74 59.51	4.60 4.55	5.81 5.65
8b	60	178–179	$C_{23}H_{20}N_2O_7S$ (468.48)	58.97 59.02	4.30 4.34	5.98 5.93
8c	72	221–223	$C_{23}H_{19}FN_2O_7S$ (486.47)	56.79 56.39	3.94 4.02	5.76 5.67
9a	58	126–128	$C_{22}H_{20}N_2O_5$ (392.41)	67.34 67.57	5.14 5.22	7.14 6.96
9b	52	172–174	$C_{21}H_{18}N_2O_5$ (378.38)	66.66 66.56	4.79 4.83	7.40 7.54
9c	70	175–177	$C_{21}H_{17}FN_2O_5$ (396.37)	63.63 63.48	4.32 4.25	7.07 7.05

Table II. Spectral data of synthesised compounds

Compd.	IR (ν, cm^{-1})	^1H NMR (δ, ppm) (DMSO-d_6) / MS
3a	2924, 1721, 1617, 1493, 1439, 1283	2.41 (s, 3H, CH_3 of coumarin), 2.51 (s, 3H, CH_3 of phenyl substituent), 3.35–3.48 (m, 6H, CH_3 of pyrazole ring), 4.88 (s, 2H, OCH_2), 6.25 (s, 1H, CH of coumarin), 7.23–7.74 (m, 7H, Ar-H) *m/z* 431.6 $(M+1)^+$ (14), 219.2 (100), 175.2 (34), 136.9 (61), 123.2 (56)
3b	2973, 1719, 1615, 1491, 1390, 1283	2.19 (s, 3H, CH_3 of coumarin), 2.50 (s, 3H, CH_3 at C_3 of pyrazole), 3.37 (s, 3H, CH_3 at C_5 of pyrazole), 4.79 (s, 2H, $OCH_{2)}$, 6.25 (s, 1H, CH of coumarin), 6.85–7.71 (m, 8H, Ar-H)
3c	2934, 1695, 1606, 1521, 1427, 1288	2.41 (s, 3H, CH_3 of coumarin), 2.51 (s, 3H, CH_3 at C_3 of pyrazole), 3.37 (s, 3H, CH_3 at C_5 of pyrazole), 4.79 (s, 2H, $OCH_{2)}$, 6.25 (s, 1H, CH of coumarin), 6.98–7.73 (m, 7H, Ar-H)
5a	3156, 3055, 2977, 1721, 1623, 1491	2.27 (s, 3H, CH_3 of phenyl substituent), 2.38 (s, 3H, CH_3 of coumarin), 3.31(s, 3H, $CH_3$of pyrazolone), 4.73 (s, 2H, $OCH_{2)}$, 6.22 (s, 1H, CH of coumarin), 6.85–7.74 (m, 7H, Ar-H), 10.32 (s, 1H, NH) *m/z* 431.6 (M^+) (10), 191.2 (26), 123.5 (11), 106.2 (34)
5b	3225, 3056, 2966, 1718, 1612, 1388	2.52 (s, 3H, CH_3 of coumarin), 2.63 (s, 3H, CH_3 of pyrazolone), 4.87 (s, 2H, OCH_2), 6.38 (s, 1H, CH of coumarin), 7.08–7.87 (m, 8H, Ar-H), 10.01 (s, 1H, NH)
5c	2927, 1717, 1385, 1263, 1156	2.41 (s, 3H, CH_3 of coumarin), 2.52 (s, 3H, CH_3 of pyrazolone), 4.73 (s, 2H, OCH_2), 6.25 (s, 1H, CH of coumarin), 6.86–7.74 (m, 7H, Ar-H), 10.3 (s, 1H, NH)
6a	3170, 1655, 1686, 1275	2.32 (s, 3H, CH_3 of phenyl substituent), 2.41 (s, 3H, CH_3 of coumarin), 4.78 (s, 2H, OCH_2), 5.12 (s, 1H, CH), 6.24 (s, 1H, CH of coumarin), 7.32–8.71 (m, 7H, Ar-H), 10.01 (s, 1H, NH) *m/z* 351.2 $(M+1)^+$ (14), 219.2 (100), 189.0 (42), 175.2 (32)
6b	3210, 1672, 1685, 1267	2.41(s, 3H, CH_3 of coumarin), 4.83 (s, 2H, $OCH_{2)}$, 4.98 (s, 1H, CH), 6.36 (s, 1H, CH of coumarin), 7.08–7.87 (m, 8H, Ar-H), 10.30 (s, 1H, NH)
6c	3175, 1654, 1692, 1270	2.34 (s, 3H, CH_3 of coumarin), 4.78 (s, 2H, $OCH_{2)}$, 5.01 (s, 1H, CH), 6.42 (s, 1H, CH of coumarin), 8.24–8.37 (m, 7H, Ar-H), 10.32 (s, 1H, NH)
7a	2981, 2807, 1716, 1687, 1511, 1273	2.34 (s, 3H, CH_3 of phenyl substituent), 2.41 (s, 3H, CH_3 of coumarin), 3.35 (s, 2H, CH_2), 4.80 (s, 2H, $OCH_{2)}$, 5.28 (s, 1H, CH), 6.24 (s, 1H, CH of coumarin), 6.97–7.75 (m, 7H, Ar-H), 10.28 (s, 1H, NH) *m/z* 425.3 $(M+1)^+$ (10), 291.1 (100), 249.1(80), 177.2 (23), 149.2 (38)
7b	3178, 3078, 2972, 1713, 1687, 1509	2.41 (s, 3H, CH_3 of coumarin), 3.41 (s, 2H, CH_2), 4.78 (s, 2H, $OCH_{2)}$, 5.31 (s, 1H, CH), 6.25 (s, 1H, CH of coumarin), 6.99–7.74 (m, 8H, Ar-H), 8.02 (s, 1H, NH)
7c	3283, 3087, 2852, 1709, 1509, 1273	2.41 (s, 3H, CH_3 of coumarin), 2.50 (s, 2H, CH_2), 5.25 (s, 2H, $OCH_{2)}$, 6.25 (s, 1H, CH), 6.87 (s, 1H, CH of coumarin), 7.71–8.32 (m, 7H, Ar-H), 10.31 (s, 1H, NH) *m/z* 429.2 $(M+1)^+$ (12), 355.2 (100), 291.1 (64), 249.1 (67), 177.2 (11)
8a	3308, 2923, 1713, 1686, 1508	2.33 (s, 3H, CH_3 of phenyl substituent), 2.40 (s, 3H, CH_3 of coumarin), 2.49–2.51 (d, 2H, CH_2), 4.80 (s, 2H, OCH_2), 5.30 (s, 1H, CH-N), 6.24 (s, 1H, CH of coumarin), 6.9–7.8 (m, 7H, Ar-H), 7.75–7.98 (t, 1H, CH), 8.30 (s, 1H, NH), 10.30 (s,1H, COOH) *m/z* 483.2 (M^+) (44), 351.2 (85), 291.1 (11), 249.1 (12), 149.2 (64)

8b	3481, 2922, 1737, 1655, 1459	2.41 (s, 3H, CH_3 of coumarin), 2.50–2.51 (d, 2H, CH_2), 4.70 (s, 2H, OCH_2), 5.31 (s, 1H, CH-N), 6.24 (s, 1H, CH of coumarin), 6.97–8.02 (m, 10H, Ar-H, CH and NH protons), 10.32 (s,1H, COOH)
8c	3408, 2965, 1711, 1686, 1507	2.41 (s, 3H, CH_3 of coumarin), 2.50–2.62 (d, 2H, CH_2), 4.78 (s, 2H, OCH_2), 5.31 (s, 1H, CH-N), 6.25 (s, 1H, CH of coumarin), 6.98–7.80 (m, 7H, Ar-H), 8.02 (t, 1H, CH), 8.33 (s, 1H, NH), 11.69 (s,1H, COOH) *m/z* 487.3 $(M+1)^+$ (29), 355.2 (100), 291.1 (26), 249.1 (20), 149.2 (27)
9a	3158, 1719, 1660, 1509, 1054	2.4 (s, 3H, CH_3 of phenyl substituent), 2.5 (s, 3H, $COCH_3$), 3.4 (s, 3H, CH_3 of coumarin), 5.3 (s, 2H, OCH_2), 6.2 (s, 1H, CH of coumarin), 7.1 (s, 1H, CH), 7.3–7.80 (m, 7H, Ar-H)
9b	3163, 1720, 1558, 1393, 1078	2.4 (s, 3H, $COCH_3$), 2.5 (s, 3H, CH_3 of coumarin), 4.8 (s, 2H, OCH_2), 6.2 (s, 1H, CH of coumarin), 6.8–7.8 (m, 9H, Ar-H and CH)
9c	3120, 1741, 1622, 1509, 1017	2.4 (s, 3H, $COCH_3$), 2.5 (s, 3H, CH_3 of coumarin), 5.3 (s, 2H, OCH_2), 6.2 (s, 1H, CH of coumarin), 7.1 (s, 1H, CH), 7.3–8.1 (m, 7H, Ar-H) *m/z* 397.1 $(M+1)^+$ (88), 355.1 (100), 193.3 (6), 163.2 (49), 122.2 (24)

*Synthesis of 1-(4-methylcoumarinyl-7-oxyacetyl)-3,5-dimethyl-4-(arylazo) pyrazoles (**3a-c**)*

A mixture of appropriate 3-(arylazo)-2,4-pentanediones (**2a-c**) (0.001 mol) and compound **1** (0.248 g, 0.001 mol) in glacial acetic acid (10 mL) was refluxed for 10 h. The resultant solution was cooled and allowed to stand overnight. The resultant solid was collected by filtration, purified by repeated washings with acetic acid and recrystallised from acetic acid.

*Synthesis of 1-(4-methylcoumarinyl-7-oxyacetyl)-3-methyl-4-(substituted phenyl) hydrazono-2-pyrazolin-5-ones (**5a-c**)*

Ethyl-2-(substituted phenyl)hydrazono-3-oxobutyrates (**4a-c**) (0.02 mol) were dissolved in glacial acetic acid (20 mL) and 4-methylcoumarinyl-7-oxyacetic acid hydrazide (0.496 g, 0.002 mol) dissolved in 20 mL of glacial acetic acid was added. The mixture was refluxed for 4 h, cooled and then allowed to stand overnight. The resultant solid was filtered, dried and then recrystallised from ethanol. The purity of all compounds was established by single spot on the TLC plates as described above.

*Synthesis of 4-methylcoumarinyl-7-oxyacetic acid [(substituted phenyl) methylene] hydrazides (**6a-c**)*

A mixture of compound **1** (2.48 g, 0.01 mol), glacial acetic acid (20 mL) and substituted benzaldehyde (0.01 mol) was refluxed for 8 h. The contents were then poured onto crushed ice. The resultant solid was filtered and recrystallised using glacial acetic acid.

*Synthesis of 2-(substituted phenyl)-3-(4-methylcoumarinyl-7-oxyacetamido)--4-thiazolidinones (**7a-c**)*

A homogenous mixture of compounds **6a-c** (0.01 mol) and thioglycollic acid (0.92 g, 0.01 mol) in 20 mL of glacial acetic acid was refluxed for 10 h. The reaction mixture was triturated with sodium bicarbonate solution (10 %). The resultant neutral solid was poured onto crushed ice. The separated product was filtered off, washed with water, dried and recrystallised from ethanol.

*Synthesis of 2-(substituted phenyl)-3-(4-methylcoumarinyl-7-oxyacetamido)--5-carboxymethyl-4-thiazolidinone (**8a-c**)*

A homogenous mixture of compounds **6a-c** (0.01 mol) and thiomalic acid (1.52 g, 0.01 mol) in glacial acetic acid (20 mL) was refluxed for 10 h. The reaction mixture was dissolved in sodium bicarbonate solution, reprecipitated by hydrochloric acid (10 %) and recrystallised from ethanol.

*Synthesis of 3-acetyl-2-(substituted phenyl)-5-(4-methylcoumarinyl-7-oxymethyl)--2,3-dihydro-1,3,4-oxadiazoles (**9a-c**)*

A mixture of compounds **6a-c** (0.01 mol) and acetic anhydride (5 mL) was refluxed for 2 h. The mixture was cooled, poured onto crushed ice and allowed to stand at room temperature overnight. The separated solid was washed with water, dried and recrystallised from acetone-ethanol (1:1).

In vitro *cytotoxic activity towards DLA cells and EAC cells*

The synthesised compounds **3a-c**, **5a-c**, **7a-c**, **8a-c** and **9a-c** were tested for their cytotoxicity *in vitro*, in comparison with 5-fluorouracil as reference drug, against DLA cells and EAC cells. Dalton's lymphoma ascites (DLA) and Ehrlich ascite carcinoma (EAC) cells were procured from Adayar Cancer Institute, Chennai, India.

DLA and EAC cells (1×10^6) were incubated with synthesised compounds at concentrations of 50 μg mL^{-1} and 100 μg mL^{-1}, respectively, in 1 mL phosphate buffered saline (incorporated with 10 μL DMSO) at 37 °C for 3 h. Viable cells were counted in a haemocytometer using the trypanblue exclusion method (15). Experiments were carried out in triplicate. Results are given in Table III.

Antioxidant activity

Free radical scavenging activity of the test compounds **3a-c, 5a-c, 7a-c, 8a-c** and **9a-c** was studied by the diphenylpicryl hydrazyl (DPPH) assay method (3–6). Methanolic solution of the synthesised compounds (1.5 mL, 0.2 mmol L^{-1}) was added to 1.5 mL (0.2 mmol L^{-1}) solution of DPPH radical in methanol (final concentration of DPPH and synthesized compounds was 0.1 mmol L^{-1}). The mixture was shaken vigorously allowed to stand for 30 min, absorbance at 517 nm was determined and the percentage of scavenging activity was calculated.

Table III. Cytotoxic activity of synthesised compounds

Compd.	Cytotoxicity (%)[a]	
	DLA cells[b]	EAC cells[c]
3a	83.0	11.3
3b	48.9	77.9
3c	89.1	29.8
5a	59.1	12.1
5b	90.8	97.5
5c	34.9	90.2
7a	68.3	14.9
7b	62.8	20
7c	48.4	9.1
8a	38.1	66.8
8b	42	97.3
8c	57.9	43.2
9a	49.6	70.1
9b	36.5	50.6
9c	63.9	46.1
Negative control	–	–
5-Fluorouracil	98.8	99.5

[a] Results are the mean of three experiments.
[b] Compounds were tested at 100 μg mL^{-1} for cytotoxicity against DLA cells.
[c] Compounds were tested at 50 μg mL^{-1} for cytotoxicity against EAC cells.

Ascorbic acid was used as the reference compound. All tests and analyses were done in three replicates and the results were averaged. Results are presented in Table IV.

RESULTS AND DISCUSSION

Chemistry

Formation of compounds **3a-**c and **5a-c** was confirmed by the presence of characteristic ring C=N stretching at ν between 1606–1623 cm^{-1} in the IR spectrum. Compound **3a**, which was representative of pyrazoles, showed *m/z* 431.6 $(M+1)^+$ and compound **5a**, which represented pyrazolones, showed *m/z* 431.6 (M^+). Similarly, elemental analysis data together with ^1H NMR data supported the proposed structure for compounds **3a-c** and **5a-c** given in Tables I and II.

Formation of 4-thiazolidinones (**7a-c**) and 5-carboxymethyl-4-thiazolidinones (**8a-c**) was confirmed by IR spectra, which showed ring C=O stretching characteristic of thiazolidinone ring in the range of ν 1709–1737 cm^{-1}. ^1H NMR for **7a-c** showed CH_2 protons of the thiazolidinone ring between δ 3.35–3.41 ppm as the singlet signal and δ 5.28–5.31

Table IV. Antioxidant activity of synthesised compounds

Compd. No.	Scavenging activity (%)[a]
3a	53
3b	83
3c	31
5a	62
5b	29
5c	–
7a	–
7b	–
7c	–
8a	36
8b	95
8c	23
9a	60
9b	59
9c	39
Negative control	–
Ascorbic acid	96

[a] Results are mean of three different experiments.
– Denotes very low antioxidant activity (scavenging activity < 10 %).

ppm for CH proton of the thiazolidinone ring as a singlet signal. Compounds **7a** and **7c** representative of thiazolidine-4-ones showed $(M+1)^+$ peaks at *m/z* of 425.3 and 429.2, respectively, in mass spectra. ^{1}H NMR for 5-carboxymethyl-4-thiazolidinones (**8a-c**) showed a singlet in the spectra in the range δ 5.30–5.31 ppm, indicating the presence of CH-N of thiazolidinones. M^+ peak and $(M+1)^+$ peak at 483.2 and 487.3 were obtained in the mass spectra of representative compounds **8a** and **8c**, respectively.

IR spectra of compounds **9a-c** had different characteristics since they showed no N-H stretching bands, but C-O stretching in the 1054–1078 cm^{-1} region, which could be attributed to C-O stretching of oxadiazole nucleus. ^{1}H NMR spectra of **9a,b,c** showed a singlet in the spectra in the range δ 7.10, 7.04, 7.09 ppm indicating CH resonance of the oxadiazoline ring in accord with the literature (10). The mass spectrum of **9c** showed the molecular ion peak at *m/z* 397.1 $(M+1)^+$.

Cytotoxicity studies

5-Fluorouracil, which was used as standard cytotoxic agent, exhibited cytotoxicity of 98.8 % against DLA cells at 100 μg mL^{-1} and 99.5 % against EAC cells at a 50 μg mL^{-1} concentration. The results of short term *in vitro* cytotoxicity studies against DLA cells showed that compounds **3a**, **3c**, **5a**, **5b**, **7a**, **7b**, **8c** and **9c** exhibited more than 50 % cytotoxicity at a 100 μg mL^{-1} concentration. Compound **5b** showed the highest cytotoxicity

of 90.8 %. The results of short term *in vitro* cytotoxicity studies against EAC cells showed that compounds **3b**, **5b**, **5c**, **8a**, **8b**, **9a** and **9b** exhibited more than 50 % cytotoxicity at a concentration of 50 μg mL^{-1}. Compounds **5b**, **5c** and **8b** showed the highest percentage of cytotoxicity of 97.5, 90.2 and 97.3 %, respectively.

Antioxidant activity

Concentration of the test compounds and ascorbic acid were of 0.1 mmol L^{-1}. Among the pyrazoles, compounds **3a** and **3b** with *p*-methyl phenyl substituent and unsubstituted phenyl substituent, respectively, showed more than 50 % antioxidant activity. It was interesting to note that thiazolidine-4-ones showed negligible antioxidant activity of less than 10 % free radical scavenging capacity. Among 5-carboxymethyl-4-thiazolidinones, compound **8b** with unsubstituted phenyl substituent showed more than 95 % antioxidant activity which was comparable to that of the standard ascorbic acid (96 %). 3-Acetyloxadiazoles **9a** and **9b** showed more than 50 % antioxidant activity. Antioxidant activity of pyrazoles and 5-carboxymethyl-4-thiazolidinones followed the following order: unsubstituted phenyl derivative > *p*-methyl phenyl derivative > *p*-fluorophenyl derivative. Pyrazolin-5-ones and 3-acetyloxadiazole showed antioxidant activity in the descending order: *p*-methyl phenyl derivative > unsubstituted phenyl derivative > *p*-fluorophenyl derivative. Irrespective of the type of heterocyclic nucleus, it was observed that *p*-fluorophenyl derivatives exhibited the lowest antioxidant activity among compounds in the respective series.

Structure activity relation

(i) Coumarin derivatives with different heterocyclic nuclei having unsubstituted phenyl group exhibited cytotoxicity against DLA cells in the descending order of potency: pyrazolin-5-one derivative > thiazolidin-4-one derivative > pyrazole derivative > 5-carboxymethyl-4-thiazolidinone derivative > 1,3,4-oxadiazole derivative, as it is evident from the percentage cytotoxicity of **5b, 7b, 3b, 8b** and **9b**, respectively (Table III). The presence of *p*-fluorophenyl substituent increased the cytotoxicity of 5-carboxymethyl--4-thiazolidinone derivative, 1,3,4-oxadiazole derivative and azopyrazoles against DLA cells compared to the compounds having unsubstituted phenyl ring or *p*-methylphenyl substituent in the respective series. The presence of *p*-fluorophenyl substituent decreased the cytotoxicity of hydrazonopyrazolin-5-ones and thiazolidin-4-one derivatives against DLA cells compared to those having unsubstituted phenyl ring or *p*-methyl phenyl substituent in the respective series.

(ii) Coumarin derivatives with different heterocyclic nuclei having unsubstituted phenyl group exhibited cytotoxicity against EAC cells in the descending order of potency: pyrazolin-5-one derivative > 5-carboxymethyl-4-thiazolidinone derivative > azo pyrazole derivative > 1,3,4-oxadiazole derivative > thiazolidin-4-one derivative, as seen from the cytotoxicity of **5b, 8b, 3b, 9b** and **7b**, respectively (Table III). The presence of *p*-fluorophenyl substituent increased the cytotoxicity of azopyrazoles and hydrazonopyrazolin--5-ones against EAC cells compared to the compounds having *p*-methylphenyl substituent in the respective series. The presence of *p*-fluorophenyl substituent decreased the cytotoxicity of thiazolidin-4-one derivative, 5-carboxymethyl-4-thiazolidinone derivative and

1,3,4-oxadiazole derivative against EAC cells compared to those having *p*-methylphenyl substituent in the respective series.

(iii) Coumarin derivatives with different heterocyclic nuclei having unsubstituted phenyl group exhibited antioxidant activity in the descending: 5-carboxymethyl-4-thiazolidinone derivative > pyrazole derivative > 1,3,4-oxadiazole derivative > pyrazolin-5-one derivative > thiazolidin-4-one derivative (Table IV). Substitution of unsubstituted phenyl group at the fourth position of 5-carboxymethyl-4-thiazolidinone derivative (**8b**) and unsubstituted phenylazo substituent at the third position of pyrazolone derivative (**3b**) imparted higher antioxidant activity than substitution with *p*-methylphenyl or *p*-fluorophenyl group. Substitution of *p*-fluoro substituent on phenyl ring produced compounds with lower antioxidant activity than the remaining compounds in the respective series, as seen for compounds **3c, 5c, 7c, 8c** and **9c.**

CONCLUSIONS

Results of antioxidant activity show that compound **8b** (0.1 mmol L^{-1}) (phenyl derivative of 5-carboxymethyl-4-thiazolidinone) exhibited the highest free radical scavenging activity (95 %), which was comparable to that of standard ascorbic acid (0.1 mmol L^{-1}) (96 %). Cytotoxicity studies against tumour cells showed compound **5b** (phenyl derivative of pyrazolin-5-one) to be a good cytotoxic agent against DLA cells at a 100 μg mL^{-1} concentration and compounds **5b** and **8b** (phenyl derivative of 5-carboxymethyl--4-thiazolidinones) to be potent cytotoxic agents against EAC cells at a 50 μg mL^{-1} concentration. Further studies aimed at development of an effective antioxidant can insolve compound **8b**, and compounds **5b** and **8b** can be subjected to further *in vivo* anticancer studies. Since antioxidants have a valuable role in the prophylaxis of cancer, it could be concluded that compound **8b** can be selected as the lead moiety in the analogue designing process of developing an ideal antineoplastic agent.

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S A Ž E T A K

Sinteza kumarinskih heterocikličkih derivata s antioksidativnim djelovanjem i *in vitro* citotoksično djelovanje na tumorske stanice

PARAMESWARAN MANOJKUMAR, THENGUNGAL KOCHUPAPPY RAVI i GOPALAKRISHNAN SUBBUCHETTIAR

Cilj rada bio je sintetizirati kumarinske heterocikličke derivate i razjasniti njihovu potencijalnu ulogu kao antioksidativnih i citotoksičnih agenasa na tumorske stanice Daltonovog limfoma (DLA) i Ehrlichove tumorske stanice (EAC). U radu je opisana sinteza kumarinskih derivata s pirazolskim, pirazolonskim, tiazolidin-4-onskim, 5-karboksimetil-4-tiazolidinonskim i 3-acetil-1,3,4-oksadiazolskim prstenom. Hidrazid 4-metilkumarinil-7-oksioctene kiseline (**1**) dao je u reakciji s derivatima arilazopropana ili hidrazono-3-oksobutirata derivate pirazola (**3a-c**) i pirazolona (**5a-c**). Heterociklizacijom Schiffovih baza **1** s tioglikolnom kiselinom, tiojabučnom kiselinom ili anhidridom octene kiseline nastali su heterociklički derivati 4-tiazolidinoni (**7a-c**), 5-karboksimetil-4-tiazolidinoni (**8a-c**) i oksadiazoli (**9a-c**). Neki od spojeva pokazali su obećavajuće rezultate u *in vitro* testovima za antioksidativno i citostatsko djelovanje na DLA i EAC stanicama.

Ključne riječi: pirazol, pirazolon, tiazolidin-4-on, oksadiazol, antioksidativno djelovanje

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