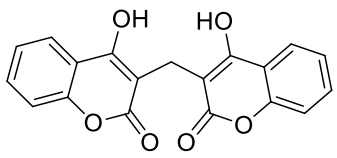
Chapter-3

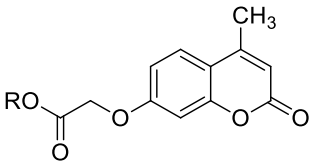
Synthesis of chromene-2-thiones from β- oxodithioesters

3.1 Introduction

Coumarins and their derivatives exhibit a wide range of biological properties such as antioxidant, anticancer, anti-inflammatory, anticoagulant, antimicrobial and anti-tubercular properties (**Fig. 1**). Dicoumarol, a coumarin derivative, acts as an anticoagulant by disturbing the action of the enzyme; vitamin K reductance[1]. Coumarin derivatives are proven to be promising candidates in the development of anti-TB agents[2]. 3-Aryl coumarins exhibit cytotoxic activity and are useful as anti-inflammatory agents. Coumarin-dihydropyrazole thio-ethanone derivatives with high antiproliferative activity have been designed as efficient telomerase inhibitors[3]. Alkenylchromanones and akenylthiocromanones show promising antimicrobial activity[4].



Dicoumarol (Anticoagulant)



Coumarin ester (Antimicrobial)

Fig. 1

Thiocoumarins show third-order nonlinear properties[5]. These properties were found to vary with variation in the extent of electron delocalisation. It also exhibits interesting photophysical properties[6]. These compounds possess extremely short S_2 state lifetimes due to intramolecular S_2 state deactivation. Thiocoumarins act as inhibitors for NADPH catalysed lipid peroxidation and also as inhibitors for TNF-α induced expression of intracellular adhesion molecule-1 (ICAM-1) on endothelial

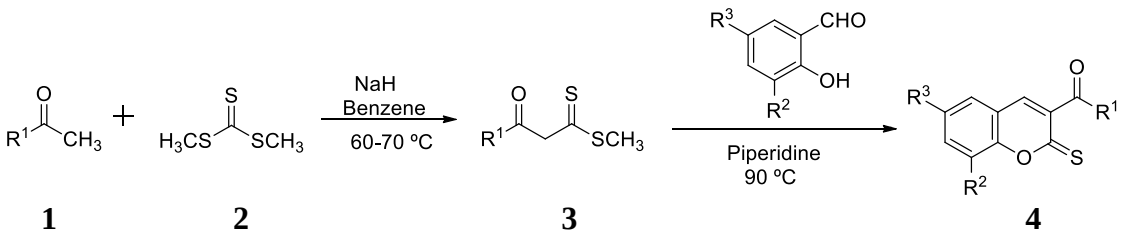
cells[7]. A number of coumarins and thiocoumarins were found to inhibit the action of zinc metalloenzyme namely carbonic anhydrase[8]. Thiocoumarins are compounds of promising antileishmanial activity[9]. These compounds act effectively against the leishmania parasite and are found to be less toxic to the human body.

Mercury triggered desulphurisation of thiocoumarin to coumarin has been used for the selective detection of Hg^{2+} ion. For this, a fluorescent probe based on intramolecular charge transfer (ICT) has been developed[10]. Moreover, this probe is applicable for fluorescence imaging of Hg^{2+} in live cells. Further studies on the structure-activity relationship have shown that the bioactivity of heterocyclic compounds depends largely on the position of sulphur atom in the heterocyclic ring[11].

3.2 Synthesis of chromene-2-thiones – a review

Various methods have been developed for the synthesis of coumarin derivatives. Wenchen *et al.* synthesised a number of 3-aryl coumarins via Perkin condensation[12]. Knoevenagel condensation reaction was found to be an efficient environment-friendly synthetic method for coumarin derivatives[13]. Brahmachari synthesised coumarin-3-carboxylic acids from 2-hydroxybenzaldehyde through Knoevenagel condensation followed by intramolecular cyclisation[14]. Cravotto *et al.* developed microwave irradiation method for the synthesis of coumarins[15]. Pechmann condensation using palladium nanoparticles was found to be an efficient method for the synthesis of coumarin derivatives[16].

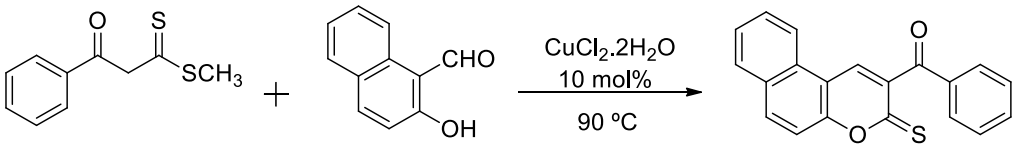
Thiocoumarins having antioxidant properties were synthesised from β-oxodithioesters by Singh *et al.*[17]. In this synthetic method, compounds having active methylene group **1** were treated with dimethyl trithiocarbonate **2** to get the β-oxodithioesters **3** as the reactive intermediate. The β-oxodithioesters thus obtained were subjected to piperidine catalysed Knoevenagel type condensation reaction with ortho hydroxy benzaldehyde to afford thiocoumarins **4** (**Scheme 1**).



1 **2** **3** **4**

Scheme 1

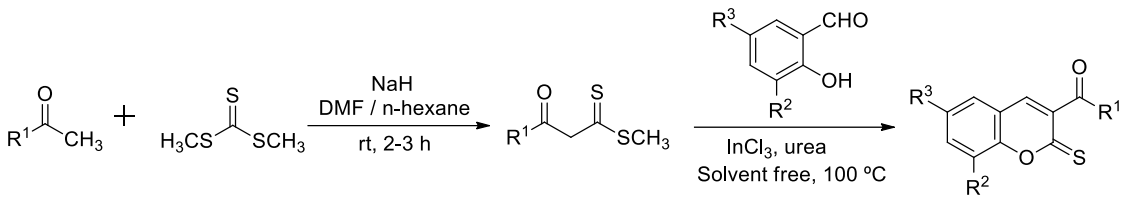
Singh *et al.* introduced a facile and efficient synthetic strategy for thiocoumarins from β-oxodithioesters[18]. Thiocoumarins **7** have been synthesised by cupric chloride catalysed condensation of β-oxodithioesters **5** with 2-hydroxy-1-naphthaldehyde **6** under solvent-free conditions (**Scheme 2**).



5 **6** **7**

Scheme 2

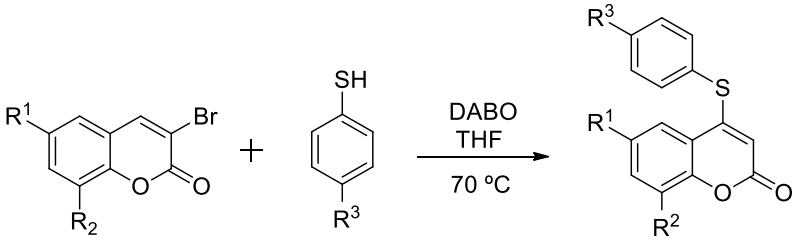
Verma *et al.* have reported a solvent-free synthesis of chromene-2-thiones **11** via ring annulation reaction between β-oxodithioesters **10** and salicylaldehyde in the presence of catalytic amounts of indium trichloride and urea[19]. The β-oxodithioesters **10** have been synthesised by the reaction between appropriate ketone **8** and trithiocarbonate **9** in the presence of NaH in a 1:4 DMF/hexane solvent mixture (**Scheme 3**).



8 **9** **10** **11**

Scheme 3

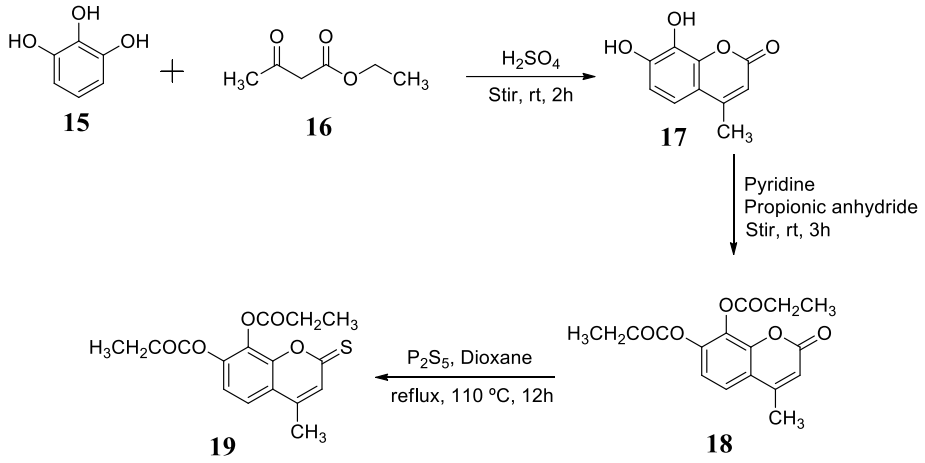
Mostardeiro devised DABCO (1,4-diazabicyclo[2.2.2]octane) assisted one-pot synthetic protocol for 4-sulphanylcoumarins from 3-bromocoumarins[20]. The reaction between 3-bromocoumarins **12** and thiophenols **13** in the presence of DABCO in THF at 70 ºC resulted in the corresponding 4-sulphanylcoumarins **14** (**Scheme 4**). The reaction proceeds through DABCO assisted thia-Michael addition of thiolate anion to coumarin and subsequent dehydrobromination affording the thiocoumarins in good yields.



12 **13** **14**

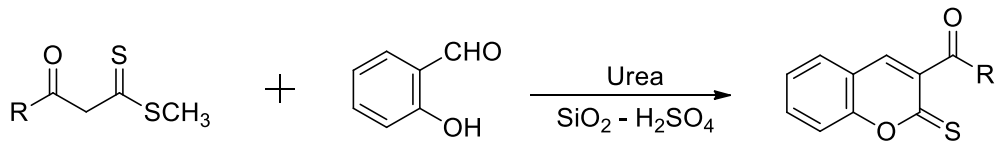
Scheme 4

7,8-Dipropoxy-4-methylthiocoumarin with interesting pharmacological properties were synthesised by Kumar and co-workers[21]. The synthesis started with the reaction between pyrogallic acid **15** and ethyl acetoacetate **16** in H_2SO_4 followed by treatment with propionic anhydride in pyridine to yield 7,8-dipropoxy-4-methylcoumarin **18**. Thionation of this compound with pentasulphide in dioxane afforded the desired product 7,8-dipropoxy-4-methylthiocoumarin **19** (**Scheme 5**). The compound exhibits anti-cholinesterase, anti-aggregatory and antioxidant properties. It also inhibits BACE1 and MAOB enzymes.



Scheme 5

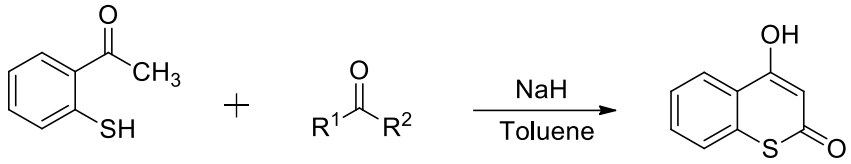
Singh *et al.* have developed a synthetic method for thiocoumarins from β-oxodithioesters[22]. According to this protocol, heating a mixture of β-oxodithioesters **20**, salicylaldehyde **21**, urea and SiO_2-H_2SO_4 at 85 ºC resulted in cyclocondensation between β-oxodithioesters and salicylaldehyde to afford the corresponding thiocoumarin **22** (**Scheme 6**). This was found to be silica-sulpuric acid promoted cyclocondensation reaction in which β-oxodithioesters act as activated β-dicarbonyl species.



20 **21** **22**

Scheme 6

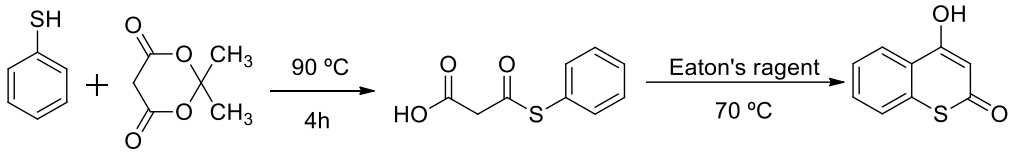
Jung developed a synthetic protocol for 4-hydroxythiocoumarin **25** by the reaction between 2-mercaptoacetophenone **23**, an acylating agent **24** and NaH in anhydrous toluene[23] (**Scheme 7**). Synthesis of 4-hydroxy-1-thiocoumarin from 2-mercaptobenzoicacid was also reported by the same group[24]. 4-Hydroxythiocoumarin was found to be a valuable synthetic precursor in organic synthesis[25].



23 **24** **25**

Scheme 7

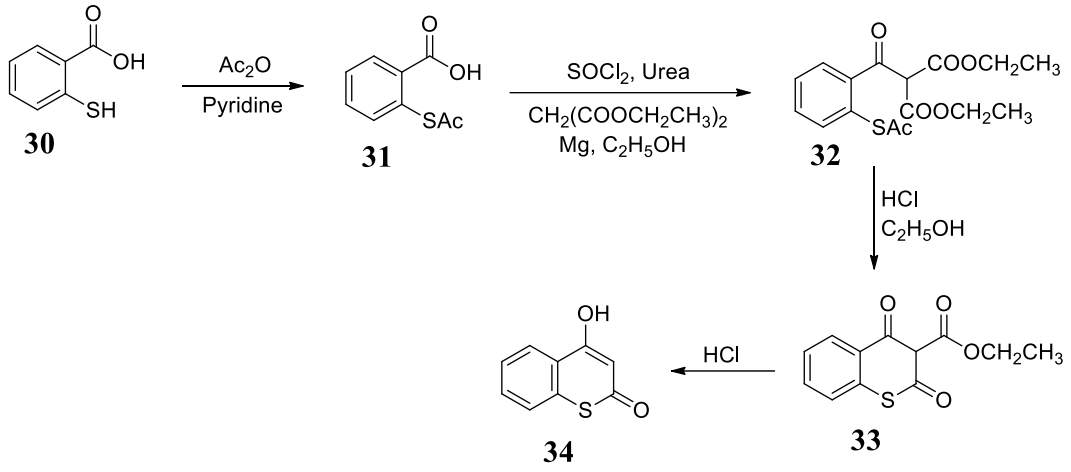
Lee *et al.* suggested an efficient, solvent-free synthetic method for 4-hydroxythiocoumarins from thiophenol and Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione)[26]. When thiophenol **26** was treated with Meldrum acid **27**, Pechmann condensation takes place affording phenylsulphonylcarbonyl acetic acid **28**. It was followed by the treatment with Eaton's reagent to yield 4-hydroxythiocoumarin **29** (**Scheme 8**).



26 **27** **28** **29**

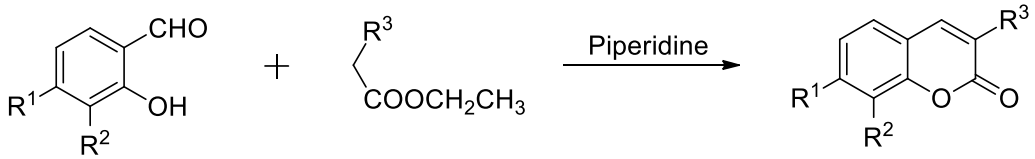
Scheme 8

An efficient and cost effective synthesis of 4-hydroxythiocoumarins from 2-mercaptobenzoic acid was reported by Jung *et al.*[24]. The synthesis consists of acetylation of 2-mercaptobenzoic acid **30** to 2-acetylmercaptobenzoic acid **31**, followed by treatment with thionylchloride in urea to get 2-acetylmercaptobenzoyl chloride. This when treated with the anion of diethyl malonate afforded ethyl 2-(2-acetylmercapto)-benzoylmalonate **32**. Subsequent treatment with hydrochloric acid resulted in the cyclisation to thiolactone followed by hydrolysis and decarboxylation affording 4-hydroxythiocoumarin **34** (**Scheme 9**).



Scheme 9

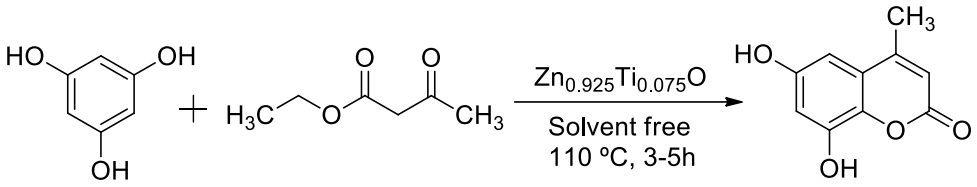
Bogdal has reported a solvent free synthesis of coumarins via Knoevenagel condensation reaction under microwave conditions[27]. Microwave irradiation of a mixture of salicylaldehyde **35**, ethyl acetate **36** and piperidine afforded coumarins **37** within 10 minutes (**Scheme 10**).



35 **36** **37**

Scheme 10

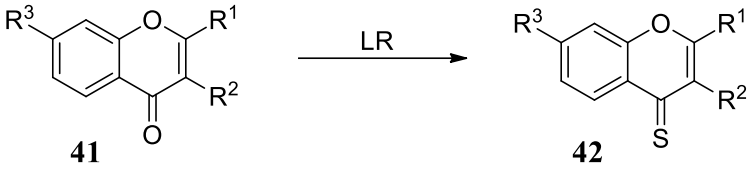
Jadhav developed a heterogeneous catalyst for the synthesis of coumarins[28]. The high surface area accompanied with the Lewis acidity of the synthesised Ti(IV)-doped ZnO matrix forming catalyst made it very active towards β-ketoesters and phenols. Phenols **38** and β-ketoesters **39** when microwave irradiated in the presence of $Zn_{0.925}Ti_{0.075}O$ catalyst undergo Pechmann condensation yielding coumarins **40** (**Scheme 11**). The reaction was carried out under solvent free conditions.



38 **39** **40**

Scheme 11

Varma and Kumar reported the synthesis of thio analogues **42** of a number of carbonyl compounds **41** such as ketones, amides, flavones, esters, lactones etc.[29]. Lawesson's reagent **43** (**Fig. 2**) was used as sulphurizing agent. The conversion was accomplished via microwave irradiation of the organic compounds with Lawesson's reagent (LR) (**Scheme 12**). The same protocol was found to be applicable to the synthesis of thiocoumarins.



41 **42**

Scheme 12

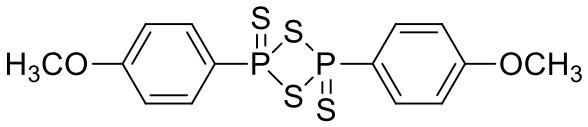
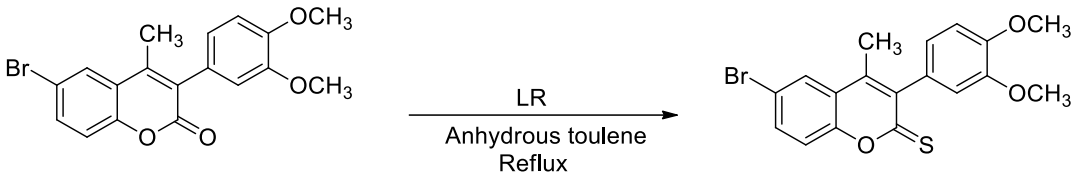


Fig. 2 *Lawesson's reagent* **43**

Roussaki *et al.* treated coumarins **44** with Lawesson's reagent in anhydrous toluene to afford thiocoumarins **45** (**Scheme 13**) possessing lipoxygenase inhibitory activity[30].

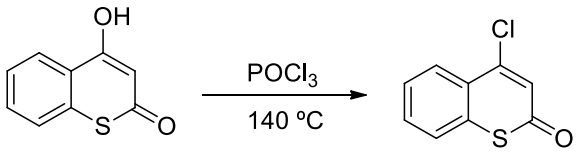


44 **45**

Scheme 13

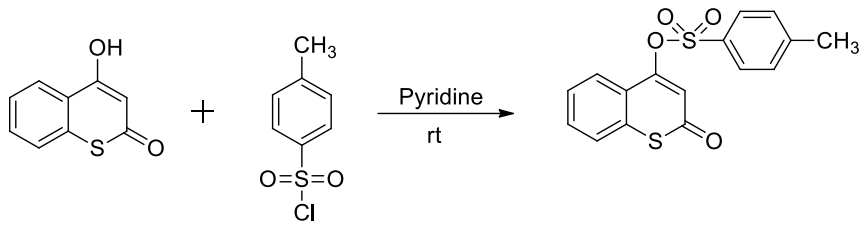
Synthetic and biological applications of thiocoumarins

Thiocoumarins undergo a number of reactions such as chlorination[31], tosylation[32], cycloaddition[33], reaction using electrochemical routes[34] etc. 4-Hydroxy thiocoumarins **46** when treated with $POCl_3$ at 140 ºC for 2.5h give 4-chlorothiocoumains **47** (**Scheme 14**). 4-Hydroxythiocoumrins **46** undergo tosylation reaction with tosyl chloride **48** in the presence of bases such as pyridine. The reaction takes place at room temperature furnishing 4-(toluenesulphonyloxy) thiocoumarin **49** (**Scheme 15**). 4-Hydroxythiocoumrins **46** undergo NaOAc catalysed domino reaction with α-bromonitroalkenes **50** in the presence of DABCO as the base in water at room temperature furnishing functionalized tricyclic 2,3-dihydrofurans **51** (**Scheme 16**). Thiocoumarins are susceptible to reactions using electrochemical routes. Highly regioselective laccase (Agaricus bisporus) catalysed reaction of 4-hydroxythiocoumrins **46** with catechols **52** in the presence of aerial oxygen as oxidant afforded 8, 9-dihydroxy-5-thiocoumestans **53** (**Scheme 17**).



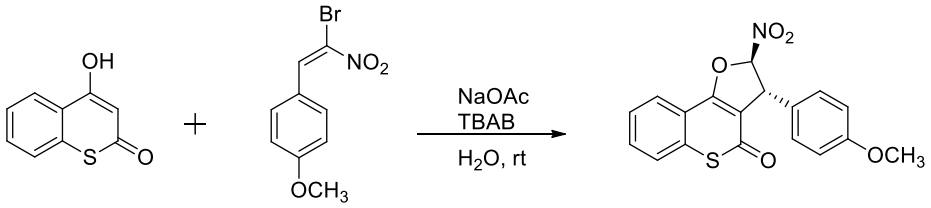
46 **47**

Scheme 14



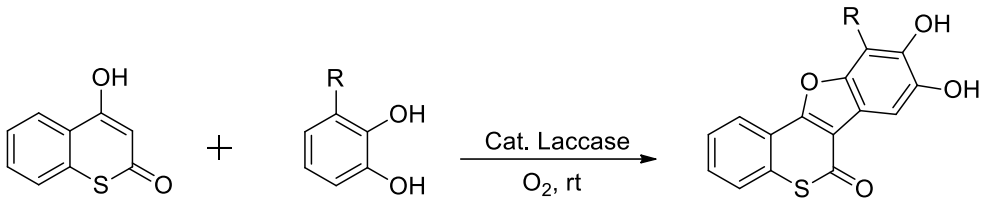
46 **48** **49**

Scheme 15



46 **50** **51**

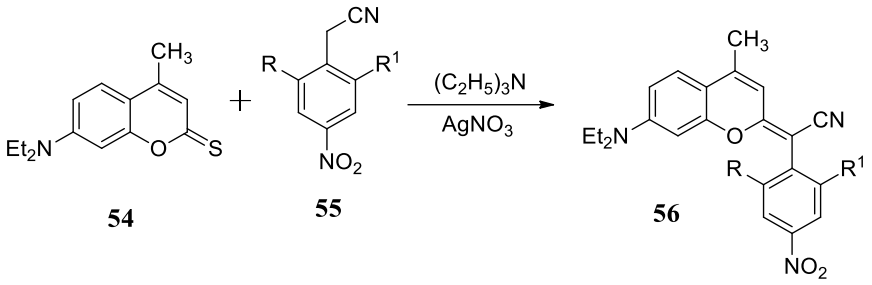
Scheme 16



46 **52** **53**

Scheme 17

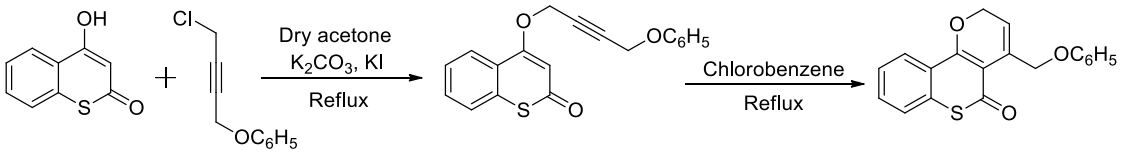
Thiocoumarins have been used for the synthesis of coumarin based chromophores **56** that can act as caging groups[35]. These have been synthesised *via* condensation of thiocoumarin **54** with arylacetonitrile **55** in triethylamine in the presence of silver nitrate (**Scheme 18**). The photophysical and optical properties can be effectively tuned by the incorporation of electron withdrawing groups.



54 **55** **56**

Scheme 18

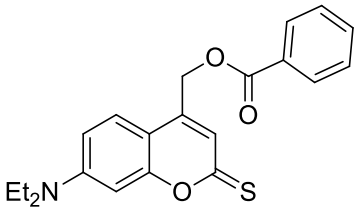
Thiocoumarin-annulated furopyrans have been synthesised by Majumdar *via* regioselective Claisen rearrangement of alkylated 4-hydroxythiocoumarins[36]. The alkylation of 4-hydroxythiocoumarin **57** was carried out by treating it with 1-aryloxy-4-chlorobut-2-yne **58** in dry acetone, potassium carbonate and potassium iodide under refluxing temperature. The subsequent treatment of the alkylated 4-hydroxythiocoumarin **59** with chlorobenzene resulted in the [3, 3] sigmatropic rearrangement followed by enolisation, [1, 5] hydrogen shift and 6π electron ring closure to furnish the desired thiocoumarin-annulated furopyrans **60** (**Scheme 19**).



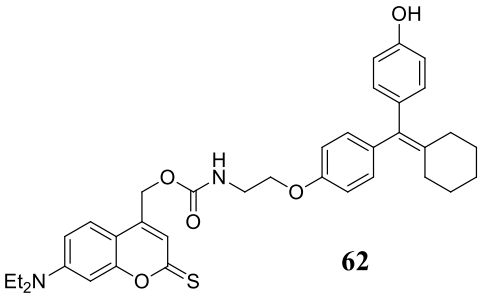
57 **58** **59** **60**

Scheme 19

Thiocoumarin based photocages **61** and **62** (**Fig. 3**) have been developed for controlling the light triggered release of various molecules in the biological systems. These photocages are extensively used in cellular signalling studies. Henning and co-workers have synthesised 7-diethylamino-4-hydroxymethyl-thiocoumarin (thio-DEACM) **63** (**Fig. 4**) as a phosphate caging group with interesting photophysical properties[37]. The thio-DEACM was found to be an excellent red sift photocage with improved photocleavage properties and showed no toxicity to biological systems.

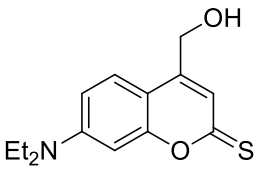


61



62

Fig. 3 *Thiocoumarin based photocages*



63

Fig. 4 *Phosphate caging group*

3.3 Results and discussion:

The synthesis of chromene-2-thiones involves two steps.

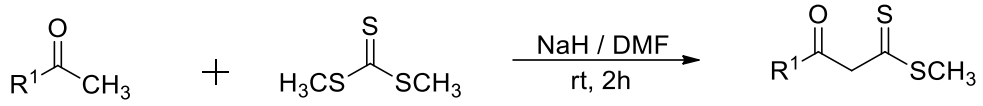
1) Synthesis of β-oxodithioesters from ketones.
2) Synthesis of chromene-2-thiones from β-oxodithioesters.

For the synthesis of a chromene-2-thione, an appropriate β-oxodithioester has to be synthesised from the corresponding ketone. The β-oxodithioesters thus prepared were converted to chromene-2-thiones. For this conversion, we have devised two protocols- conventional thermal method (method a) and microwave heating method (Method b).

3.3.1 Synthesis of β-oxodithioesters

β-Oxodithioesters have been selected as synthons for the synthesis of thiocoumarins. In this method, a mixture of ketones **64** (1 eq.), dimethyl

trithiocarbonate **65** (1 eq.) and sodium hydride (2.4 eq.) in N,N-dimethylformamide (20 mL) was stirred at room temperature for 2h. This is followed by acidification of the reaction mixture to afford the β-oxodithioesters **66** in good yields (**Scheme 20**). The results of the synthesis are summarised in **Table 1**.



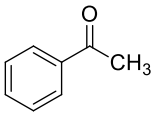
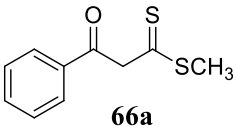
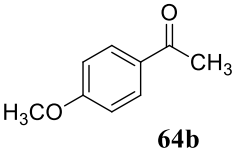
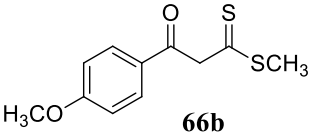
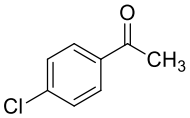
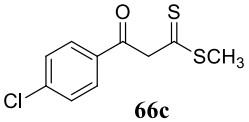
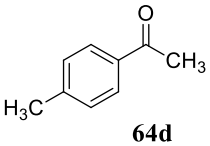
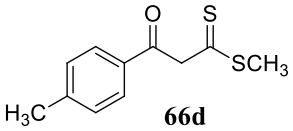
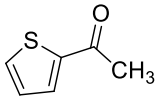
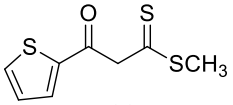
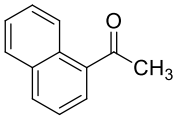
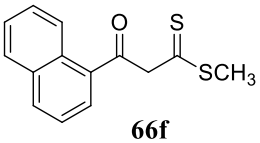
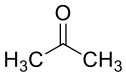
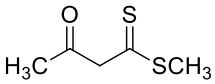
64 **65** **66**

Scheme 20

Methyl 3-oxo-3-phenylpropanedithioate **66a** was prepared from acetophenone **64a**. For this conversion, acetophenone **64a** was treated with dimethyl trithiocarbonate **65** and sodium hydride in N,N-dimethylformamide at room temperature for 2h. After the completion of the reaction (monitored by TLC), the reaction mixture was acidified with HCl, extracted using ethyl acetate and purified using column chromatography to afford the dithioester **66a** in 92% yield. ^{1}H NMR spectra of **66a** exhibited a peak at δ 2.6 ppm corresponding to proton at -SCH_3. Aromatic protons appeared at δ 6.96-7.9 ppm. Protons at -CH_2 appeared as two hydrogen singlet at δ 2.98 ppm. ^{13}C NMR peaks appeared in the aromatic region from δ 128.6 ppm to δ 136.7 ppm. The peak at δ 225.1 ppm was attributed to thiocarbonyl carbon. The carbon of -SCH_3 group gave a signal at δ 20.6 ppm. The data is in agreement with that reported in the literature[38].

β-Oxodithioesters **66b-g** were prepared using the same protocol (**Scheme 20**) (**Table 1**). The structure of the synthesised compounds has been established based on their analytical and spectral data. These are described in the experimental section.

Table. 1 β-Oxodithioesters (**66**) synthesised:

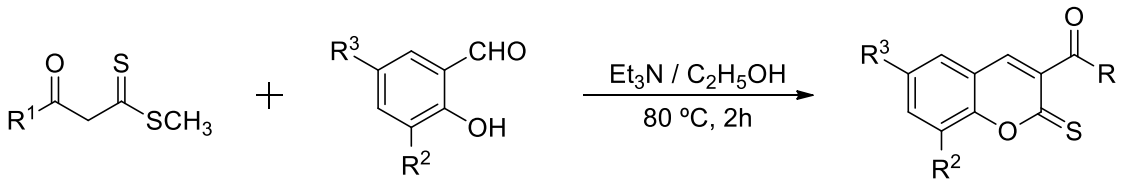
Entry	Ketone	β- Oxodithioester	Yield (%)
1	 **64a**	 **66a**	92
2	 **64b**	 **66b**	88
3	 **64c**	 **66c**	90
4	 **64d**	 **66d**	84
5	 **64e**	 **66e**	82
6	 **64f**	 **66f**	90
7	 **64g**	 **66g**	80

3.3.2 Synthesis of chromene-2-thiones

Chromene-2-thiones have been synthesised *via* the conventional thermal method (**method a, Scheme 21**) as well as the microwave heating method (**method b, Scheme 22**).

Synthesis of chromene-2-thiones: Method a

The β-oxodithioesters **66** (1 eq.) were treated with 2-hydroxybenzaldehydes **67** (1 eq.) in the presence of triethylamine (2 eq.) in ethanol (10 mL) under reflux conditions of 80 ºC for 2h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to 5 °C and filtered to get the chromene-2-thione **68** as a yellow solid in good yields (**Scheme 21**). The results of the synthesis are summarised in **Table 2**.



66 **67** **68**

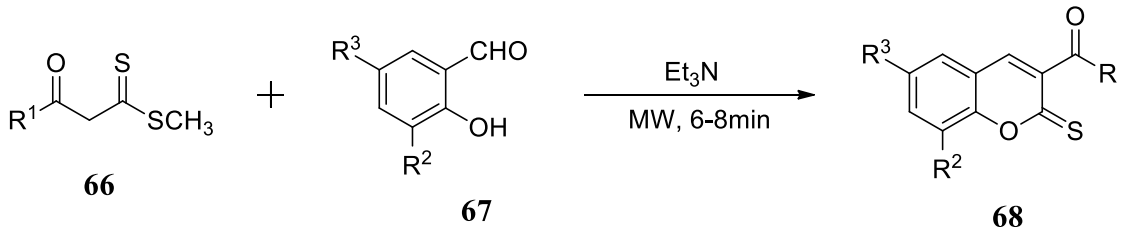
Scheme 21

The β-Oxodithioester **66a** when treated with salicylaldehyde **67a** in the presence of triethylamine in ethanol under reflux conditions for 2h afforded chromene-2-thione **68a** in 80% yield. The ^{1}H NMR spectrum of **68a** exhibited one proton multiplet at δ 7.65–7.70 ppm corresponding to ethylenic proton. The peaks at δ 7.36–7.95 ppm denoted aromatic protons. The ^{13}C NMR spectrum showed a downfield signal at δ 193.6 ppm due to the thiocarbonyl carbon. The carbonyl carbon signal appeared at δ 192.3 ppm. The peaks at δ 125.8-133.9 ppm are due to aromatic carbons.

Optimisation of the above cyclocondensation reaction was carried out by changing the base, solvent etc. It was observed that triethylamine in ethanol gives the best yields. After optimisation of the reaction condition, this protocol (**Scheme 21**) has been extended to other dithioesters and 2-hydroxybenzaldehydes to afford the 2H-chromene-2-thiones **68b-l** in good yields (**Table 2**).

Synthesis of chromene-2-thiones: Method b

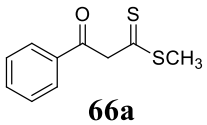
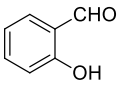
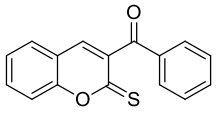
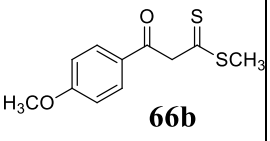
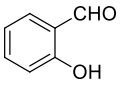
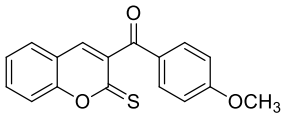
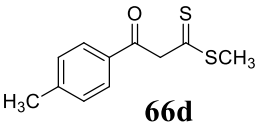
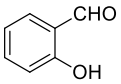
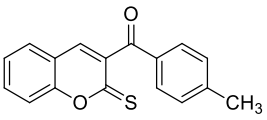
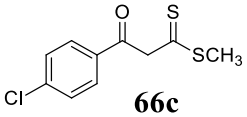
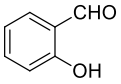
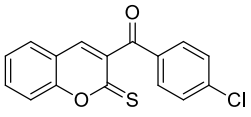
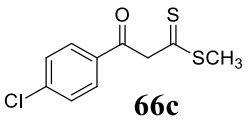
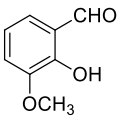
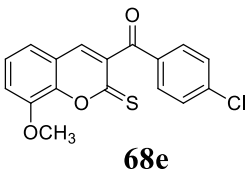
A solvent-free microwave-assisted synthetic protocol has been developed for the synthesis of chromene-2-thiones from β-oxodithioesters. Compared to conventional heating protocol, in microwave heating method, the reaction time was decreased to a few minutes without compromising the yield. A mixture of β-oxodithioester **66** (1 eq.), 2-hydroxybenzaldehydes **67** (1 eq.) and triethylamine (2 eq.) was irradiated under microwave power of 400W at 140 ºC for 6-8 min. The reaction mixture was cooled and the solid obtained was recrystallized from ethanol to afford the chromene-2-thiones **68** as yellow solids in excellent yields (**Scheme 22**).

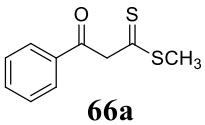
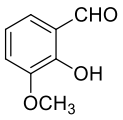
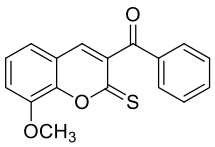
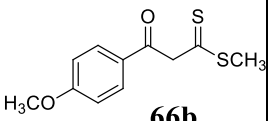
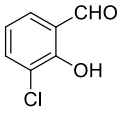
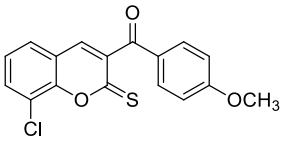
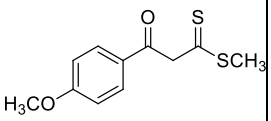
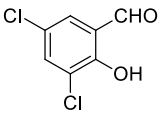
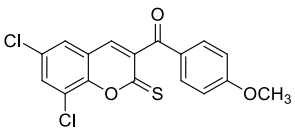
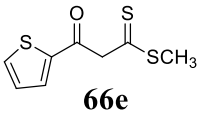
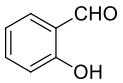
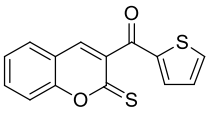
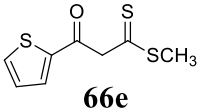
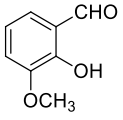
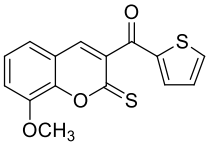


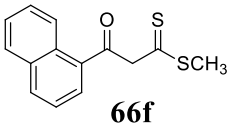
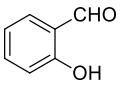
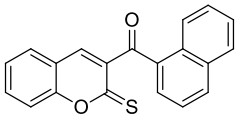
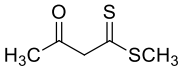
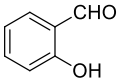
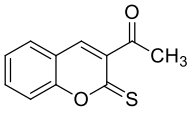
Scheme 22

A mixture of β-oxodithioester **66a**, salicylaldehyde **67a** and triethylamine upon irradiation under microwave conditions for 6-8 min. afforded the chromene-2-thione **68a** in 87% yield. The ^{1}H NMR spectrum of **68a** exhibited one proton multiplet at δ 7.65–7.70 ppm corresponding to ethylenic proton. The peaks at δ 7.36–7.95 ppm denoted aromatic protons. The ^{13}C NMR spectrum showed a downfield at δ 193.6 ppm due to thiocarbonyl carbon. The carbonyl carbon gave a signal at δ 192.3 ppm. The peaks at δ 125.8-133.9 ppm were due to aromatic carbons. Other chromene-2-thiones **68b–l** were prepared using the same protocol **(Table 2)**. The structure of the synthesised compounds was established based on their analytical and spectral data. It was found that the nature of the substituents on the dithioester or the aldehyde moiety had little effect on the overall yield of the product formed. The microwave-assisted synthetic protocol is an improvement over conventional thermal method as the reaction time was substantially reduced without compromising the yield of the reaction.

Table. 2 Chromene-2-thiones (**68**) synthesised:

Entry	β-Oxodithioester	Aldehyde	Chromene-2-thione	Yield (%)	
				Method a*	Method b+
1	 **66a**	 **67a**	 **68a**	80	87
2	 **66b**	 **67a**	 **68b**	92	95
3	 **66d**	 **67a**	 **68c**	92	97
4	 **66c**	 **67a**	 **68d**	85	88
5	 **66c**	 **67b**	 **68e**	85	89

6	 **66a**	 **67b**	 **68f**	90	92
7	 **66b**	 **67c**	 **68g**	80	85
8	 **66b**	 **67d**	 **68h**	82	86
9	 **66e**	 **67a**	 **68i**	86	88
10	 **66e**	 **67b**	 **68j**	84	85

11	 **66f**	 **67a**	 **68k**	75	82
12	 **66g**	 **67a**	 **68l**	88	92

A mixture of β-oxodithioester **66, 2-hydroxybenzaldehyde **67** and triethylamine in ethanol was refluxed at 70 ºC for 2h. The reaction mixture was poured into ice cold water, extracted using ethyl acetate, distilled off the solvent and the residue was purified using column chromatography to afford chromene-2-thione **68** as a yellow solid in good yields.*

*†A mixture of β-oxodithioester **66,** 2-hydroxybenzaldehyde **67** and triethylamine was irradiated under microwave power of 400W at 140 ºC for 6-8 min. The reaction mixture was cooled and the solid obtained was recrystallized from ethanol to afford the chromene-2-thione **68** as yellow solids in excellent yields.*

3.4 Characterisation

The structure of the synthesised 2H-chromene-2-thiones was confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectra and single crystal XRD. 3-Benzoyl-2H-chromene-2-thione **(68a) (Fig. 5)** was taken as a representative molecule for the general discussion.

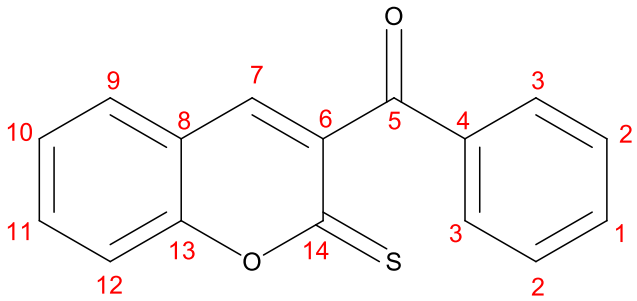


Fig. 5 *3-Benzoyl-2H-chromene-2-thione (**68a**)*

The FT-IR spectrum of 3-benzoyl-2H-chromene-2-thione (**68a**) gives major peaks at 1244, 1606, 1658, 3035, 3048 cm^{-1}. The peak at 1658 cm^{-1} is attributed to =C-H stretching vibration. C-O stretching vibration was indicated by a signal at 1244 cm^{-1}. The absorptions at 3035 cm^{-1} and 3048 cm^{-1} are due to C-H stretching vibrations. C=C stretching vibration was indicated by a signal at 1606 cm^{-1}.

The structure of 3-benzoyl-2H-chromene-2-thione (**68a**) was confirmed by the ^{1}H NMR spectrum (**Fig. 6)**. Three proton multiplet at δ 7.45–7.55 ppm corresponds to protons at positions C1 and C2. The protons at C3 are denoted by two proton multiplet at δ 7.93–7.95 ppm. The proton at C7 is observed as one proton multiplet at δ 7.65–7.70 ppm. The one proton multiplet at δ 7.36–7.40 ppm is attributed to the proton at C9. Three proton multiplet at δ 7.58–7.61 ppm corresponds to protons at positions C10, C11 and C12.

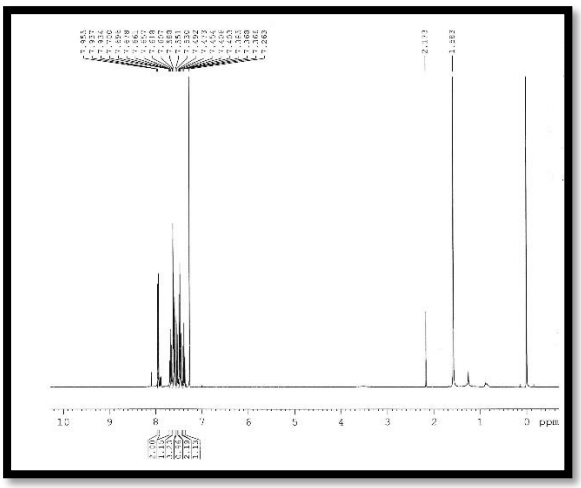


Fig. 6 ^{1}H NMR spectrum of 3-benzoyl-2H-chromene-2-thione (**68a**)

The ^{13}C NMR spectrum of 3-benzoyl-2H-chromene-2-thione (**68a**) (**Fig. 7**) is in agreement with the expected structure. The downfield signal at δ 193.6 ppm is due to thiocarbonyl carbon C14. The carbonyl carbon C5 shows signal at δ 192.3 ppm. The ethylenic carbons C6 and C7 give peaks at δ 128.7 ppm and δ 135.7 ppm respectively. The phenyl carbons give signals in the range δ 116.7- 157 ppm

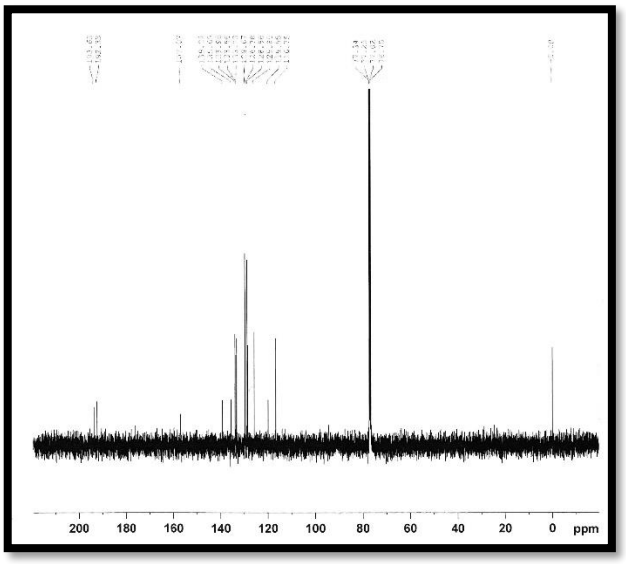


Fig. 7 *^{13}C NMR spectrum of 3-benzoyl-2H-chromene-2-thione (***68a***)*

The structure of 3-benzoyl-2H-chromene-2-thione (**68a**) was further confirmed by the mass spectral analysis. The molecular ion peak (M^+) was observed at m/z 266.

The proposed structure of 3-benzoyl-2H-chromene-2-thione (**68a**) was confirmed by single crystal X-ray analysis. The product **68a** was dissolved in dichloromethane and crystals were obtained by slow evaporation of the solvent. A single crystal of **68a** was analysed by X-ray crystallographic experiments. The details are summarised in **Table 3**. The crystal belongs to the monoclinic crystal system with space group P2(1)/n. **Fig. 8** shows the thermal plot of chromene-2-thione **68a** with the thiocarbonyl (C_{16}- S_1) bond distance 1.63 Å. C_{16}-O_2 and C_{15}-O_2 distances are 1.36 Å and 1.38 Å respectively.

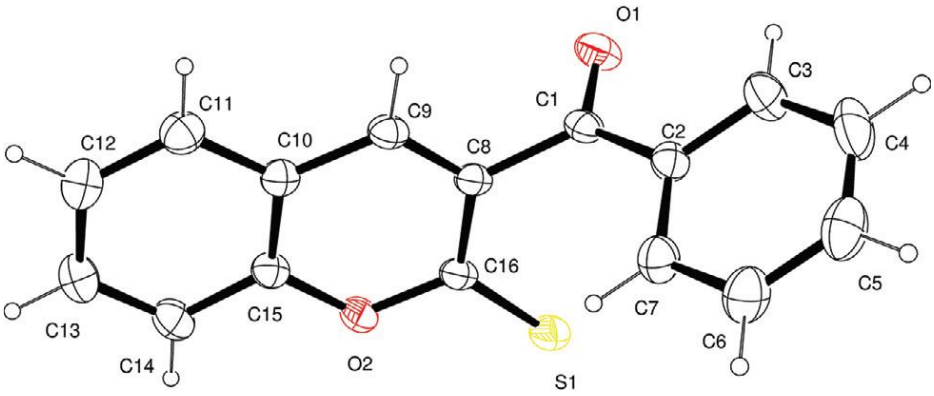


Fig. 8 *Single crystal XRD of 3-benzoyl-2H-chromene-2-thione (***68a***)*

Table. 3 Crystal data and measurement details for compound **68a**:

CCDC deposit No.	1414126
Empirical formula	$C_{16}H_{19}O_2S$
Formula weight	266.30
Temperature	296(2)K
Wavelength	0.71,073 Å
Crystal system, space group	Monoclinic, P2(1)/n
Unit cell dimensions	a=4.7346(3) Å, $\alpha=90^0$ b=13.1448(8) Å, $\beta=96.024(4)^0$ c=21.4250(13) Å, $\gamma= 90^0$
Volume	1326.03(14) $Å^3$
Z, Calculated density	4, 1.334 mg/m^3
Absorption coefficient	0.237 mm^{-1}
F(000)	552
Crystal size	0.2 x 0.15 x 0.15 mm
Theta range for data collection	1.91 to 25.00 deg.

3.5 Biological studies - *In vitro* cytotoxicity

In vitro cytotoxic activity of the synthesised chromene-2-thiones **68** against two cancerous cell lines: DLA (Dalton's Lymphoma Ascites cells), EAC (Ehrlich Ascites Carcinoma cells) was studied. 5-Fluorouracil was used as the positive control. The tumour cells aspirated from the peritoneal cavity of tumour-bearing mice were washed thrice with phosphate buffered saline (PBS) or normal saline. Cell viability was determined by the trypan blue exclusion method[39]. Viable cell suspension (1

$\times 10^6$ cells in 0.1 mL) was added to tubes containing various concentrations of the test compound in DMSO (dimethyl sulphoxide) and the volume was made up to 1 mL using PBS. The Control tube contained only cell suspension. The assay mixture was incubated for 3h at 37 °C. The cell suspension was then mixed with 0.1 mL of 1% trypan blue and kept for 2–3 min and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue; live cells do not take up the dye. The number of stained and unstained cells was counted separately.

The percentage of cell death was calculated using the following equation:-

$$\%\text{Inhibition} = \frac{\text{No . of dead cells}}{\text{No . of live cells + No . of dead cells}} \text{ X } 100$$

IC_{50} (50% inhibitory concentration) value for each cell line was determined as the average of two independent experiments and the results are shown in **Table 4**.

These results indicate that synthetic compound **68** exhibits significant cytotoxic activity against the two cell lines studied. Out of the compounds studied; **68a**, **68b**, **68d, 68e**, **68f**, **68i, 68j, 68k** and **68l** were found to have good cytotoxic activity. The activity is higher against DLA than EAC cell lines and found that the activity of the compounds; **68a**, **68b**, **68k** and **68l** was better than 5-fluorouracil, the drug used as the standard.

The compounds; **68a** and **68i** were also screened for their toxicity against normal cell lines (rat spleen cells). For this, rat was sacrificed using carbon dioxide anaesthesia and the spleen tissue was dissected. It was then smashed to single cell suspension in Dulbecco's modified Eagle Medium (DMEM) containing antibiotics and filtered using mesh cloth. The collected cells were washed thrice and suspended in a known volume of DMEM containing antibiotics and counted. Viable cell suspension (1×10^6 cells in 0.1 mL) was added to tubes containing various concentrations of the test compound and made up to 1 mL using DMEM media. Control tubes contained only cell suspension (without additives). These tubes were incubated for 3h at 37 °C and the cell viability was studied using the trypan blue exclusion method. The results are shown in **Table 5**.

The *in vitro* cytotoxicity study revealed that the synthesised chromene-2-thiones **68** showed very low lethality of normal cell lines even when a high drug concentration was used. Thus, it was proved that the chromene-2-thiones are selective in killing only cancerous cell lines.

The *in vitro* cytotoxicity studies established the anticancer activity of chromene-2-thiones which was theoretically supported by *in silico* molecular docking studies.

Table. 4 Cytotoxic activity of chromene-2-thiones against DLA and EAC cell lines:

Entry	Chromene-2-thiones (**68**)	IC_{50} values (μg/mL)	
		DLA	EAC
1	**68a**	33	33.8
2	**68b**	27.7	22
3	**68c**	9.25	15.5
4	**68d**	24	30
5	**68e**	20	32.6
6	**68f**	25	34
7	**68g**	18	24
8	**68h**	12	17
9	**68i**	22	26
10	**68j**	21	28
11	**68k**	>100	>100
12	**68l**	30	36
13	5-fluorouracil	27.2	48

Table. 5 Cytotoxic activity of chromene-2-thiones against normal cell lines:

Drug concentration (μg/mL)	% Cytotoxicity	
	68a	**68i**
10	4.67±0	4.88±0.05
20	4.67±0	6.83±0.04
50	4.67±0	9.91±1.44
100	9.5±1.23	13.7±1.89
200	13±1.32	21.8±1.01

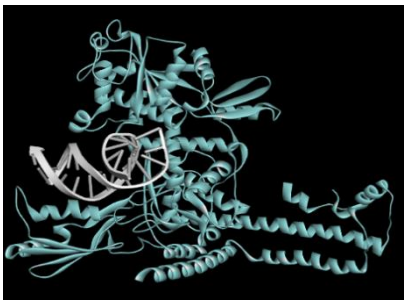
3.6 Molecular docking studies on anticancer activity

Molecular docking studies of sthe ynthesised 2H-chromene-2-thiones **68** were performed using the docking tool Autodock. Autodock is a docking programme that predicts the binding modes of small molecules with the 3D structure of large receptor molecules. This software theoretically calculates the inhibitory activity of molecules against various microbials and tumours. This is accomplished by calculating the binding energy and binding modes of the molecules with proteins. The binding energy is the amount of energy used by the ligand to bind to the protein. The higher the negative value for binding energy, the better the binding efficiency of the ligand with the target. The inhibition constant value gives the concentration of ligand required to inhibit the protein. The lower the value of the inhibition constant, the better the efficiency of the ligand. Higher negative binding energy values accompany lower inhibition constant.

The docked conformation and the interaction between the ligand and the receptor were analysed using the tool Discovery Studio Visualizer. The position of the active site where binding takes place was specified using the Grid option. The structure of the target proteins was obtained from Protein Data Bank (PDB). Active sites of the proteins were predicted using Castp. The chemical modelling tool,

Chemsketch is used for drawing the structure of the ligand. Three dimensional structures in .pdb format were retrieved from the tool CORINA. The position of hydrogen binding active sites was specified using the Grid option. The molecules having the least binding energy were selected as the lead molecule.

In the present study, the anticancer activity of 3-benzoyl-2H-chromene-2-thione (**68a**) was theoretically studied by *in silico* molecular docking method. Human DNA topoisomerase II a (alpha) and II b (beta) were selected as target proteins (**Fig. 9**). DNA topoisomerase II alpha and II beta are the nuclear enzymes that play a major role in DNA replication. These proteins are responsible for relieving torsional strain in DNA. Therefore, the molecules that can inhibit the activity of these proteins are said to possess good anticancer properties. Prior to docking studies, the proteins were prepared by the removal of water molecules and ligand atoms and also by the addition of polar hydrogen atoms after which the energy of the structure was computed. Since the proteins selected consisted of many active sites, we concentrated on the binding properties of the ligand with active sites of the protein present in the central region.



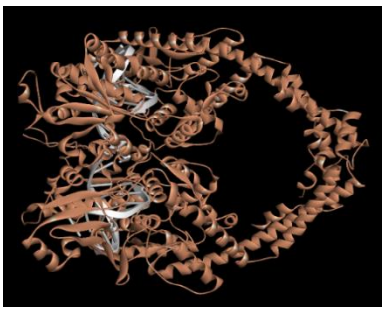


Fig. 9a ***Fig. 9b***

Fig. 9. *(**9a**) Structure of human DNA topoisomerase II alpha (PDB id: 4fm9); (**9b**) Structure of human DNA topoisomerase II beta (PDB id: 3qx3)*

The binding modes of the 3-benzoyl-2H-chromene-2-thione (**68a**) with the target proteins; human DNA topoisomerase II alpha and beta have been calculated (**Fig. 10**). The binding modes of **68a** with human DNA topoisomerase II alpha include conventional hydrogen bonds, carbon-hydrogen bonds, pi-alkyl, pi-sulphur and pi-sigma interactions. The binding modes of **68a** with human DNA topoisomerase II beta include conventional hydrogen bonds, Vander Waals interactions, pi-donor hydrogen bonds, pi-pi stacked, amide-pi stacked and pi-alkyl interactions. The various

interactions of the ligands with active site residues, the binding energies and inhibition constant values are summarized in **Table 6 (Fig. 11)**.

The compound **68a** showed a binding energy of -8.63 Kcal/mol and an inhibition constant of 474.58 nM with human DNA topoisomerase II alpha whereas the binding energy with human DNA topoisomerase II beta was found to be -8.87 Kcal/mol with an inhibition constant of 313.57 nM. The highest binding energy and lowest inhibition constant value were obtained for ligand **68a** with the protein, human DNA topoisomerase II b (beta). The cut-off value for binding energy being −5 Kcal/mol, the values obtained in the docking study established that the compound exhibit good anticancer properties. The binding energy values and the inhibition constant values showed that the 3-benzoyl-2H-chromene-2-thione (**68a**) is a good inhibitor for the proteins, human DNA topoisomerase II a (alpha) and human DNA topoisomerase II b (beta). Moreover, it was also proved that **68a** is a better inhibitor for the protein, human DNA topoisomerase II b (beta) than for human DNA topoisomerase II a (alpha).

Table. 6 Binding energies and possible interactions of the ligand **68a** with target proteins:

Ligand	Protein	Binding energy (Kcal/mol)	Inhibition constant (nM)	H-bonds	Other interactions
Compound **(68a)**	DNA topoisomerase II alpha (PDB id: 4fm9)	-8.63	474.58	LEU A:685 LEU A:592	C - H bond Pi-sigma Pi-sulfur Pi-alkyl
	DNA topoisomerase II beta (PDB id: 3qx3)	-8.87	313.57	GLN A:778 ARG A:503	Van der Waals Pi-Donor H-bond Pi-pi stacked Amide-pi stacked Pi-alkyl

Fig. 10a

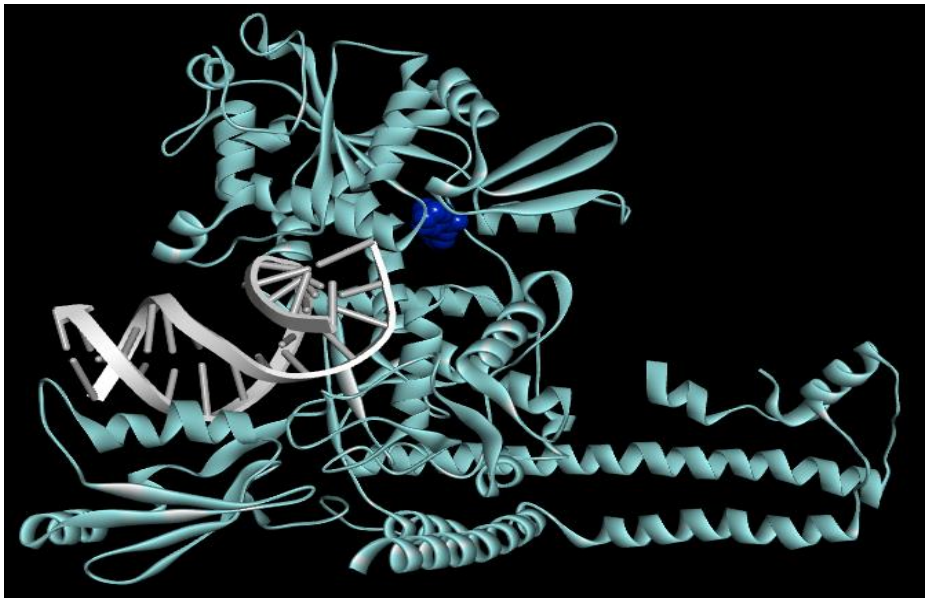


Fig. 10b

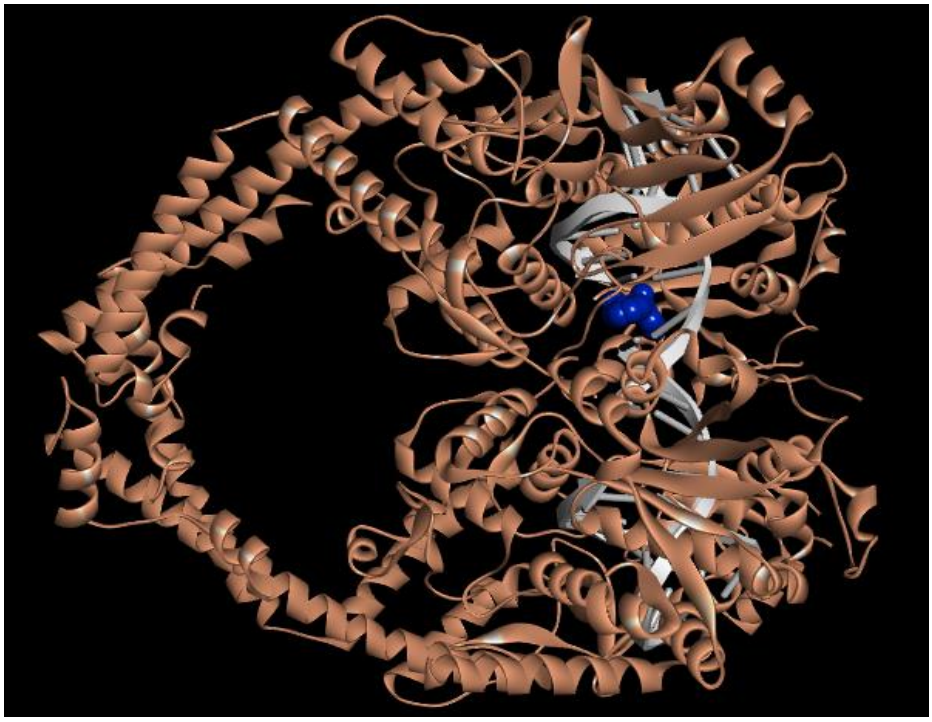


Fig. 10 ***(10a)*** *Bound conformation of human DNA topoisomerase II alpha and ligand* **68a**;
(10b) *Bound conformation of human DNA topoisomerase II beta and ligand* **68a**

Fig. 11a

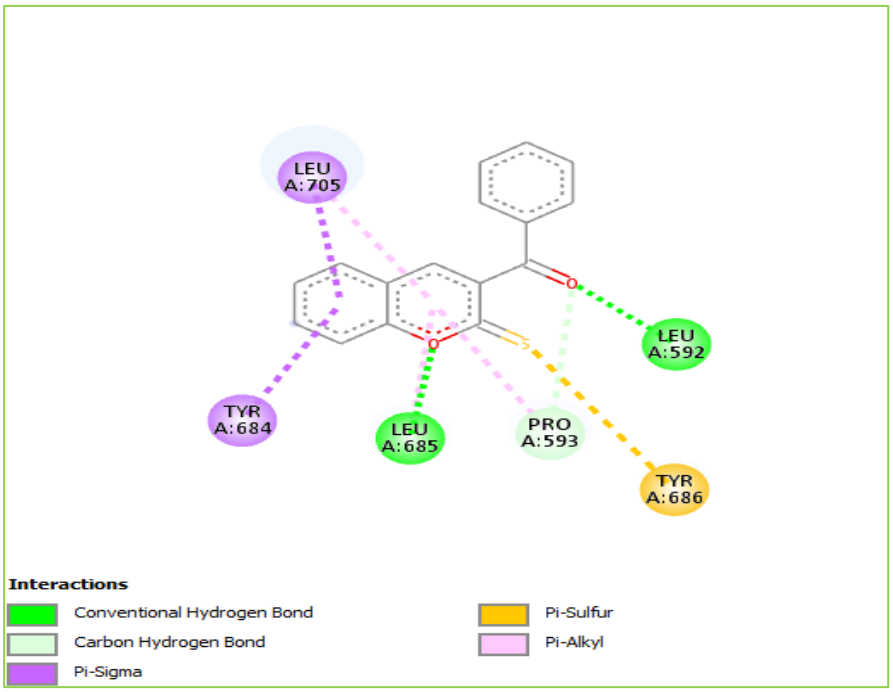


Fig. 11b

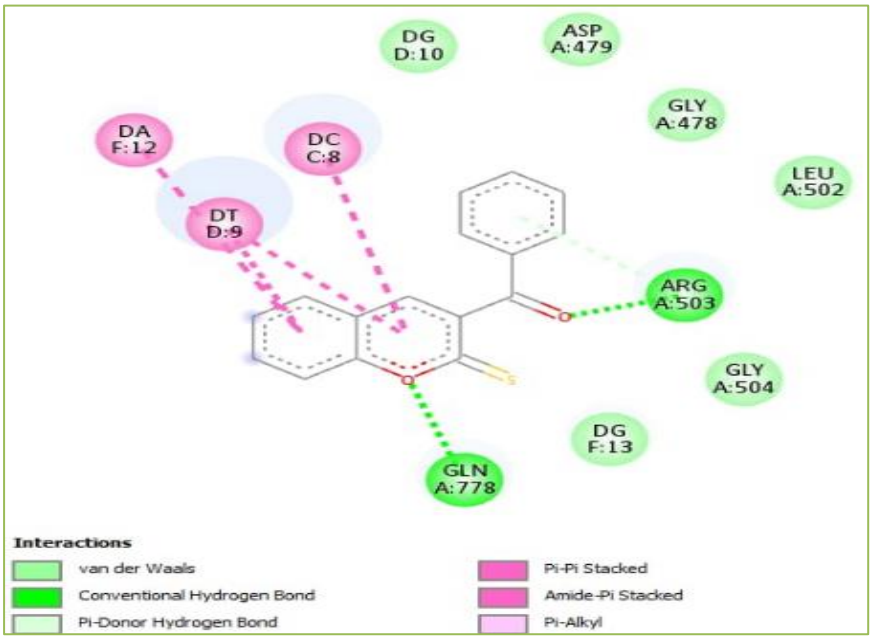


Fig. 11 ***(11a)*** *Interaction between human DNA topoisomerase II alpha and ligand* **68a***;*

(11b) *Interaction between human DNA topoisomerase II beta and ligand* **68a**

3.7 Experimental details

3.7.1 Materials and methods

All the chemicals used for the synthesis were purchased from Sigma-Aldrich, Bangalore, India. Organic solvents were purchased from Spectrochem, India. The solvents were used without further purification. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra were recorded at 100 MHz on a Bruker Avance III NMR spectrometer (Germany). Chemical shifts are quoted in parts per million (ppm) relative to TMS (δ= 0) as the internal standard in $CDCl_3$. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. Coupling constant (*J*) values are given in hertz (Hz). Elemental analysis was performed on a Vario EL III (Elementar, Germany) and all elements were within ± 0.1-0.4% of the theoretical values. Reactions were monitored by thin-layer chromatography (TLC) using TLC sheets coated with UV fluorescent silica gel Merck 60 F254 plates and were visualised using UV lamp. Chromatography was carried out using silica gel 60-120 mesh (Merck, India) and different solvents as mobile phases. Cell viability studies were performed by Trypan Blue exclusion method and carried out at Amala Cancer Research Centre, Thrissur. Molecular docking studies were carried out with the help of Green Clones Naturals Pvt. Ltd., Ernakulam.

3.7.2 Synthesis of β-oxodithioester

To a suspension of NaH (50% suspension in mineral oil, 0.96 g, 20 mmol, 2.4 eq.) in anhydrous DMF (20 mL), dimethyl trithiocarbonate **65** (10 mmol, 1 eq.) was added followed by appropriate ketone **64** (10 mmol, 1 eq.). The reaction mixture was then stirred at room temperature for 2h. It was then acidified with 5N HCl (10 mL) and extracted using ethyl acetate (3 × 20 mL). The organic layer was washed with water (2 × 25 mL), dried over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure. The residue obtained was purified using column chromatography, where the residue was passed through a silica gel column packed in hexane. The hexane was used as the eluent and evaporation of the eluent afforded the β-oxodithioesters **66** in excellent yields. The spectral and analytical data are in good agreement with those reported in the literature[40].

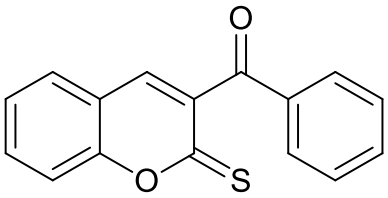
3.7.3 Synthesis of chromene-2-thiones

Two synthetic procedures (***Method a***, ***method b***) have been adopted for the synthesis of 2H-chromene-2-thiones. These methods are described below.

Method a: A mixture of β-oxodithioester **66** (10 mmol, 1 eq.), salicylaldehyde **67** (10 mmol, 1 eq.), and triethylamine (20 mmol, 2 eq.) in rectified spirit (10 mL) was refluxed at 80 °C for 2h. The reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was cooled to 5 °C and filtered to get the chromene-2-thione **68** as a yellow solid. Yield 80-92%

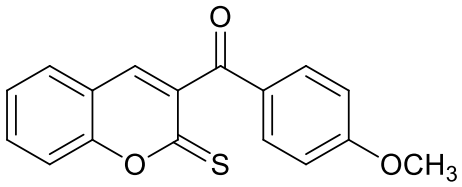
Method b: β-oxodithioester **66** (10 mmol, 1 eq.), salicylaldehyde **67** (10 mmol, 1 eq.) and triethylamine (20 mmol, 2 eq.) was taken in a glass vial and the mixture was irradiated under microwave power of 400 W at 140 °C for 6-8 min. The reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was cooled to room temperature and the solid obtained was recrystallized from ethanol to afford the chromene-2-thione **68** as a yellow solids. Yield 82-97%

a) 3-Benzoyl-2H-chromene-2-thione (**68a**)



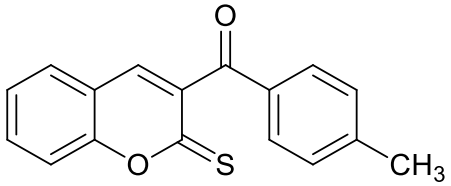
Yellow crystals. m.p. 171–172 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.40 (m, 1H), 7.45–7.55 (m, 3H), 7.58–7.61 (m, 3H), 7.65–7.70 (m, 1H), 7.93–7.95 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 116.7, 119.9, 125.8, 128.5, 128.7, 129.6, 133.5, 133.6, 133.9, 135.7, 139.2, 157, 192.3, 193.6; IR (KBr): 1244, 1606, 1658, 3035, 3048 cm^{-1}; 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, 72.12; H, 3.74; S, 11.92%.

b) 3-(4-Methoxybenzoyl)-2H-chromene-2-thione (**68b**)



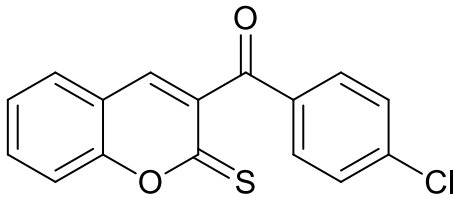
Yellow crystals. m.p. 188–189 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.86 (s, 3H), 6.93 (d, *J* = 7.4 Hz, 2H), 7.34–7.38 (m, 1H), 7.50–7.76 (m, 4H), 7.90 (d, *J* = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.0, 114.5, 117.1, 120.4, 126.2, 128.8, 128.9, 132.6, 133.4, 133.5, 139.9, 157.4, 164.7, 191.2, 194.2; IR (KBr): 1240, 1593, 1656, 2942, 3017, 3058 cm^{-1}; 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, 68.84; H, 4.02; S, 10.77%.

c) 3-(4-Methylbenzoyl)-2H-chromene-2-thione (**68c**)



Yellow crystals. m.p. 170–172 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.42 (s, 3H), 7.28 (d, *J* = 7.2 Hz, 2H), 7.36–7.40 (m, 1H), 7.52–7.59 (m, 3H), 7.64–7.69 (m, 1H), 7.84 (d, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.2, 117.1, 120.3, 126.6, 128.9, 129.9, 130.1, 130.2, 133.5, 133.7,139.8, 145.5, 157.4, 192.3, 194.1; IR (KBr): 1242, 1608, 1660, 2946, 3045, 3055 cm^{-1}; 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, 72.76; H, 4.35; S, 11.48%.

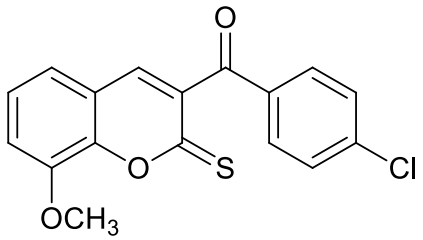
d) 3-(4-Chlorobenzoyl)-2H-chromene-2-thione (**68d**)



Yellow crystals. m.p. 185–186 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32–7.36 (m, 1H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.52 (m, 3H), 7.60–7.70 (m, 3H), 7.86

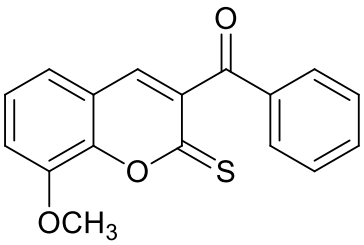
(d, *J* = 8.4, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 116.8, 119.7, 125.8, 128.6, 129.4, 133.2, 134.0, 133.6, 135.6, 138.8, 139.5, 157.3, 191.4, 193.5; IR (KBr): 1236, 1606, 1656, 3030, 3088 cm^{-1}; MS: *m/z* 300.5 (M^+). Anal. Calcd for $C_{16}H_{19}ClO_2S$: C, 63.90; H, 3.02; S, 10.66. Found: C, 63.86; H, 3.04; S, 10.57%.

e) 3-(4-Chlorobenzoyl)-8-methoxy-2H-chromene-2-thione (**68e**)



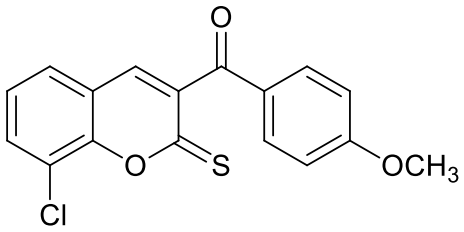
Yellow crystals. m.p. 177–178 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 3H), 7.04–7.12 (m, 2H), 7.16–7.24 (m, 1H), 7.34 (d, *J* = 5.6 Hz, 2H), 7.57 (s, 1H), 7.77 (d, *J* = 6.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.5, 114.8, 119.3, 120.4, 125.7, 129.3, 130.6, 134.3, 134.5, 139.4, 140.6, 146.4, 147.2, 191.6, 192.4; IR (KBr): 1230, 1607, 1656, 2972, 3042, 3064 cm^{-1}; MS: *m/z* 330 (M^+). Anal. Calcd for $C_{17}H_{11}ClO_3S$: C, 61.73; H, 3.35; S, 9.69. Found: C, 61.76; H, 3.30; S, 9.63%.

f) 3-Benzoyl-8-methoxy-2H-chromene-2-thione (**68f**)



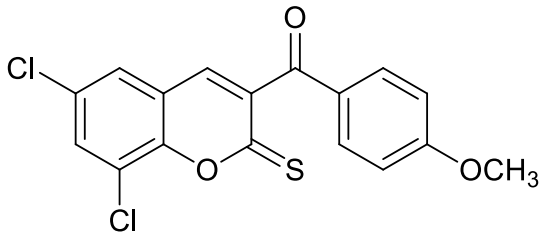
Yellow crystals. m.p. 142–143 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.05 (s, 3H), 7.12–7.22 (m, 2H), 7.25–7.32 (m, 1H), 7.42–7.48 (m, 2H), 7.56–7.60 (m, 2H), 7.93 (d, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.5, 114.7, 119.9, 120.5, 125.5, 128.3, 128.6, 129.3, 133.5, 133.6, 139.2, 146.5, 147.2, 192.6, 192.7; IR (KBr): 1272, 1603, 1667, 3955 cm^{-1}; 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, 68.47; H, 4.79; S, 10.78%.

g) 3-(4-Methoxybenzoyl)-7-chloro-2H-chromene-2-thione (**68g**)



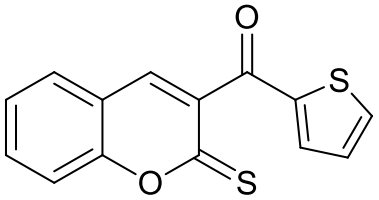
Yellow crystals. m.p. 188–190 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.83 (s, 3H), 6.92 (d, 2H, *J* = 6.6 Hz), 7.29 (d, 1H, *J* = 1.8 Hz), 7.42 (d, 1H, *J* = 1.8 Hz), 7.63 (d, 1H, *J* = 8.7 Hz), 7.87 (d, 1H, *J* = 8.7 Hz), 7.97 (d, 2H, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 54.5, 112.8, 120.5, 123.5, 124.0, 124.5, 126.7, 128.4, 129.8, 129.9, 135.8, 148.3, 162.5, 187.5, 194.7; IR (KBr): 1232, 1602, 1657, 3049, 3064 cm^{-1}; MS: *m/z* 330 (M^+). Anal. Calcd for $C_{17}H_{11}ClO_3S$: C, 61.73; H, 3.35; S, 9.69. Found: C, 61.77; H, 3.32; S, 9.63%.

h) 3-(4-Methoxybenzoyl)-6,8-dichloro-2H-chromene-2-thione (**68h**)



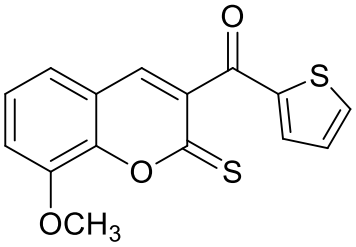
Yellow crystals. m.p. 196–198 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.83 (s, 3H), 6.92 (d, 2H, *J* = 6.6 Hz), 7.39 (s, 1H), 7.47 (s, 1H), 7.51 (s, 1H), 7.97 (d, 2H, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 54.5, 112.9, 120.4, 123.5, 124.0, 124.5, 126.7, 128.4, 129.8, 129.9, 135.8, 148.3, 162.5, 187.5, 193.5; IR (KBr): 1230, 1608, 1655, 3049, 3062 cm^{-1}; MS: *m/z* 364 (M^+). Anal. Calcd for $C_{17}H_{10}Cl_2O_3S$: C, 55.90; H, 2.76; S, 8.78. Found: C, 55.85; H, 2.79; S, 8.73%.

i) 3-(2-Acetylthiophene)-2H-chromene-2-thione (**68i**)



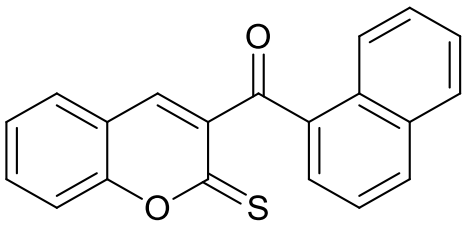
Yellow crystals, m.p. 176–178 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12–7.14 (m, 1H), 7.36–7.40 (m, 1H), 7.59–7.69 (m, 4H), 7.76 (d, *J* = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$):δ 116.6, 119.7,125.9, 128.2, 128.7, 133.3, 134.6, 135,2, 135.7, 138.7, 143.5, 156.9, 184.3, 193.3; IR (KBr): 1265, 1554, 1642, 3052 cm^{-1}; MS, *m/z* : 372 (M^+). Anal. calcd. for $C_{14}H_8O_2S_2$: C, 61.74; H, 2.96, S, 23.55. Found: C, 61.69; H, 2.19, S, 23.47.

j) 3-(2-Acetylthiophene)-8-methoxy-2H-chromene- 2-thione (**68j**)



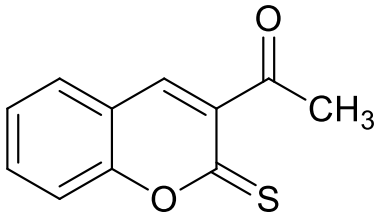
Yellow crystals. m.p. 167–168 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.86 (s, 3H), 7.08–7.16 (m, 2H), 7.28 (s, 1H), 7.30–7.38 (m, 1H), 7.63 (d, *J* = 3.6 Hz, 1H), 7.8 (d, *J* = 3.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.2, 112.5, 119.6, 120.4, 121.4, 125.6, 128.4, 133.8, 135.4, 138.8, 143.2, 145.6, 146.7, 185.1, 192.6; 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, 59.54; H, 3.28; S, 21.24%.

k) 3(1-Naphthyl)-2H-chromene-2-thione (**68k**)



Yellow crystals. m.p. 184–186 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38–7.45 (m, 3H), 7.54 (d, J = 6.3 Hz, 1H), 7.59–7.73 (m, 4H), 7.79 (s, 1H), 7.85 (d, J = 5.4 Hz, 1H), 7.93 (d, J = 6.0 Hz, 1H), 8.05 (d, J = 6.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 111.7, 119.8, 120.0, 124.2, 125.8, 126.2, 126.8, 128.4, 128.5, 128.7, 130.4, 131.1, 133.2, 133.4, 133.7, 134.1, 140.0, 157.1, 193.6, 194.1; IR (KBr): 1232, 1610, 1652, 3025, 3056 cm^{-1}; MS: m/z 316 (M^+). Anal. Calcd for $C_{20}H_{12}O_2S$: C, 75.93; H, 3.82; S, 10.14. Found: C, 75.85; H, 3.88; S, 10.17.%.

l) 3-Acetyl-2H-chromene-2-thione (**68l**)



Yellow crystals. m.p. 178–179 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.43 (s, 3H), 7.36–7.40 (m, 2H), 7.52–7.58 (m, 2H), 7.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5, 114.9, 119.8, 127.4, 128.4, 129.6, 131.0, 134.9, 157.2, 191.7, 193.7; IR (KBr): 1215, 1608, 1652, 2972, 3018, 3062 cm^{-1}; MS: m/z 204 (M^+). Anal. Calcd for $C_{11}H_8O_2S$: C, 64.69; H, 3.95; S, 15.70. Found: C, 64.75; H, 4.02; S, 15.66%.

3.8 Conclusion

In this chapter, two synthetic protocols for chromene-2-thiones from β-oxodithioesters have been described. The first one is the conventional heating method and the second is the solvent-free metal-free microwave heating method. Both synthetic protocols provided functionalized chromene-2-thiones in good yields. Compared to the conventional heating method, higher yield with lesser reaction time were achieved in the microwave heating method. The products were obtained in 6-8 min. with 85-97% yield. Moreover, simple and mild reaction conditions are the characteristics of this synthetic strategy. The reaction takes place in an environmentally benign condition using solventless microwave heating. Therefore it can be concluded that the microwave heating method is more advantageous than the conventional heating method.

The structure of the chromene-2-thiones was confirmed by elemental analysis, FT-IR, NMR, mass spectroscopic studies and single crystal X-ray analysis.

The synthesised chromene-2-thiones were screened for *in vitro* cytotoxicity against two cancerous cell lines- DLA and EAC. The *in vitro* cytotoxic analysis proved that most of the chromene-2-thiones are active against DLA and EAC cell lines and were found to be promising candidates for cancer chemotherapy. The compounds were also screened for their toxicity against normal cell lines. The *in vitro* cytotoxic analysis of chromene-2-thiones against normal cell lines revealed that they are selective in killing only the cancerous cell lines.

Use of chromene-2-thiones as anticancer drugs have been theoretically proved *via in silico* molecular docking studies. The results of molecular docking studies were in agreement with *in vitro* cytotoxic studies. The molecular docking studies showed that the chromene-2-thiones exhibited various interactions with human DNA topoisomerase II alpha and II beta. These interactions are responsible for the enhanced cytotoxicity of these compounds. The binding energy and inhibition constant for ligand-protein interaction being lowest for DNA topoisomerase II beta, it can be concluded that chromene-2-thiones are better inhibitors for DNA topoisomerase II beta.

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