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Original article

Novel 3-alkanoyl/aroyl/heteroaroyl-2*H*-chromene-2-thiones: Synthesis and evaluation of their antioxidant activities

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ABSTRACT

A facile, convenient and high yielding synthesis of a combinatorial library of 3-alkanoyl/aroyl/heteroaroyl-2*H*-chromene-2-thiones has been developed by the condensation of easily accessible β-oxodithioesters and salicylaldehyde/substituted 2-hydroxybenzaldehydes under solvent-free conditions. The assessment of radical scavenging capacity of the compounds towards the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured and these compounds were found to scavenge DPPH free radical efficiently. Five selected compounds were able to protect curcumin from the attack of sulfur free radical generated by radiolysis of glutathione (GSH). The newly synthesized compounds exhibited profound antioxidant activities. Five of them rendered comparatively high antioxidant capacity.

1. Introduction

Coumarins constitute an important class of oxygen heterocycles found abundantly in numerous naturally occurring products, including edible vegetables and fruits [1]. They are used as additives in food and cosmetics, optical brightening agents and dispersed fluorescent and laser dyes [2]. The coumarins display a remarkable array of biochemical and pharmacological actions, such as antifungal, antihypertensive, antioxidant, anti-inflammatory, and antimicrobial activity [3–8]. Some coumarins are also reported as chemotherapeutic agents [9–12].

There has been, in recent years, a major rekindling of interest in the studies on the defensive effects of coumarins as antioxidants [13,14]. Nishiyama et al. reported the stronger antioxidative activities of hydrocoumarins than α-tocopherol for the oxidation of tetralin and linoleic acid in a homogeneous solution [15].

It is therefore of utmost importance that the synthesis of coumarin and its derivatives should be achieved by a simple and effective method. The Pechmann reaction for the synthesis of coumarin involves the condensation of phenols with β-ketonic esters in the presence of variety of acidic condensing agents [16–19] and exchange resins [20]. Applications of microwaves [21] and ionic liquids [22] for the synthesis of coumarins have also been reported. Knoevenagel condensation is reported recently to proceed with high selectivity and reactivity in the formation of coumarins over solid base catalysts [23]. Microwave irradiation accelerates these reactions several-fold with better yields of the products [24].

However, to the best of our knowledge the new methodologies in all these reported methods use only the β-ketoesters such as ethyl acetoacetate or methyl acetoacetate and thus structural variations are limited to those derived from these esters. As a result, the structures of known coumarins either in improved yields or by the application of new techniques are always reported. Recently, β-oxodithioesters [25] instead of β-ketoesters have been applied in solvent-free multicomponent reactions for synthesis of dihydropyrimidines [26]. In conjunction with our works related with the synthesis and biological evaluation of heterocycles [26,27], we have investigated the cyclocondensation of β-oxodithioesters and 2-hydroxy benzaldehyde/substituted salicylaldehydes to yield the 3-alkanoyl/aroyl/heteroaroyl-2*H*-chromene-2-thiones **5a–o** in good to excellent yields.

Furthermore, scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured for all the newly prepared compounds and discussed herein. The antioxidant evaluation was supplemented by sulfur free radical reactivity assay using the technique of radiolysis of glutathione (GSH) and

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measuring the protective indices of inhibition of curcumin depletion.

2. Results and discussion

2.1. Chemistry

The syntheses of 3-alkanoyl/aroyl/heteroaroyl-2*H*-chromene-2-thiones **5** are summarized in Scheme 1.

β-Oxodithioesters **3a**–**g** are selected as the appropriate synthons of the present investigation. They are generally prepared by reacting any active methylene compound with CS_2 in the presence of a suitable base followed by alkylation with either dimethylsulfate or methyl iodide. However, this method gives poor yields and also mixtures of dithioesters and ketene dithioacetals. Thus improved yields of β-oxodithioesters are obtained by treating active methylene compounds **1a**–**g** with (*S*,*S*)-dimethyl trithiocarbonate. Subsequently, these intermediates were treated for Knoevenagel type condensations with ortho hydroxyl substituted benzaldehydes to afford the desired 3-alkanoyl/aroyl/heteroaroyl-2*H*-chromene-2-thiones **5** in good to excellent yields (Scheme 1).

Several catalytic and noncatalytic conditions were evaluated for this cyclocondensation reaction. We found the best yields of the desired coumarins by using piperidine as the base and no other organic solvent was used as a medium of the reaction.

Previously we have carried out the coumarin synthesis by heating β-oxodithioester **3a** (2.5 mmol) with 2-hydroxy benzaldehyde **4a** (2.5 mmol) at 90 °C for 45 min in the presence of piperidine (2.5 mmol). After completion of the reaction (monitored by TLC), water was added, and the product was extracted with ethyl acetate. After the organic layer was dried (Na_2SO_4) and evaporated, the residue was recrystallized from ethyl acetate and hexane to give the desired coumarin product **5a** as yellow crystals in 93% yield. The formation of compound **5a** was confirmed by the spectral and analytical data.

It is noteworthy to mention that using different solvents such as DMSO, DMF, THF and acetonitrile did not improve the yields and thus we have optimized the reaction condition at 90 °C for 2 h under solvent-free condition. After optimizing the reaction condition, the same process was successfully extended to different β-oxodithioesters (**3b**–**g**) and different substituted 2-hydroxybenzaldehydes to afford coumarin (**5b**–**o**) in good to excellent yields. The results are summarized in Table 1. The reaction proceeds smoothly in a short time and the methodology was found equally facile in all the aryl, alkyl and heteroaryl particularly, 4-pyridyl substituted β-oxodithioesters. The functional groups either electron withdrawing or electron donating groups at the 3- and 5-positions were introduced in this coumarin ring systems successfully.

2.2. Antioxidant assay

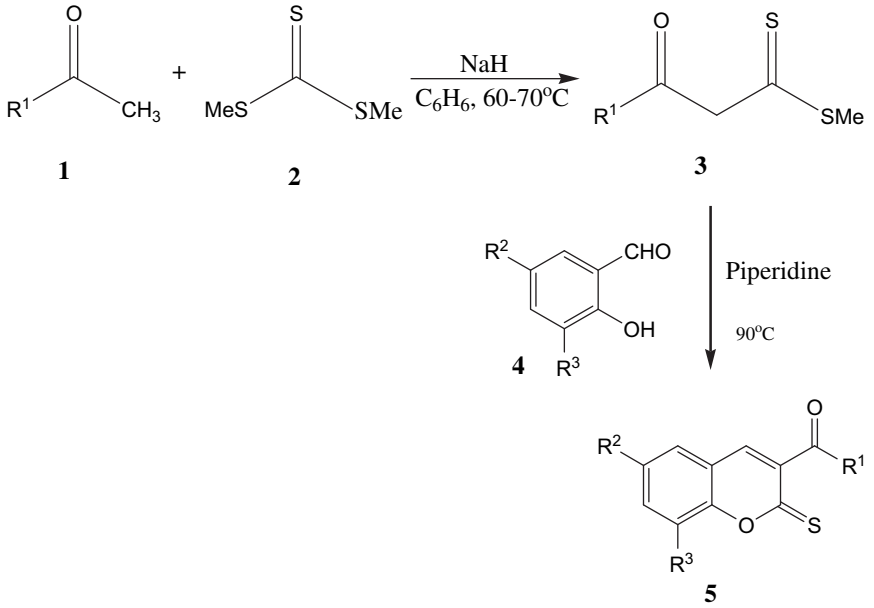
2.2.1. DPPH free radical scavenging assay

The use of stable free radical DPPH• in evaluating the free radical scavenging activity of extracts from plants, food materials and single compounds [28] is one of the most frequently used methods. The assay is based on the assessment of scavenging capacity of antioxidants towards the stable free radical DPPH• which is indicated by the bleaching of purple coloured methanol solution of DPPH• to the corresponding yellow coloured hydrazine [29] either by hydrogen atom- or electron donation [30].

The use of DPPH in antioxidant assays is well documented probably because of its rapid reaction and reliability. Initially, free radical scavenging activities of the newly prepared compounds **5a**–**o** were evaluated based on the DPPH decoloration range. Three compounds **5a**, **5f** and **5m** were omitted in the discussion part for clarity as their antioxidant activity from the initial measurements of these compounds using DPPH was found almost negligible (below 5%).

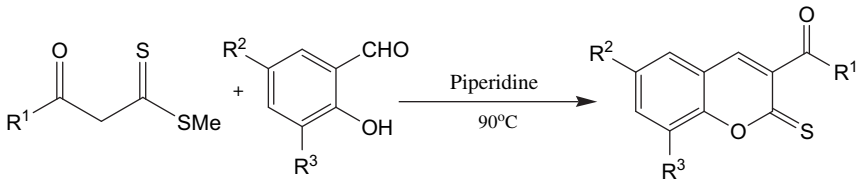
Thus the wide range of activities of the 12 tested compounds excluding **5a**, **5f** and **5m** were found from 6.41% to 92.81% at a concentration of 250 μg/mL. Compound **5l** was found to be the most potent antioxidant with the least values of IC_{50} (inhibition concentration) and the least active among the series being compound **5b** with the largest values of IC_{50} (Table 2 and Fig. 1).

The IC_{50} values were found to vary from 31.20 μg/mL to 1579.25 μg/mL showing a wide range of variations in the reactivity of the samples. Compound **5l** having the least IC_{50} value of 31.20 μg/mL and the highest corresponding value of AEAC (ascorbic acid equivalent antioxidant capacity), was followed by **5e**, **5i**, **5g** and **5j** in the



Scheme 1. Synthesis of 3-alkanoyl/aroyl/heteroaroyl-2*H*-chromene-2-thiones **5a**–**o**.

Table 1
Piperidine catalyzed preparation of coumarins under solvent-free conditions.[a]



3a-g **4a-c** **5a-o**

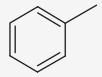
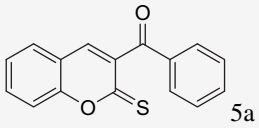
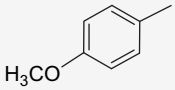
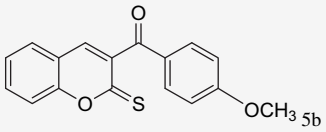
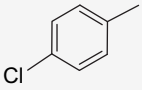
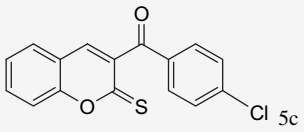
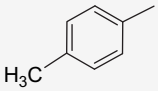
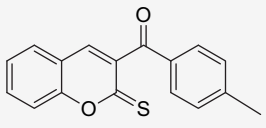
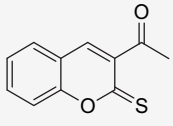
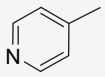
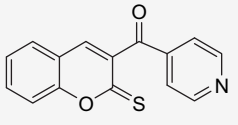
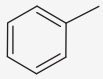
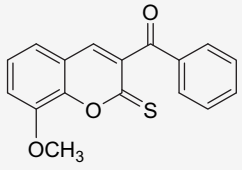
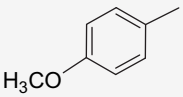
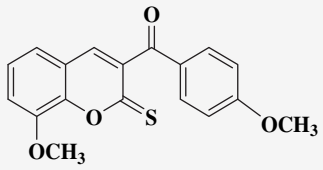
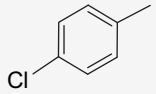
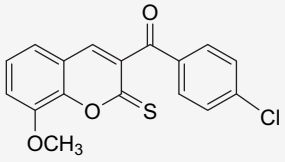
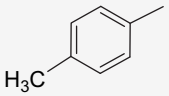
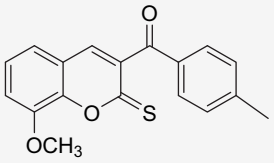
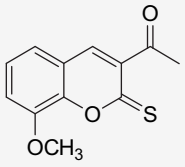
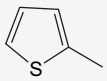
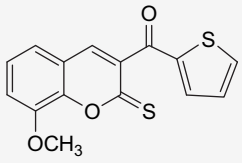
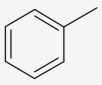
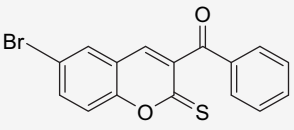
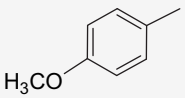
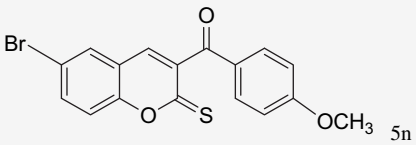
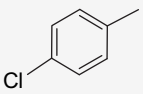
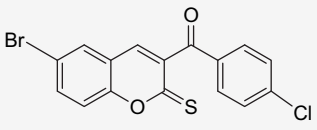
Entry	R^1	R^2	R^3	Product (**5**)	Yield[b] (%)
1.		H	H	 5a	93
2.		H	H	 5b	95
3.		H	H	 5c	90
4.		H	H	 5d	95
5.	CH_3	H	H	 5e	91
6.		H	H	 5f	85
7.		H	OCH_3	 5g	93
8.		H	OCH_3	 5h	91

Table 1 (*continued*)

Entry	R^1	R^2	R^3	Product (**5**)	Yield[b] (%)
9.		H	OCH_3	 5i	95
10.		H	OCH_3	 5j	92
11.	CH_3	H	OCH_3	 5k	89
12.		H	OCH_3	 5l	87
13.		Br	H	 5m	91
14.		Br	H	 5n	85
15.		Br	H	 5o	89

[a] Reaction conditions: **3** (2.5 mmol), **4** (2.5 mmol), piperidine (2.5 mmol), 90 °C, 1.0–2.0 h.
[b] Isolated yield.

activity test. Compound **5b** with the IC_{50} value of 1579.25 μg/mL showed the lowest DPPH• scavenging activity (Table 2). The wide variations in free radical scavenging activities may be due to the variations in the proton–electron transfer by the compounds due to difference in their structures and stability.

2.2.2. Sulfur free radical reactivity assay

Increase in the depletion of curcumin content with increase in dose of γ-radiation was observed in the control reaction solutions. Curcumin depletion by free radicals (GS•) in the reactions is given in Fig. 2. The reaction solutions supplemented with five compounds **5e**, **5g**, **5i**, **5j** and **5l**, which were selected through assessment of IC_{50} and AEAC values from the DPPH free radical scavenging assay exhibited substantial reduction in depletion of curcumin with variations. Reaction solutions supplemented with compounds **5l** and **5e** at a concentration of 10 μg/mL inhibited curcumin depletion by 2.41 μM and 3.27 μM respectively at a dose of 50 Gy. The samples showed varying protective indices (Table 3). Calculations were done using a concentration of 10 μg/mL for the five samples. A relative assessment of the PIs (protective indices) of the five tested

Table 2
Free radical scavenging capacities of coumarin derivatives measured in DPPH assay.

Sl. no.	Sample	Mean $IC_{50} \pm SE$ (μg/mL)
1	**5b**	1579.25 ± 0.64
2	**5c**	537.58 ± 0.79
3	**5d**	551.57 ± 0.80
4	**5e**	35.01 ± 0.56
5	**5g**	98.72 ± 0.97[a]
6	**5h**	603.43 ± 0.57
7	**5i**	95.57 ± 0.91[a]
8	**5j**	233.71 ± 0.70
9	**5k**	358.67 ± 0.76
10	**5l**	31.20 ± 0.40
11	**5n**	1112.83 ± 0.90
12	**5o**	850.41 ± 0.69

[a] Values of IC_{50} are expressed as means ± SE ($n = 3$). Values followed by same letters do not differ significantly at $P > 0.05$ as measured by Tukey HSD test.

compounds at 100 Gy revealed that the activity order was as **5l** > **5e** > **5i** > **5g** > **5j** (Fig. 3). However, the order of PIs changes with variation in radiation dose, and decrease with increasing radiation dose.

Aqueous solution of glutathione (GSH) generates sulfur free radicals (GS•) when irradiated with γ-radiation. The GS• free radical resulting from the irradiation of GSH oxidizes the chrome yellow compound curcumin present in the reaction solution and depletes its colour. Inhibition of curcumin depletion by substitution of 60% methanol solution of the selected compounds suggests that they possess certain entities which contribute to free radical scavenging activities and antioxidant properties. Substitution of the reaction solutions with the samples protect the curcumin molecules in the solution from getting depleted due to GS• free radicals even at a high dose of 250 Gy.

The PI values decrease as the dose increases as the curcumin molecules are not able to compete with the sulfur free radicals (GS•) produced after a certain threshold dose. More damages are caused to the curcumin molecules as the generation of free radicals increase with increasing dose. The compounds **5l** and **5e** showed comparatively good response in terms of DPPH stable radical scavenging and inhibition of curcumin depletion by GS• radicals as compared to the other samples (Table 3). The variation in PI, IC_{50} and AEAC values may be attributes of variation in molecular structures and nature of the compounds. Interestingly, the 2-acetyl thiophene substituted compound **5l** was found to be the most potent antioxidant in the series of the newly prepared compounds.

3. Conclusion

In conclusion, we have successfully demonstrated the synthesis and antioxidant evaluation of 3-alkanoyl/aroyl/heteroaroyl-2*H*-chromene-2-thiones. β-Oxodithioesters have been utilized as reactive intermediates to afford the desired coumarins by treating them with salicylaldehyde/substituted 2-hydroxybenzaldehydes in presence of piperidine under solvent-free conditions. The antioxidant activities of the 12 newly prepared compounds were measured using DPPH and we found them to be efficient DPPH free radical scavengers. Further, five selected compounds were found as protectors of curcumin from the attack of sulfur free radical generated by radiolysis of glutathione (GSH).

4. Experimental

4.1. General

Melting points are uncorrected and were determined in capillary tubes on an apparatus containing silicon oil. The IR spectra were recorded on a Perkin Elmer 983 spectrometer in KBr pellets with absorption given in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded respectively on a Varian EM-390 (300 MHz and 75.5 MHz) spectrometer. The chemical shifts (δ ppm) and the coupling constants (Hz) are reported in the standard fashion with reference to internal tetramethyl silane (TMS). The MS spectra were recorded on a Jeol JMSD-300 spectrometer. Elemental analyses were performed on a Carlo Erba's108 microanalyzer.

4.2. General procedure for the synthesis of β-oxodithioesters (*3a–g*) [25]

NaH (0.05 mol) was added slowly to a solution of the appropriate ketone (**1**; 0.025 mol) in dry benzene (50 mL) followed by

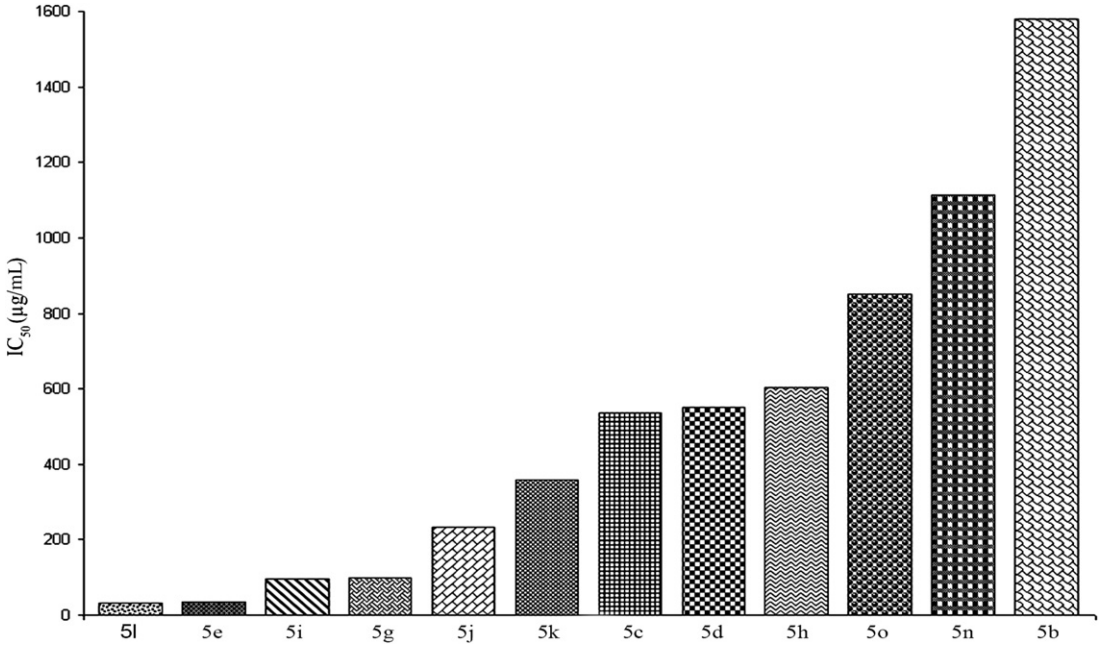


Fig. 1. Variations in IC_{50} of 12 selected compounds.

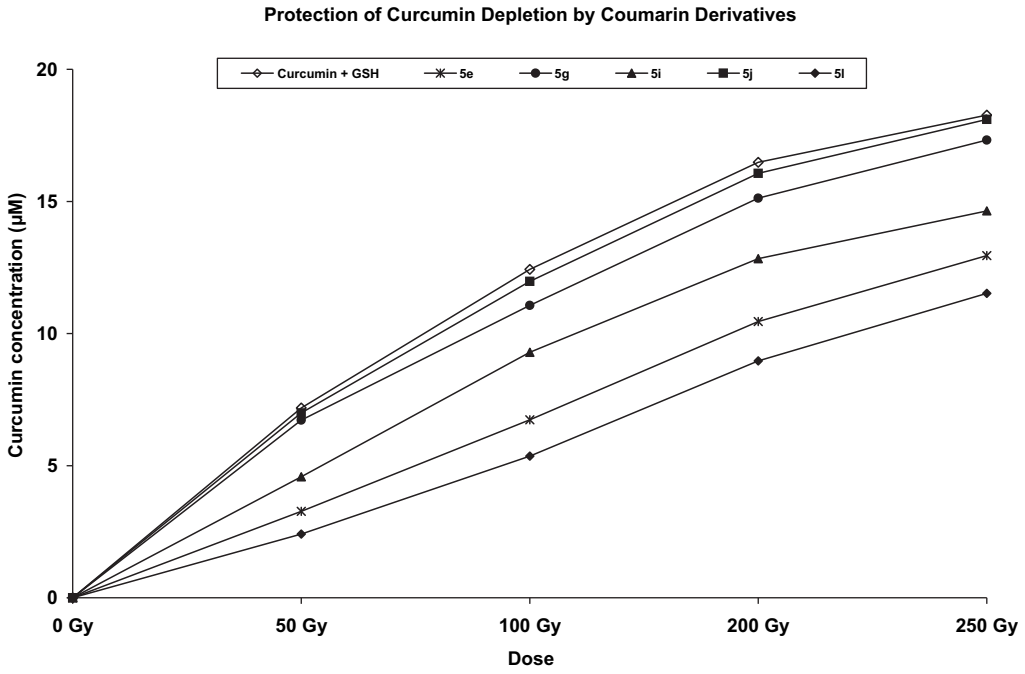


Fig. 2. Depletion of curcumin in control caused by sulfur free radical (GS˙) and its protection by 60% methanol solutions of the selected compounds.

the addition of dimethyl trithiocarbonate (**2**; 0.027 mol) dropwise over a period of 1–2 h. The mixture was stirred for 30 min at room temperature and then refluxed for 2 h. The mixture was allowed to cool, poured into ice-cold water (250 mL). The aqueous layer was separated, washed with benzene (200 mL), acidified with 3 N hydrochloric acid or 20% acetic acid, and extracted with chloroform (2×150 mL). The extract was dried with sodium sulfate and evaporated to give the products **3a**–**g**. Purification was done by column chromatography on silica gel using hexane as eluent. Spectral data of the unreported compound **3f** is given below:

Methyl-3-oxo-3-(pyridine-4-yl)propanedithioate (***3f***): Yellow crystals. m.p. 71–72 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.7 (s, 3H); 6.9 (s, $1H_{olefin}$); 7.9 (d, $J = 6$ Hz, 2H); 8.3 (d, $J = 6.3$ Hz, 2H); 15.0 (s, 1H, OH); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 22.5, 65.1, 119.9, 120.5, 145.8, 156.5, 191.9, 220.5; IR (KBr) (ν max, cm^{-1}): 1263, 1585; MS: $m/z = 211$ (M^+). Anal. Calcd. for $C_9H_9ONS_2$: C, 51.16; H, 4.29; S, 30.35. Found: C, 51.15; H, 4.27; S, 30.37.

4.3. General procedure for the synthesis of 2H-chromene-2-thiones (*5a*–*o*)

Salicyldehyde/substituted salicyldehyde (2.5 mmol), dithioester (2.5 mmol), and piperidine (10 mol %) were heated at 90 °C with stirring for 1–2 h. Then the progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, water was added, and the product was extracted with ethyl acetate. After the organic layer was dried (Na_2SO_4) and evaporated, the residue was recrystallized by ethyl acetate and hexane to products **5a**–**o**. In cases where further purification was required, the crude products were subjected to column chromatography on SiO_2, using increasing amounts of ethyl acetate in hexanes as eluent.

3-Benzoyl-2H-chromene-2-thione (***5a***). Yellow crystals. m.p. 170–171 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.37–7.42 (m, 1H), 7.45–7.56 (m, 3H), 7.59–7.66 (m, 3H), 7.68–7.71 (m, 1H), 7.94–7.96 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 116.7, 119.9, 125.9, 128.6, 128.7, 129.6, 133.2, 133.6, 133.9, 135.6, 139.1, 157, 192.3, 193.6; IR (KBr) (ν max, cm^{-1}): 1246, 1604, 1662, 3032, 3052; MS m/z 266 (M^+). Anal. Calcd. for $C_{16}H_{10}O_2S$: C, 72.16; H, 3.78; S, 12.04. Found: C, 72.10; H, 3.72; S, 11.84.

3-(4-Methoxybenzoyl)-2H-chromene-2-thione (***5b***). Yellow crystals. m.p. 187–189 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.85 (s, 3H), 6.93 (d, $J = 8.7$ Hz, 2H), 7.36–7.43 (m, 1H), 7.53–7.71 (m, 4H), 7.89 (d, $J = 8.7$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 55.5, 114, 116.7, 120, 125.9, 128.4, 128.6, 132.2, 133, 133.2, 139.5, 157, 164.3, 190.8, 193.8; IR (KBr) (ν max, cm^{-1}): 1242, 1597, 1654, 3018, 3055;

Table 3
Ascorbic acid equivalent antioxidant capacity (AEAC) and protective indices (PI) of the five effective coumarin derivatives.

Sl. No.	Samples	AEAC (mg/g)	PI (%) at 100 Gy
1	**5e**	110.78 ± 0.14	45.83 ± 0.03
2	**5g**	38.70 ± 0.38[a]	10.94 ± 0.19
3	**5i**	39.98 ± 0.37[a]	25.23 ± 0.04
4	**5j**	16.38 ± 0.30	3.65 ± 0.09
5	**5l**	121.01 ± 0.10	56.93 ± 0.02

[a] Values of AEAC and PI are expressed as means ± SE ($n = 3$). Values followed by same letters in the same column do not differ significantly at $P > 0.05$ as measured by Tukey HSD test.

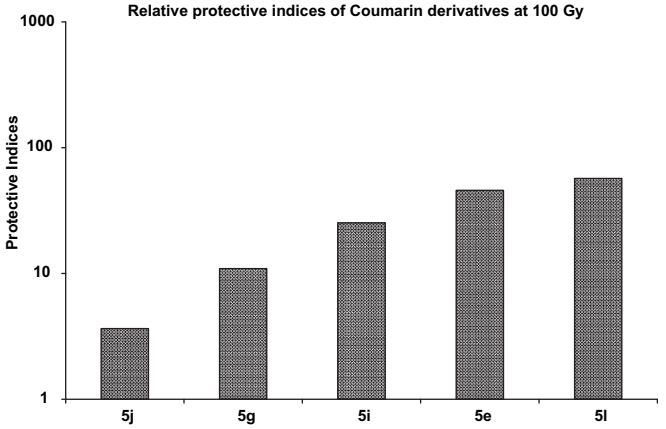


Fig. 3. Relative protective indices (PI) of best five effective compounds against GS˙ free radical induced curcumin depletion at 100 Gy.

MS: $m/z = 296$ (M^+). Anal. Calcd. for $C_{17}H_{12}O_3S$: C, 68.90; H, 4.08; S, 10.82. Found: C, 68.88; H, 3.98; S, 10.79.

*3-(4-Chlorobenzoyl)-2H-chromene-2-thione (**5c**)*. Yellow crystals. m.p. 185–186 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.31–7.35 (m, 1H), 7.42 (d, $J = 8.4$ Hz, 2H), 7.51 (m, 3H), 7.59–7.66 (m, 3H), 7.68–7.71 (m, 1H), 7.94–7.96 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 116.7, 119.9, 125.9, 128.7, 129.6, 133.2, 133.6, 133.9, 135.6, 139.1, 157, 192.3, 193.6; IR (KBr) (ν max, cm^{-1}): 1234, 1604, 1660, 3036, 3091; MS: $m/z = 300.5$ (M^+). Anal. Calcd. for $C_{16}H_{19}O_2SCl$: C, 63.90; H, 3.02; S, 10.66. Found: C, 63.88; H, 3.01; S, 10.59.

*3-(4-Methylbenzoyl)-2H-chromene-2-thione (**5d**)*. Yellow crystals. m.p. 172–173 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.43 (s, 3H), 7.26 (d, $J = 8.1$ Hz, 2H), 7.36–7.41 (m, 1H), 7.58–7.60 (m, 4H), 7.83 (d, $J = 8.4$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 21.8, 116.6, 119.9, 125.6, 125.8, 128.5, 129.5, 129.8, 133.1, 139.3, 145.1, 157, 191.9, 193.7; IR (KBr) (ν max, cm^{-1}): 1238, 1604, 1666, 3043, 3059; MS: $m/z = 280$ (M^+). Anal. Calcd. for $C_{17}H_{12}O_2S$: C, 72.83; H, 4.31; S, 11.44. Found: C, 72.78; H, 4.28; S, 11.40.

*3-Acetyl-2H-chromene-2-thione (**5e**)*. Yellow crystals. m.p. 178–179 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.43 (s, 3H), 7.36–7.41 (m, 2H), 7.58–7.60 (m, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 20.5, 113.9, 119.6, 128.4, 128.6, 129.8, 131.1, 134.5, 156.3, 191.7, 193.7; MS: $m/z = 204$ (M^+). Anal. Calcd. for $C_{11}H_8O_2S$: C, 64.69; H, 3.95; S, 15.70. Found: C, 64.78; H, 4.08; S, 15.70.

*3-(4-Acetylpyridine)-2H-chromene-2-thione (**5f**)*. Yellow crystals. m.p. 179–181 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.66–7.80 (m, 4H), 7.99 (d, $J = 6$ Hz, 1H), 8.18 (d, $J = 6.6$ Hz, 1H), 8.29–8.35 (m, 1H), 8.51 (s, 1H), 8.81–8.85 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 115.1, 116.5, 121.7, 122.0, 127.3, 129.3, 129.4, 130.7, 135.7, 142.3, 150.9, 192.2, 192.2; MS: $m/z = 267$ (M^+). Anal. Calcd. for $C_{15}H_9NO_2S$: C, 67.40; H, 3.39; N, 5.24; S, 12.00. Found: C, 67.37; H, 3.37; N, 5.29; S, 11.99.

*3-Benzoyl-8-methoxy-2H-chromene-2-thione (**5g**)*. Yellow crystals. m.p. 142–143 °C .^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 4.03 (s, 3H), 7.14–7.20 (m, 2H), 7.27–7.34 (m, 1H), 7.45–7.49 (m, 2H), 7.59–7.62 (m, 2H), 7.94 (d, $J = 5.7$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm) 56.3, 114.6, 119.6, 120.6, 125.8, 128.7, 129.6, 133.6, 133.9, 139.5, 146.8, 147.0, 192.4, 192.9; IR (KBr) (ν max, cm^{-1}): 1274, 1602, 1664, 3951; MS: $m/z = 298$ (M+). Anal. Calcd. for $C_{17}H_{14}O_3S$: C, 68.44; H, 4.73; S, 10.75. Found: C, 68.45; H, 4.77; S, 10.72.

*3(4-Methoxybenzoyl)-8-methoxy-2H-chromene-2-thione(**5h**)*. Yellow crystals. m.p. 140–142 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.80 (s, 3H), 3.92 (s, 3H), 6.85 (d, $J = 6.6$ Hz, 2H), 7.04–7.11 (m, 2H), 7.19–7.24 (m, 1H), 7.48 (s, 1H), 7.83 (d, $J = 6.6$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm) 55.6, 56.3, 114.1, 114.42, 119.5, 120.7, 125.8, 128.6, 132.2, 133.3, 139.8, 146.8, 146.9, 164.3, 190.9, 193.0; IR (KBr) (ν max, cm^{-1}): 1274, 1593, 1651, 3051; MS $m/z = 326$ (M^+). Anal. Calcd. for $C_{18}H_{14}O_4S$: C, 66.24; H, 4.32; S, 9.82. Found: C, 66.25; H, 4.31; S, 9.85.

*3(4-Chlorobenzoyl)-8-methoxy-2H-chromene-2-thione (**5i**)*. Yellow crystals. m.p. 175–176 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.95 (s, 3H), 7.07–7.13 (m, 2H), 7.19–7.26 (m, 1H), 7.37 (d, $J = 5.4$ Hz, 2H), 7.54 (s, 1H), 7.78 (d, $J = 6.3$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 56.3, 114.7, 119.7, 120.6, 125.9, 129.1, 130.9, 134.1, 134.1, 139.0, 140.3, 146.8, 147.1, 191.2, 192.7; MS $m/z = 330$ (M^+). Anal. Calcd. for $C_{17}H_{11}O_3SCl$: C, 61.73; H, 3.35; S, 9.69. Found: C, 61.75; H, 3.31; S, 9.65.

*3(4-Methylbenzoyl)-8-methoxy-2H-chromene-2-thione (**5j**)*. Yellow crystals. m.p. 163–165 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.43 (s, 3H), 4.03 (s, 3H), 7.13–7.32 (m, 5H), 7.57 (s, 1H), 7.84 (d, $J = 6.3$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 21.8, 56.3, 114.5, 119.5, 120.7, 125.7, 129.5, 129.8, 133.2, 133.4, 139.7, 145.1, 146.8, 192.0, 192.9; IR (KBr) (ν max, cm^{-1}): 1276, 1604, 1658, 3058; MS $m/z = 312$ (M^+). Anal. Calcd. for $C_{18}H_{16}O_3S$: C, 69.21; H, 5.16; S, 10.26. Found: C, 69.25; H, 5.13; S, 10.29.

*3-Acetyl-8-methoxy-2H-chromene-2-thione (**5k**)*. Yellow crystals. m.p. 145–146 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 2.66 (s, 3H), 3.93 (s, 3H), 7.07–7.23 (m, 3H), 7.66 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 30.0, 56.3, 114.9, 120.1, 120.5, 125.8, 135.4, 140.3, 146.6, 147.1, 192.6, 199.4; IR (KBr) (ν max, cm^{-1}) 1276, 1602, 1693, 3093; MS $m/z = 234$ (M^+). Anal. Calcd. for $C_{12}H_{10}O_3S$: C, 61.52; H, 4.30; S, 13.69. Found: C, 61.55; H, 4.31; S, 13.65.

*3(2-Acetylthiophene)-8-methoxy-2H-chromene-2-thione (**5l**)*. Yellow crystals. m.p. 165–167 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.83 (s, 3H), 6.74–6.79 (m, 2H), 7.06–7.09 (t, 1H), 7.25 (s, 1H), 7.40–7.42 (m, 1H), 7.63 (d, $J = 3.9$ Hz, 1H), 7.83 (d, $J = 2.7$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 56.1, 111.5, 119.7, 120.2, 121.2, 125.1, 128.1, 134.7, 135.5, 138.7, 143.3, 144.7, 146.4, 185.6, 192.8; MS $m/z = 302$ (M^+). Anal. Calcd. for $C_{15}H_{10}O_3S_2$: C, 59.58; H, 3.33; S, 21.21. Found: C, 59.55; H, 3.31; S, 21.18.

*3-Benzoyl-6-bromo-2H-chromene-2-thione (**5m**)*. Yellow crystals. m.p. 180–181 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.19 (s, 1H), 7.33–7.43 (m, 3H), 7.53–7.56 (m, 1H), 7.64–7.69 (m, 2H), 7.85 (d, $J = 5.4$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 118.3, 118.6, 121.4, 128.8, 129.6, 130.6, 131.7, 134.2, 135.4, 135.9, 140.1, 155.8, 191.8, 192.8; IR (KBr) (ν max, cm^{-1}): 1234, 1596, 1662, 3064; MS $m/z = 345$ (M^+). Anal. Calcd. for $C_{16}H_9O_2SBr$: C, 55.67; H, 2.63; S, 9.29. Found: C, 55.69; H, 2.61; S, 9.31.

*3-(4-Methoxybenzoyl)-6-bromo-2H-chromene-2-thione (**5n**)*. Yellow crystals. m.p. 208–209 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 3.8 (s, 3H), 6.87 (d, $J = 6.6$ Hz, 2H), 7.33 (d, $J = 6.6$ Hz, 1H), 7.40 (s, 1H), 7.26–7.67 (m, 2H), 7.83 (d, $J = 6.6$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 55.6, 114.2, 118.3, 118.5, 121.5, 128.3, 130.5, 131.3, 132.2, 135.7, 140.4, 155.7, 164.5, 190.3, 193.0; IR (KBr) (ν max, cm^{-1}): 1236, 1593, 1643, 3051; MS $m/z = 375$ (M^+). Anal. Calcd. for $C_{17}H_{11}O_3SBr$: C, 54.41; H, 2.95; S, 8.55. Found: C, 54.45; H, 2.93; S, 8.57.

*3-(Chlorobenzoyl)-6-bromo-2H-chromene-2-thione (**5o**)*. Yellow crystals. m.p. 195–196 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 7.41–7.55 (m, 3H), 7.73–7.87 (m, 5H); ^{13}C NMR (75.5 MHz, $CDCl_3$, δ ppm): 118.4, 118.7, 121.3, 129.1, 129.2, 130.7, 130.9, 132.1, 133.8, 136.1, 139.7, 155.8, 190.6, 192.7; IR (KBr) (ν max, cm^{-1}): 1234, 1587, 1662, 3053; MS $m/z = 377$ (M^+). Anal. Calcd. for $C_{16}H_8O_2SClBr$: C, 50.62; H, 2.12; S, 8.45. Found: C, 50.67; H, 2.09; S, 8.43.

4.4. Antioxidant assay

4.4.1. Materials and methods

4.4.1.1. DPPH free radical scavenging assay. Methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) was used as a reagent for the spectrophotometric assay with modifications [31–33]. The reaction mixture in triplicates consisted of 100 μM DPPH in absolute methanol with different concentrations (0–1000 μg/mL) of the compounds. A negative control with the same DPPH concentration in methanol without sample was used. Absorbance was read against a blank at 515 nm after incubation of the reaction mixtures for 30 min in dark at room temperature. Percentage decoloration of DPPH was determined by comparison with methanol treated control and IC_{50} was calculated in μg/mL. Ascorbic acid equivalent antioxidant capacity (AEAC) in mg/g was calculated for relatively effective compounds from the IC_{50} as

$$\text{AEAC(mg Ascorbic acid/g Sample)} = IC_{50\ (\text{ascorbate})}/IC_{50\ (\text{sample})} \times 1000.$$

The IC_{50} of ascorbic acid used for calculation of AEAC was 3.82 μg/mL.

4.4.1.2. Sulfur free radical reactivity assay. Glutathione (GSH) was used for *in vitro* generation of sulfur free radical (GS$^\bullet$) by γ-

radiolysis, and curcumin was used as a reference to assess its reactivity with the GS• radicals [34,35]. The reaction solution contained 20 μM curcumin and 15 mM glutathione (GSH) in 60% methanol [36]. Various concentrations of samples (5–20 μg/mL) were added to the reaction solution and the final volume was adjusted with 60% methanol. The control reaction contained 20 μM curcumin and 15 mM glutathione without samples and the reaction volume was adjusted with 60% methanol. These solutions were irradiated with 0, 50, 100, 200, and 250 Gy respectively at a dose rate of 6.62 kGy/h using a ^{60}Co gamma chamber – GC 5000 (Board of Research on Isotope Technology, Bhabha Atomic Research Center, Mumbai). The gamma irradiation generates sulfur free radicals from glutathione which could deplete the curcumin molecules in the control. Curcumin protection by the samples was monitored by recording the absorbance at 425 nm using a UV/vis spectrophotometer (Beckman DU 640) against a blank. Curcumin depletion in the reaction solution with or without samples was calculated and protective indices (PI) were deduced as:

$$\text{Protective index}(\%) = [\text{curcumin depletion (without sample} - \text{with sample})/\text{initial curcumin concentration}] \times 100.$$

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