

Synthesis of Novel Thiazolidin-4-one Derivatives of 7-Hydroxy-4-methyl Coumarinyl Acetic Hydrazide and its Activity on Antioxidant and *In Vitro* Cytotoxic against DLA Cells

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Abstract

The main aim of the present work was to synthesis a series of novel thiazolidin-4-one derivatives of 7-hydroxy-4-methyl coumarinyl acetic hydrazide. The synthesis majorly involves six steps, where the synthesis of coumarin molecules with thiazolidin-4-one was reported with its activity study. Here ten novel derivatives of compounds were synthesized and evaluated for their antioxidant and *in vitro* cytotoxicity against DLA cells. Some of the compounds showed promising activity for the antioxidant and *in vitro* cytotoxic activity against DLA and thus these compounds represent a new class of promising lead compounds.

Keywords: Thiazolidin-4-one; Coumarin; Antioxidant activity; *In vitro* cytotoxicity study

Introduction

Coumarin are naturally occurring compounds present in many members of plant kingdom, containing lactone ring having 1 benzopyran-2-one ring system and possess a wide variety of biological activity [1,2]. Similarly thiazolidinone are saturated form of thiazole, that have an atom of sulfur at position 1, an atom of nitrogen at position 3 and a carbonyl group at 2, 4 or 5 [3]. Also it is well documented that coumarin with pyrazolin-5-ones, 4-thiazolidinones and 1,3,4-oxadiazoles display pronounced antioxidant and antineoplastic activity [4,5]. Keeping in view of the biological importance of coumarin derivatives and thiazolidin-4 one derivatives, novel hybrid molecules of thiazolidin-4-one derivatives incorporated with coumarin nucleus and having various arylidene substituents at 5th position of thiazolidin-4-one nucleus were designed. Then the designed hybrid molecules were synthesized and were screened for their antioxidant and *in vitro* cytotoxicity studies against DLA cells.

Experimental

Melting point was determined in open capillary tube and was uncorrected. IR spectra were recorded on Shimadzu FT-IR at Chemistry Lab, College of Women, Trivandrum 695036, Mass spectra were recorded on Brukerultraflexxtreme MALDI system at Rajiv Gandhi Centre for Biotechnology, Trivandrum and ¹H NMR spectra was recorded on Bruker Ultra shield DPX 400 at Sophisticated Test and Instrumentation Centre, Cochin.

The purity of all compounds was established by single spot on the TLC plates. The solvent system used was methanol: toluene (7:3).

The starting material 4-methylcoumarinyl-7-oxyacetic acid hydrazide was prepared according to the literature procedure [6-8]. Synthesis of 4-methylcoumarinyl-7-oxyacetic acid hydrazide 1(a-d) were prepared according to procedure [7,8]. 2-(substituted phenyl)-3-(-4-methylcoumarinyl-7-oxyacetamido)-4-thiazolidin-4-one 2(a-d) were prepared [8,9]. Synthesis of 5-(arylmethylidene)-2-(substituted phenyl)-3-(-4-methylcoumarinyl-7-oxyacetamido)-4-thiazolidin-4-one 3(a-j) were according to procedure [10-12] (Scheme 1).

Synthesis of 4-methylcoumarinyl-7-oxyacetic acid hydrazide 1(a-d)

mixture of 4-methylcoumarinyl-7-oxyacetic hydrazide (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 poured into crushed ice. The resultant solid was filtered and recrystallized using glacial acetic acid [8,9].

Synthesis of 2-(substituted phenyl)-3-(-4-methylcoumarinyl-7-oxyacetamido)-4-thiazolidin-4-one 2(a-d)

A homogenous mixture of 4-methylcoumarinyl-7-oxyacetic acid [(substituted phenyl)methylene] hydrazide 1(a-d) (0.01 mol) and thioglycolic 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 10 per cent sodium bicarbonate solution. The neutral solid that resulted was pored into crushed ice. The separated product was filtered off, washed with water, dried and recrystallized from ethanol [8,9].

Synthesis of 5-(arylmethylidene)-2-(substituted phenyl)-3-(-4-methylcoumarinyl-7-oxyacetamido)-4-thiazolidin-4-one 3(a-j)

A mixture of 2-(substituted phenyl)-3-(-4-methylcoumarinyl-7-oxyacetamido)-4-thiazolidin-4-one 2(a-d) (2g, 0.01 mol) and substituted benzaldehyde (0.01 mol) were added to a solution of anhydrous sodium acetate (2 g, 0.02 mol) in acetic acid (30 ml) and refluxed for 5 h at 120°C and cooled to room temperature. The solid product was filtered from the mixture, washed with water, dried and crystalized from acetic acid [10,12].

The purity of all compounds was established by single spot on the TLC plates. The solvent system used was methanol: toluene (7:3).

The newly synthesized compounds were characterized [13] on the basis of physical data (Table 1).

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Antioxidant Activity Studies

The compounds synthesized will be screened for antioxidant activity by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical method and the activities will be compared with that of ascorbic acid which will be used as the standard.

1.5 ml methanolic solution of the synthesized compounds (0.2 mM) was added to 1.5 ml (0.2 mM) solution of DPPH radical in methanol (final concentration of DPPH and synthesized compounds was 0.1 mM). The mixture was shaken vigorously and allowed to stand for 30 min. After this the absorbance at 515 nm was determined and

the percentage of scavenging activity was calculated using the formula shown below. Ascorbic acid was used as the reference compound. All tests and analyses were undertaken on two duplicate and the results were averaged [14].

$$\text{Scavenging activity (\%)} = \left\{ \frac{(Ab+As)-Am}{Ab} \right\} \times 100\%$$

Ab: Absorbance of 0.1 mM methanolic solution of DPPH at 515 nm; As: Absorbance of 0.1 mM methanolic solution of test compound at 515 nm; Am: Absorbance of methanolic mixture of the drug and DPPH at 515 nm.

The antioxidant activity of the newly synthesized compounds is given in Table 2.

In Vitro Cytotoxicity Studies by Trypan Blue Exclusion Method

The synthesized compounds 3(a-j) were tested for their cytotoxicity *in vitro*, in comparison with cyclophosphamide as reference drug, against DLA cells. Dalton's lymphoma ascites (DLA) cells were procured from Adayar Cancer Institute, Chennai, India.

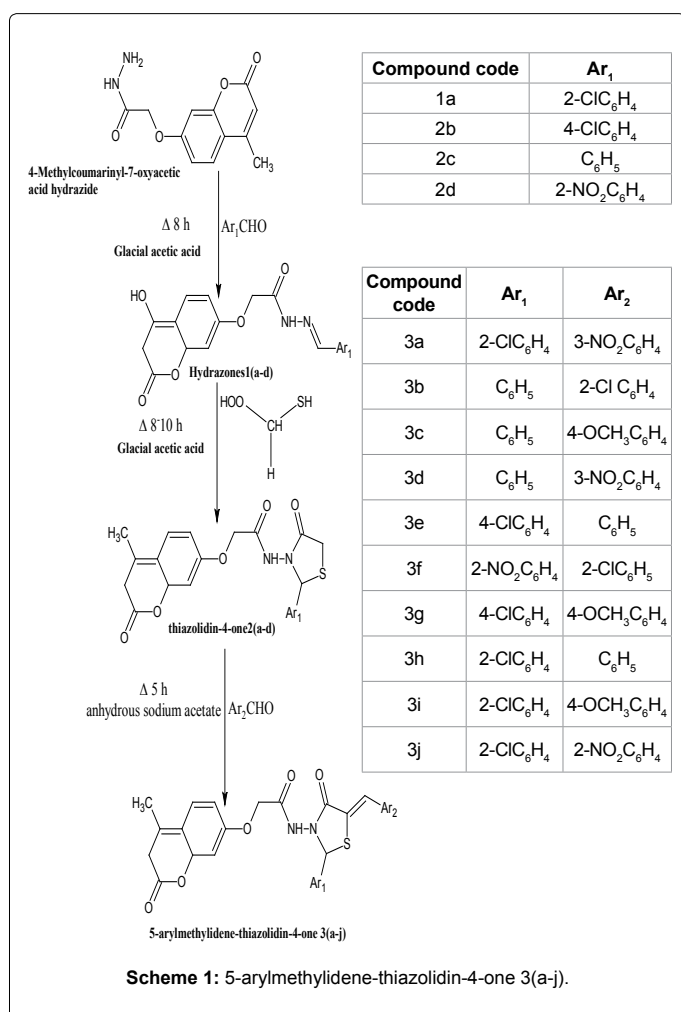
Tumour cells were aspirated from the peritoneal cavity of mice and added to test tube containing PBS. The cells were washed with PBS and centrifuged three times. The cells were diluted with PBS and the viability was checked using trypan blue stain. The cells were counted in haemocytometer. The cell count should be '100' in the four large sized quadrants. If the cell count is above or below 100, it is made to 100 by adding extra cells or diluting with PBS as required. If the cell count is 100, then number of cells in the diluted sample is 1 million/ml. The synthesized compounds were dissolved in dimethylsulphoxide to get concentration of 20 mg/ml. After this, experiment was set up by incubating 10 µl of sample solution with 0.1 ml of diluted DLA cells. In each case, volume of the mixture was made up to 900 µl using PBS and incubated at 37°C for 3 h. After incubation, 0.1 ml of trypan blue was added and the number of dead cells was determined using haemocytometer. Cyclophosphamide was used as the standard compound [9,15]. The percentage cytotoxicity was calculated by using the under mentioned formula:

$$\% \text{Cytotoxicity} = \left[\frac{\text{No. of dead cells}}{\text{No. of viable cells} + \text{Dead cells}} \right] \times 100$$

The *in vitro* cytotoxicity studies of the newly synthesized compounds are given in Table 3.

Results and Discussion

The IR spectrum of compound 3a showed absorption band at 3375 cm⁻¹ due to NH stretch. The absorption band for thiazolidinone C=O stretch was observed at 1637 cm⁻¹. The other prominent absorption



Compound	Ar ₁	Ar ₂	Molecular formula	Molecular weight	Yield (%)	Melting point (°C)	Rf value
3a	o-ClC ₆ H ₄	m-NO ₂ C ₆ H ₄	C ₂₈ H ₂₀ ClN ₃ O ₇ S	577.99	67	280-330	0.602
3b	C ₆ H ₅	o-ClC ₆ H ₄	C ₂₉ H ₂₁ ClN ₂ O ₆ S	532.99	56	200-210	0.541
3c	C ₆ H ₅	p-OCH ₃ C ₆ H ₄	C ₂₉ H ₂₄ N ₂ O ₆ S	528.57	63	280-340	0.633
3d	C ₆ H ₅	m-NO ₂ C ₆ H ₄	C ₂₈ H ₂₁ ClN ₂ O ₅ S	543.54	70	220-260	0.604
3e	p-ClC ₆ H ₄	C ₆ H ₅	C ₂₈ H ₂₁ ClN ₂ O ₅ S	532.99	54	200-250	0.571
3f	o-NO ₂ C ₆ H ₄	o-ClC ₆ H ₄	C ₂₉ H ₂₃ ClN ₂ O ₆ S	577.99	59	190-230	0.664
3g	p-ClC ₆ H ₄	p-OCH ₃ C ₆ H ₄	C ₂₈ H ₂₁ ClN ₂ O ₅ S	563.02	62	210-240	0.695
3h	o-ClC ₆ H ₄	C ₆ H ₅	C ₂₈ H ₂₁ ClN ₂ O ₆ S	532.99	57	170-220	0.553
3i	o-ClC ₆ H ₄	p-OCH ₃ C ₆ H ₄	C ₂₉ H ₂₃ ClN ₂ O ₆ S	563.02	62	200-230	0.658
3j	o-ClC ₆ H ₄	o-NO ₂ C ₆ H ₄	C ₂₈ H ₂₀ ClN ₃ O ₇ S	577.99	71	190-240	0.609

Table 1: Physical data of 5-arylmethylidene thiazolidin-4-one 3 (a-j).

Compound code	Absorbance			Percentage scavenging activity (0.1 mM)
	Ab	As	Am	
3a	1.11	0.179	0.6495	58
3b		0.000	0.474	57
3c		0.066	0.066	61
3d		0.055	0.055	46
3e		0.064	0.503	63
3f		0.079	0.453	66
3g		0.063	0.484	62
3h		0.000	0.375	66
3i		0.119	0.455	70
3j		0.007	0.007	60
Ascorbic acid		0.000	0.056	95

Table 2: DPPH Radical scavenging activity 3(a-j).

Compound code	Percentage cytotoxicity at 200 µg/ml
3a	45
3b	40
3c	40
3d	70
3e	65
3f	32
3g	31
3h	36
3i	52
3j	27
Cyclophosphamide	45

Table 3: Cytotoxicity of newly synthesized compounds against DLA cells 3 (a-j).

bands in IR spectrum were observed at 1562 cm⁻¹ (C=C stretch) 1348 cm⁻¹ (symmetric NO₂ stretch) and 1271 cm⁻¹ (coumarinyl C-O-C stretch).

The ¹H NMR Spectrum of 3a showed a triplet at 1.639 corresponding CH₃ protons of coumarin. The two OCH₂ protons are resonated at value of 2.410. The thiazolidinone CH groups show singlet peak at value 6.221. Aromatic protons and alkenic protons resonated as multiple at 6.98-8.725.

Further evidence for the formation of 5-arylidene derivatives of thiazolidin-4-one was obtained by recording its mass spectrum. The mass spectrum of the compound 3a showed molecular ion peak at m/z 579.367(M+1)⁺ in conformity with the molecular formula C₂₈H₂₀ClN₃O₇S.

The IR spectrum of compound 3b showed absorption band at 3377 cm⁻¹ due to NH stretch. The absorption band for thiazolidinone C=O stretch was observed at 1714 cm⁻¹. The other prominent absorption bands in IR spectrum were observed at 1554 cm⁻¹ (C=C stretch) 1087 cm⁻¹ (C-Cl stretch) and 1274 cm⁻¹ (coumarinyl C-O-C stretch).

The ¹H NMR Spectrum of 3b showed a triplet at 2.407 corresponding CH₃ protons of coumarin. The two OCH₂ protons are resonated at value of 4.847. The thiazolidinone CH groups show singlet peak at value 5.322. Aromatic protons and alkenic protons resonated as multiple at 6.990-8.745.

Further evidence for the formation of 5-arylidene derivatives of thiazolidin-4-one was obtained by recording its mass spectrum. The mass spectrum of the compound 3b showed molecular ion peak at m/z 534.105 (M+1)⁺ in conformity with the molecular formula C₂₈H₂₁ClN₃O₇S [16].

Antioxidant Activity Studies

The antioxidant activity of the newly synthesized compounds was evaluated by their DPPH radical scavenging activity at 0.1 mM concentration. Among the ten newly synthesized compounds 3(a-j) screened for DPPH free radical scavenging activity, compound 3i (p-methoxyphenylmethylidene substituent at 5th position and o-chloro phenyl at 2nd position) exhibited the highest free radical scavenging capacity of 70% with the series of newly synthesized compounds. Compounds 3b (o-chlorophenyl methylidene substituent at 5th position and phenyl substituent at 2nd position), 3a (m-nitrophenylidene substituent at 5th position and phenyl substituent at 2nd position), 3c (p-methoxy phenylmethylidene substituent at 5th position and phenyl substituent at 2nd position), 3g (p-methoxyphenylmethylidene substituent at 5th position and m-chlorophenyl substituent at 2nd position), 3e (phenylmethylidene substituent at 5th position and p-chlorophenyl substituent at 2nd position), 3j (o-nitrophenylmethylidene substituent at 5th position and p-chlorophenyl substituent at 2nd position), 3f (o-chlorophenylmethylidene substituent at 5th position and o-nitrophenyl substituent at 2nd position) and 3h (phenylmethylidene substituent at 5th position and o-chlorophenyl substituent at 2nd position) exhibiting moderate free radical scavenging activity ranging from 57-66%. Compound 3d (m-nitrophenylmethylidene substituent at 5th position and phenyl at 2nd position) showed the lowest free radical scavenging capacity of 46%. Ascorbic acid which was used as standard antioxidant in the present study showed the highest free radical scavenging activity of 95% at 0.1 mM concentration.

In Vitro Cytotoxicity Studies

Cytotoxicity studies against Dalton's Lymphoma Ascites (DLA) tumour cells were carried out with all the newly synthesized compounds 3(a-j). The results of the short term invitro cytotoxicity studies showed the compounds 3d (m-nitrophenylmethylidene substituent at 5th position and phenyl substituent at 2nd position), 3e (phenylmethylidene substituent at 5th position and o-chlorophenyl substituent at 2nd position) and compound 3i (o-methoxy phenylmethylidene substituent at 5th position and o-chlorophenyl substituent at 2nd position) exhibiting percentage of cytotoxicity of 70%, 65% and 52% respectively which was superior when compared with that of the standard cyclophosphamide which showed a percentage cytotoxicity of 45% at 200 µg/ml. Compound 3a (m-nitrophenylmethylidene substituent at 5th position and o-chlorophenyl substituent at 2nd position) showed a percentage of 45% which was comparable with the percentage cytotoxicity exhibited by standard cyclophosphamide at 200 µg/ml. Rest of the compounds showed percentage cytotoxicity ranging from 27%-40%.

Conclusion

The present work comprises the synthesis of ten novel hybrid molecules which are thiazolidin-4-one derivatives incorporated with coumarin nucleus and having various arylidene substituents at 5th position of thiazolidin-4-one nucleus.

After analysing the results following conclusions are made:

- Among the synthesized compounds 3i (p-methoxyphenylmethylidene substituent at 5th position and o-chlorophenyl at 2nd position) showed the highest antioxidant activity of 70%.
- Among the synthesized compounds 3d (m-nitrophenylmethylidene substituent at 5th position and phenyl at 2nd position), 3e (phenylmethylidene substituent at 5th

position and p-chlorophenyl at 2nd position) and 3i (p-methoxyphenylmethylidene substituent at 5th position and o-chlorophenyl at 2nd position) showed the percentage cytotoxicity of 70%, 65% and 52% respectively, which was more than that of the standard cyclophosphamide, that showed a percentage cytotoxicity of 45% against DLA cells.

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