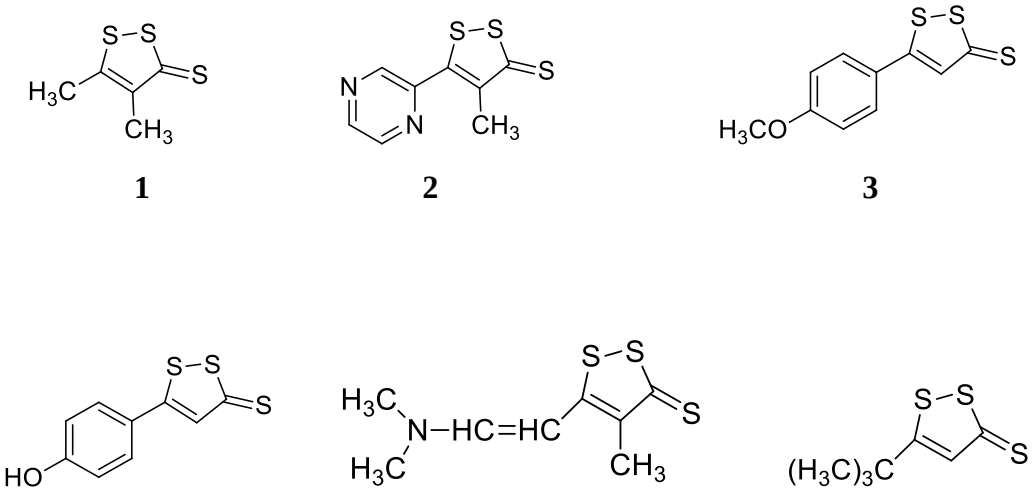
Chapter 4

Synthesis of 1,2-dithiole-3-thiones from β-oxodithioesters

4.1 Introduction

1,2-Dithiole-3-thiones are pseudoaromatic polysulphur containing heterocyclic compounds with wide applications in medicinal chemistry as drugs. The 4,5-dimethyl-1,2-dithiole-3-thione **1** was the first synthetic compound in this category. Oltipraz **2** is a 1,2-dithiole-3-thione derivative with antischistosomal properties and can effectively inhibit HIV-1 replication[1]. It exhibits chemopreventive activity by inducing the Phase II enzyme activities and thus decreasing the multiplication of tumour cells[2]. It protects cells from mutagenic effects by detoxifying cell metabolites[3].



1 **2** **3**

4 **5** **6**

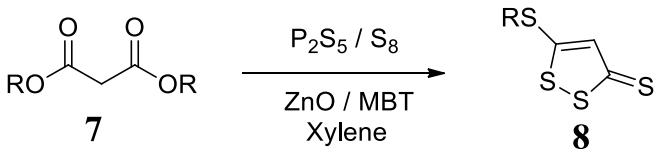
Fig.1 *1,2-dithiole-3-thione derivatives*

1,2-Dithiiole-3-thiones (**Fig. 1**) can induce antioxidant genes and were found to be chemopreventive[4]. Anethole dithiolethione [5-(4-methoxypheny)-3H-1,2-dithiole-3-thione] **3** was proved to be an efficient corrosion inhibitor for copper[5]. 5-(4-Hydroxypheny)-3H-1,2-dithiole-3-thione (ADT-OH) **4** was found to be specific for the prevention of bladder cancer[6]. The *in vitro* and *in vivo* antifungal studies proved that the 3H-1,2-dithiole-3-thione are fungitoxic[7]. The compounds such as 5-(N,N-dimethyl-2-aminoethenyl)-4-methyl-3H-1,2-dithiole-3-thione **5** and 5-*t*-butyl-3H-1,2-dithiole-3-thione **6** were found to be better chemopreventive agents than oltipraz. These compounds can effectively prevent hepatic tumorigenesis[8].

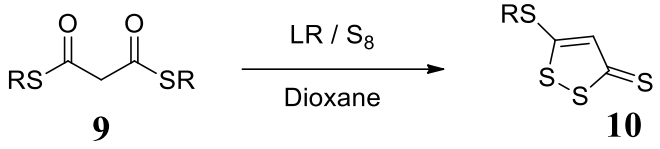
4.2 Strategies for 1,2-dithiole-3-thiones synthesis

Various synthetic methods have been developed for the synthesis of 1,2-dithiole-3-thiones. The reported synthetic protocols involve sulphonation of the substrate followed by cyclisation to get 1,2-dithiole-3-thiones. Reagents such as S_8, P_2S_5, P_4S_{10}, S_2Cl_2, Lawesson's reagent etc. were used as sulphonating agents.

Aimar *et al.* reported the synthesis of 5-alkylthio-3H-1,2-dithiole-3-thione **8** from dialkyl malonate esters **7** via sulphurisation using P_2S_5/S_8 mixture in boiling xylene in the presence of ZnO/mercaptobenzothiazole as catalyst[9] (**Scheme 1**). An alternate procedure for the synthesis of 5-alkylthio-3H-1,2-dithiole-3-thione from dithiomalonate has also been devised by the same group. Treatment of dithiomalonate **9** with Lawesson's reagent/S_8 in dioxane afforded 5-alkylthio-3H-1,2-dithiole-3-thione **10** (**Scheme 2**)[10]. It was found that Lawesson's reagent/S_8 mixture in dioxane gave a better yield than P_2S_5/S_8 mixture in boiling xylene.



Scheme 1



9 **10**

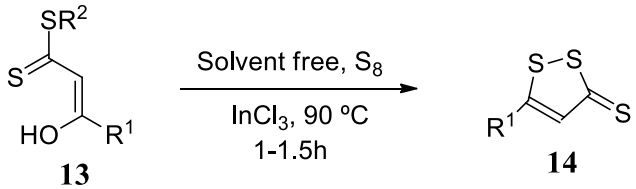
Scheme 2

Curphey devised a synthetic protocol for dithiolethiones from 3-oxo esters using a mixture of phosphorus pentasulphide and hexamethyldisiloxane (P_4S_{10}/HMDO) as a thionating agent[11]. It was found that the reagent mixture can effectively carry out the thionation of several organic compounds such as ketones, amides, lactams, esters and lactones to corresponding organosulphur compounds. For the synthesis of dithiolethiones, 3-oxoester **11** was refluxed with a mixture of P_4S_{10}/HMDO in xylene when 3-oxoester undergo thionation affording corresponding dithiolethione **12** (**Scheme 3**). The yield was found to be better than the thionation using Lawesson's reagent or P_4S_{10} alone.

11 **12**

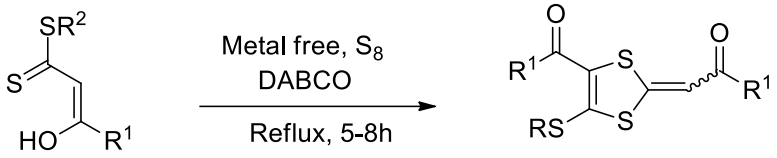
Scheme 3

1,2-dithioles as well as 1,3-dithioles have been synthesised from α-enolic dithioesters[12]. Treatment of α-enolic dithioesters **13** with elemental sulphur in the presence of catalytic amounts of $InCl_3$ afforded 1,2-dithioles **14** under solvent-free conditions (**Scheme 4**). A metal-free conversion of α-enolic dithioesters **15** to 1,3-dithioles **16** has been achieved by refluxing it with DABCO in toluene. This resulted in the self-coupling of dithioesters *via* C-S bond formation affording 1,3-dithioles (**Scheme 5**).



13 **14**

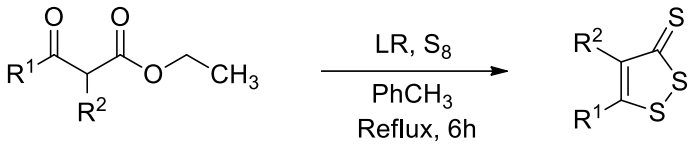
Scheme 4



15 **16**

Scheme 5

β-Keto esters have been converted to dithiolethiones by treatment with elemental sulphur and Lawesson's reagent[13]. Refluxing a mixture of β-oxo ester or β-keto ester **17**, S_8 and Lawesson's reagent (LR) in toluene afforded dithiolethiones **18** in good yields (**Scheme 6**). The synthesised dithiole thiones were found to induce glutathione production in cells.

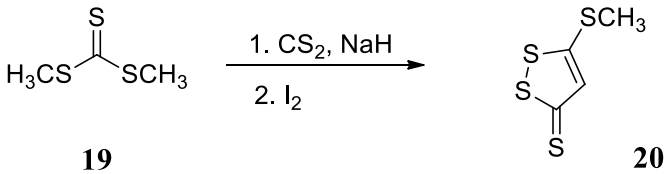


17 **18**

Scheme 6

1,2-Dithiole-3-thiones are susceptible to addition reactions with activated acetylenes such as dibenzoyl acetylene and dimethyl acetylenedicarboxylate resulting in 2-thio formyl methylene-1,3-dithioles, thiopyran spiro-1,3-dithioles and bis-1,3-dithiolylidene-2-butenes[14].

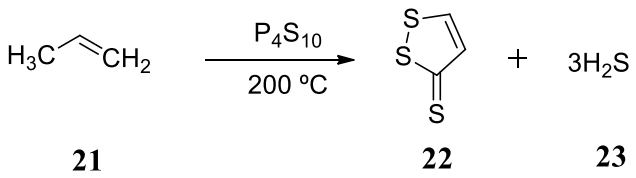
Lu *et al.* devised a one-pot synthetic method for 1,2-dithiole-3-thiones[15]. In this method dimethyl trithiocarbonate **19** was treated with carbon disulphide and sodium hydride in THF for 6h. Then it was quenched with iodine to yield 1,2-dithiole-3-thione **20** (**Scheme 7**).



19 **20**

Scheme 7

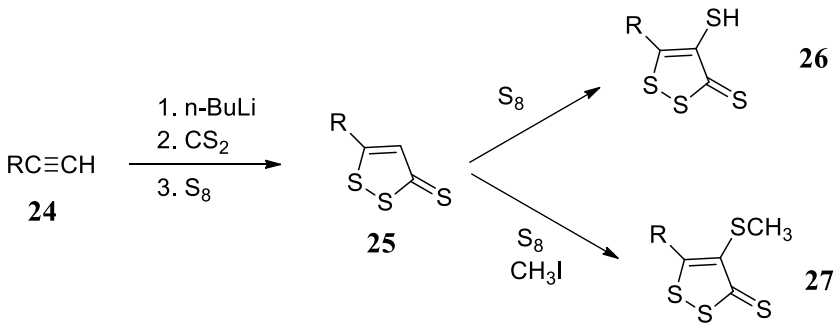
1H-1,2-dithiole-1-thiones and 3H-1,2-dithiole-3-thiones have been synthesised from compounds with allylic methyl group **21**[16]. Dehydrogenation and sulphurisation required for this conversion were achieved by treating the compound having an allylic methyl group with sulphur or phosphorous pentasulphide (**Scheme 8**).



21 **22** **23**

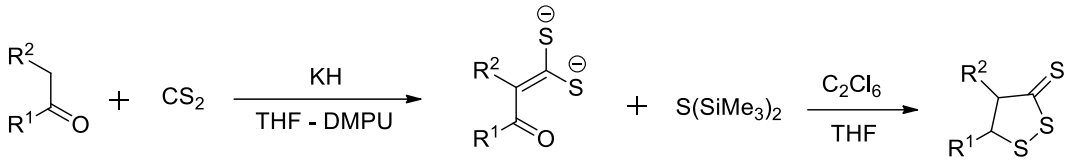
Scheme 8

Adams successfully synthesised 4-mercapto-1,2-dithiole-3-thiones from compounds with the terminal alkynyl group having acidic hydrogen **24**[17]. The compound was treated with n-butyllithium (n-BuLi) to achieve deprotonation of terminal alkynes. This was followed by treatment with carbon disulphide and sulphur when it undergo sulphurisation and ring closure affording an anionic intermediate which on acidification afforded 1,2-dithiole-3-thiones **25**. Treatment of the intermediate with alkylating agents afforded 4-alkylthio-1,2-dithiole-3-thiones **26** and **27** (**Scheme 9**).



Scheme 9

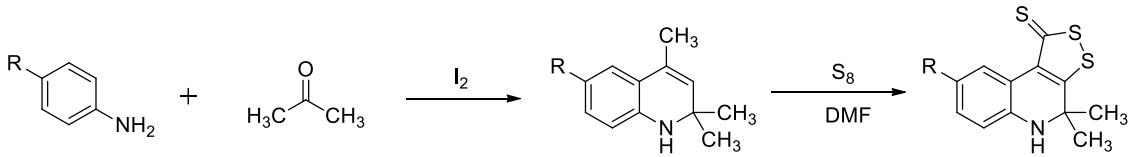
Curphey suggested a synthetic strategy for 1,2-dithiol-3-thiones from ketone[18]. Ketones **28** were treated with CS_2 **29** and 2 equivalents of KH in THF- DMPU (N, N'-dimethylpropyleneurea) to yield dianions of 3-oxodithioic acids **30**. The dianions produced were then treated with hexamethyldisilathiane **31** and hexachloroethane in THF to afford 4,5-disubstituted 1,2-dithiole-3-thiones **32** (**Scheme 10**).



28 **29** **30** **31** **32**

Scheme 10

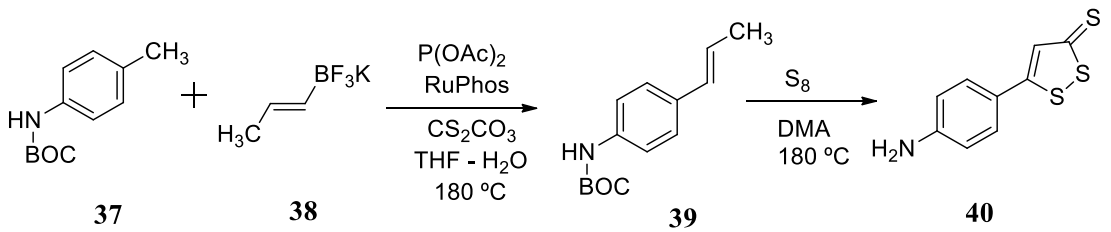
Kartsev succeeded in synthesising 4,4-dimethyl-4,5-dihydro-1H-[1,2]dithiolo[3,4-c]quinolone-1-thiones from aryl amines. First, aryl amines **33** were treated with acetone **34** in the presence of the catalytic amount of iodine to obtain 2,2,4-trimethyl-1,2-dihyroquinolines **35**. Subsequent treatment of the dihydroquinolines with a 5-fold excess of sulphur in DMF resulted in the synthesis of 4,4-dimethyl-4,5-dihydro-1H-[1,2]dithiolo[3,4-c]quinolone-1-thiones **36** in good yields[19] (**Scheme 11**).



33 **34** **35** **36**

Scheme 11

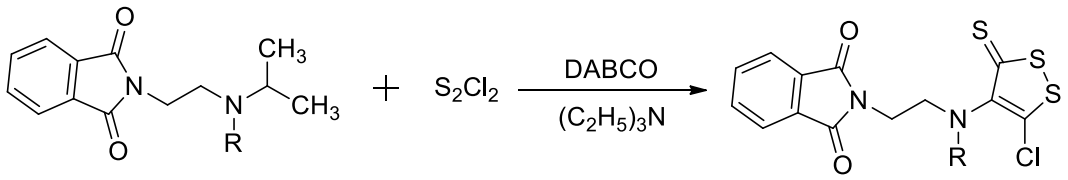
Hammers synthesised 5-(4-aminophenyl)-3H-1,2-dithiole-3-thione (ADT-NH_2) from 4-chloroaniline[20]. BOC protected 4-chloroaniline **37** was treated with potassium trans-1-propenyltrifuoroborate **38** in the presence of palladium acetate as the catalyst to yield tert-butyl(E)-(4-(prop-1-en-1-yl)phenyl)carbamate **39**. The subsequent treatment with elemental sulphur and DMA at 180 ºC resulted in the synthesis of dithiolethione which upon deprotection afforded ADT-NH_2 **40** (**Scheme 12**).



37 **38** **39** **40**

Scheme 12

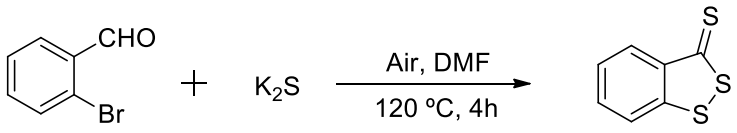
5-Chloro-1,2-dithiole-3-thiones were synthesised by Valverde using N-(2-phthalimidoethyl)-N-alkyl isopropyl amines as synthons (**Scheme 13**). In this synthetic procedure, a mixture of N-(2-phthalimidoethyl)-N-alkyl isopropyl amines **41** and DABCO in chloroform was treated with S_2Cl_2 **42**. The reaction was carried out at room temperature for three days. After that, it was treated with triethylamine at room temperature for two days to afford 5-chloro-1,2-dithiole-3-thiones **43**.



41 **42** **43**

Scheme 13

An efficient, catalyst-free synthesis for 3H-benzo[1,2]-dithiole-3-thiones has been proposed by Jin *et al.*[21]. The synthetic protocol involves the intramolecular heteroannulation reaction between 2-halobenzaldehyde **44** and potassium sulphide **45**. 2-Halobenzaldehyde was treated with potassium sulphide in DMF in the presence of air at 120 ºC for 4h (**Scheme 14**). Potassium sulphide induces aromatic substitution of 2-halobenzaldehyde and it also provides elemental sulphur by oxidation with air affording 3H-benzo[1,2]-dithiole-3-thiones **46** in good yields.

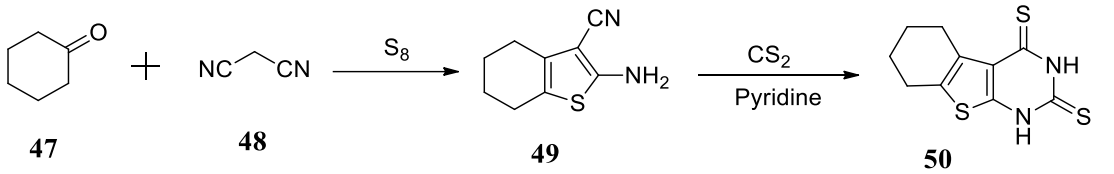


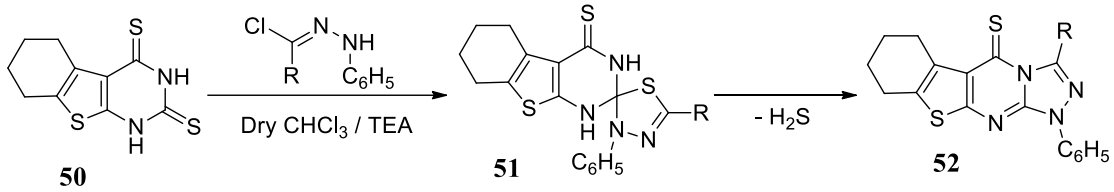
44 **45** **46**

Scheme 14

Pyrimidine-2,4-dithiones have been synthesised by Hafez *et al.* using 3-(2-aminothiophene)-carbonitriles as precursors[22]. The reaction between cyclic ketone **47**, malononitrile **48**, triethylamine and elemental sulphur in absolute alcohol resulted in the formation of 3-(2-aminothiophene)-carbonitriles **49**. It was then refluxed with carbon disulphide in dry pyridine to afford thieno[2,3-d]pyrimidine-2,4-dithiones **50** in excellent yields. The thienopyrimidine-2,4-dithiones **50** when treated with

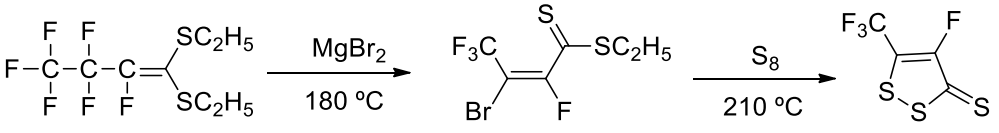
hydrazonyl chlorides undergo a 1,3-dipolar cycloaddition reaction furnishing 1,3-disubstituted triazolo[1,2,4]thieno[2,3-d]pyrimidines **52**. This cycloaddition was found to be highly chemoselective that takes place at C-2 of the thienopyrimidine-2,4-dithiones yielding triazolo[1,2,4]thieno[2,3-d]pyrimidines exclusively (**Scheme 15**). The synthesised compounds were found to exhibit antibacterial activity against *E. coli, S. aureus* and *P. putida.* These were also found to be active against human immunodeficiency virus-1 (HIV-1).



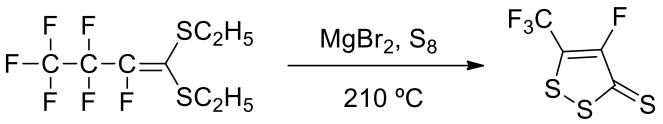


Scheme 15

Timoshenko *et al.* synthesised 4-fluoro-5-trifluoromethyl-3*H*-1,2-dithiole-3-thione from perfluoroketene diethyl thioacetal[23]. The synthesis was achieved in two steps. In the first step, perfluoroketene diethyl thioacetal **53** was heated with magnesium bromide at 180 ^{o}C to afford β-bromo-β-trifluoromethyl dithiocrotonic ester **54**. In the second step, β-bromo-β-trifluoromethyl dithiocrotonic ester **54** was treated with elemental sulphur at 210 ºC to get 4-fluoro-5-trifluoromethyl-3*H*-1,2-dithiole-3-thione **55** (**Scheme 16**). The direct conversion of perfluoroketene diethyl thioacetal **53** to 4-fluoro-5-trifluoromethyl-3*H*-1,2-dithiole-3-thione **55** was achieved by heating it at 210 ºC with magnesium bromide and elemental sulphur (**Scheme 17**).



Scheme 16



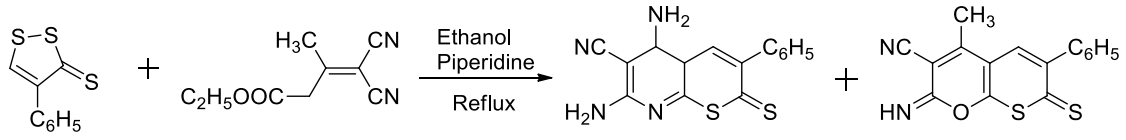
53 **55**

Scheme 17

Synthetic and biological applications of 1,2-dithiole-3-thiones

The 1,2-dithiole-3-thiones are susceptible to addition reactions with activated acetylenes such as dibenzoyl acetylene and dimethyl acetylene dicarboxylate resulting in 2-thio formyl methylene-1,3-dithioles, thiopyranspiro-1,3-dithioles and bis-1,3-dithiolylidene-2-butenes[14]. The dithiolethiones are also susceptible to nucleophilic attack yielding a wide range of products depending on the ring substituents and the nucleophile[24]. Position 5 of the dithiole ring was found to be the centre for nucleophilic attack.

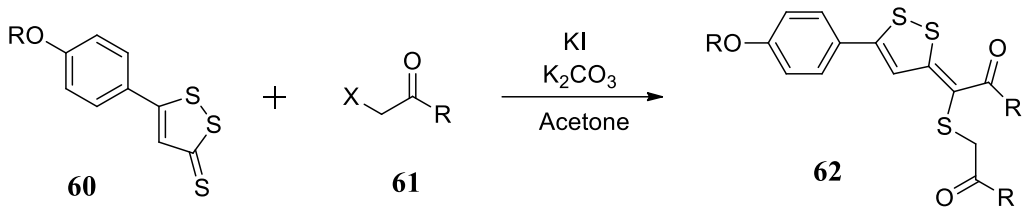
1,2-Dithiole-3-thiones were found to react readily with compounds containing active methylene groups yielding 1,2-dithiole-3-ylidenes[25]. 1,2-Dithiole-3-thiones **56** are susceptible to ring opening-ring closure reactions with α,β-unsaturated nitriles **57** to yield pyridine derivatives **58** and derivatives of pyrane **59** (**Scheme 18**).



56 **57** **58** **59**

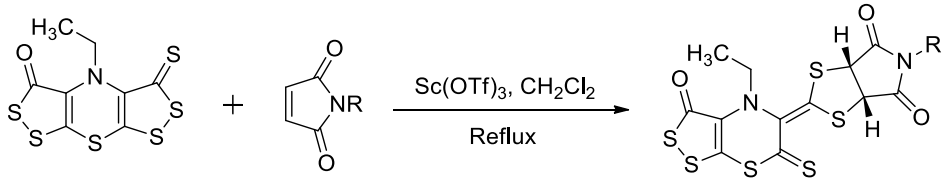
Scheme 18

Couto put forward a single step conversion of 3H-1,2-dithiole-3-thiones **60** to (E)-3[1-(alkylthio)alkylidene]-3H-1,2-dithiole **62** *via* treatment with α-haloketones **61** in the presence of potassium iodide[26] (**Scheme 19**). The conversion was accomplished due to the electrophilic character of the thiocarbonyl group present at position 3 of the 1,2-dithiole. The conversion was carried out both through conventional and microwave methods and it was proved that microwave irradiation provided better yields.



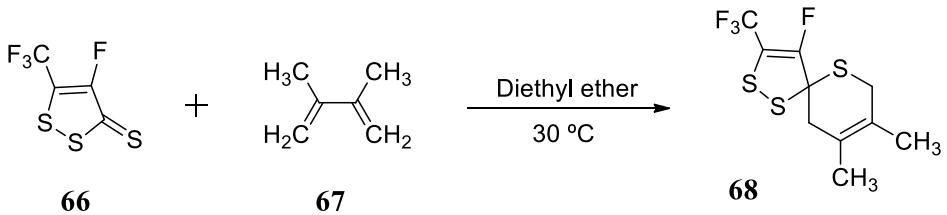
Scheme 19

Furtes *et al.* synthesised pyrrolidine fused 1,3-dithiolanes from polycyclic dithiolethiones[27]. Bisdithioloketothione **63** undergo cycloaddition reaction with maleimides **64** in the presence of scandium triflate catalyst in dichloromethane to afford pyrrolidine fused 1,3-dithiolanes **65** (**Scheme 20**). The presence of a number of sulphur atoms in the ring and the conjugated α,β-unsaturated thione groups make these compounds highly sensitive to mercury (II) ion. These compounds with complex structures are useful as selective indicators in the detection of mercury (II) ions.

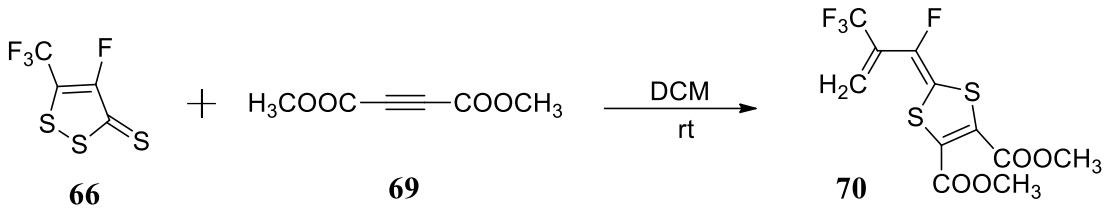


Scheme 20

4-Fluoro-5-trifluoromethyl-3*H*-1,2-dithiole-3-thiones **66** act as dienophiles as well as 1,3-dipoles[23]. These compounds undergo cycloaddition reaction with dienes such as 2,3-dimethylbuta-1,3-diene **67** in diethyl ether at 30 ºC yielding cycloadducts **68** (**Scheme 21**). This reaction clearly showed its dienophilic nature. As 1,3-dipoles, these compounds **66** undergo cycloaddition reaction with dimethyl acetylenedicarboxylate (DMAD) **69** in dichloro methane (DCM) at room temperature affording thioketones **70** as cycloadducts (**Scheme 22**).



Scheme 21



66 **69** **70**

Scheme 22

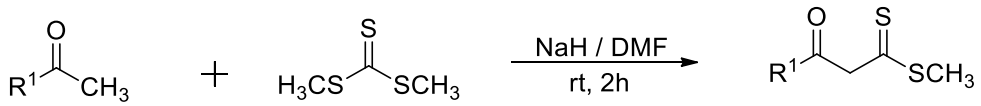
4.3 Results and discussion

A number of synthetic protocols for 1,2-dithiole-3-thiones have been reported in the literature. This involved the use of various reagents and solvents which are not so eco-friendly. In the present work, an eco-friendly solvent-free, metal-free, microwave heating method for 1,2-dithiole-3-thiones has been developed. *In vitro* anticancer properties of the synthesised compounds were studied in cancer cell lines as well as in normal cell lines. *In silico* molecular docking studies supported *in vitro* anticancer studies.

The synthesis of 1,2-dithiole-3-thiones involves two steps.

1) Synthesis of β-oxodithioesters from ketones.
2) Synthesis of 1,2-dithiole-3-thiones from β-oxodithioesters.

β-Oxodithioesters have been selected as synthons for 1,2-dithiole-3-thiones. In this method, a mixture of ketones **71** (1 eq.), dimethyl trithiocarbonate **72 (**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 **73** in good yields (**Scheme 23**).

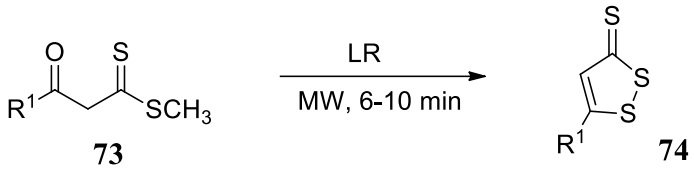


71 **72** **73**

Scheme 23

1,2-dithiole-3-thiones were synthesised from β-oxodithioester **73**. In this synthetic method, β-oxodithioester **73** (1 eq.) was mixed with Lawsson's reagent (LR) (1 eq.) and irradiated under microwave of power 400W at 140 ºC for 6-10 min to afford 1,2-dithiole-3-thiones **74** in good yields (**Scheme 24**).

The characteristic feature of this synthetic protocol is that the reaction was carried out *via* the microwave method in the absence of any base or solvent. The only reagent used along with β-oxodithioesters was Lawsson's reagent which is a selective sulphurising agent. The reaction proceeds by an *in situ* sulphurisation of the β-carbonyl group of the β-oxodithioesters followed by cyclisation with the expulsion of the methyl moiety to afford 1,2-dithiole-3-thiones **74**.



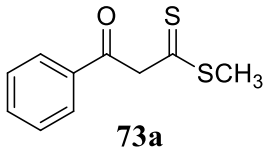
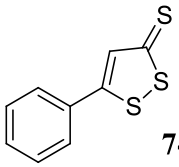
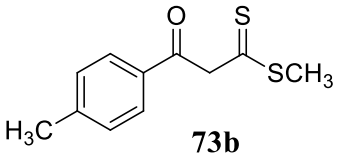
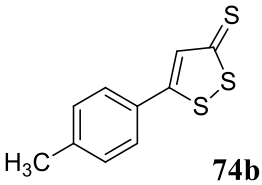
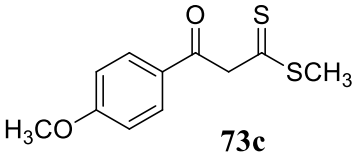
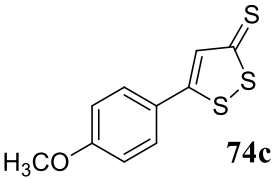
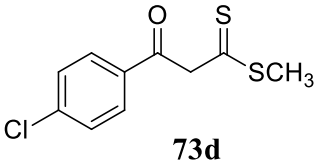
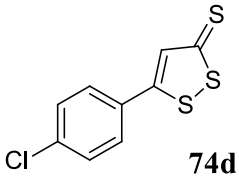
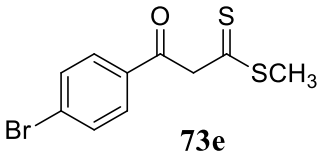
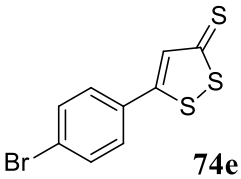
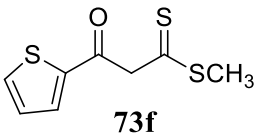
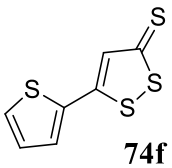
73 **74**

Scheme 24

The β-oxodithioester **73a** was mixed with Lawsson's reagent (LR) and irradiated under microwave conditions for 6-10 min. After completion of the reaction (monitored by TLC), the reaction mixture was dissolved in dichloromethane (DCM) and purified by column chromatography using silica gel as stationary phase and hexane: chloroform (8:2) as eluent to afford 5-phenyl-3H-1,2-dithiole-3-thione **74a** in 90% yield. ^{1}H NMR spectrum of **74a** exhibited peaks corresponding to aromatic protons in the range δ 7.37–7.59 ppm. ^{13}C NMR peaks appeared in the aromatic region from δ 126.9-172.9 ppm and the thiocarbonyl carbon appears at δ 215.2 ppm.

1,2-Dithiole-3-thiones **74b-f** were prepared using the same protocol. The structure of the synthesised compounds has been established based on their analytical and spectral data. The results of the synthesis are summarised in **Table 1**. Yields of the 1,2-dithiole-3-thiones obtained from bromo and chloro substituted β-oxodithioesters are lower than other thiones. This might be due to the nucleophilic substitution of the halogen atom present on the aromatic ring by the sulphur atom during the sulphurisation process.

Table. 1 1,2-Dithole-3-thiones synthesised:

Entry	β-oxodithioester	1,2-dithiole-3-thione	Yield (%)
1	 **73a**	 **74a**	90
2	 **73b**	 **74b**	93
3	 **73c**	 **74c**	92
4	 **73d**	 **74d**	88
5	 **73e**	 **74e**	86
6	 **73f**	 **74f**	90

4.4 Characterisation

The structure of the synthesised 1,2-dithiole-3-thiones was confirmed by FT-IR, ^{1}H NMR and ^{13}C NMR spectral data. 5-Phenyl-3H-1, 2-dithiole-3-thione **(74a) (Fig. 2)** was taken as a representative molecule for the general discussion.

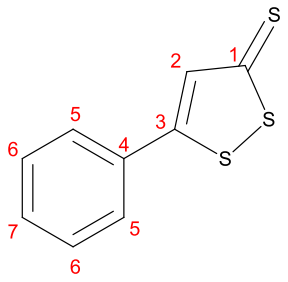


Fig. 2 *5-phenyl-3H-1,2-dithiole-3-thione (***74a***)*

The FT-IR spectrum of 5-phenyl-3H-1,2-dithiole-3-thione (**74a**) showed major peaks at 1014, 1107, 1174, 1305, 1512, 1589 and 3026 cm^{-1} (**Fig. 3**). The peaks at 3026 cm^{-1} and 1305 cm^{-1} corresponds to C-H stretching and bending vibrations respectively. Presence of the thiocarbonyl group is indicated by a strong peak at 1107 cm^{-1}. Two peaks at 1512 cm^{-1} and 1589 cm^{-1} represent carbon-carbon double bonds of 5-phenyl-3H-1,2-dithiole-3-thione.

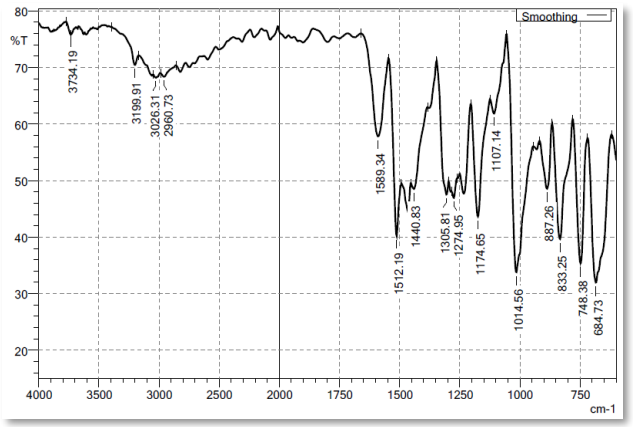


Fig. 3 *FT-IR spectrum of 5-phenyl-3H-1,2-dithiole-3-thione (***74a***)*

Structure of 5-phenyl-3H-1,2-dithiole-3-thione (**74a**) was confirmed by the ^{1}H NMR spectrum (**Fig. 4**). The proton at position C2 is observed as one proton singlet at δ 7.37 ppm. Two proton multiplet at δ 7.59-7.57 ppm corresponds to the protons at position C5. The protons at C6 are observed as two proton multiplet at δ 7.43-7.39 ppm. One proton multiplet δ 7.49-7.47 ppm denotes the C7 aromatic proton.

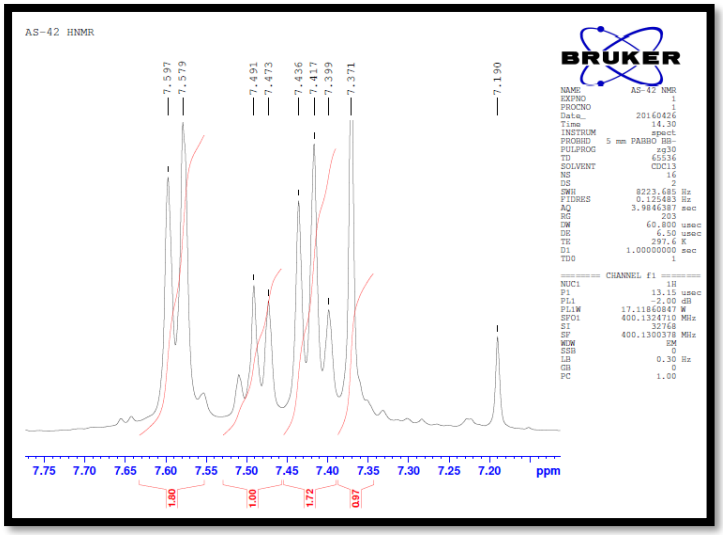


Fig. 4 *^{1}H NMR spectrum of 5-phenyl-3H-1,2-dithiole-3-thione (**74a**)*

The ^{13}C NMR spectrum of 5-phenyl-3H-1,2-dithiole-3-thione (**74a**) (**Fig. 5**) is in agreement with both FT-IR and ^{1}H NMR data. The downfield peak at δ 215.2 ppm is attributed to thiocarbonyl carbon C1. The ethylenic carbons C2 and C3 give peaks at δ 135.9 and 172.9 ppm respectively. The signals at δ 126.9, 129, 132 and 133.0 ppm correspond to phenyl carbons C7, C5, C6 and C4 respectively.

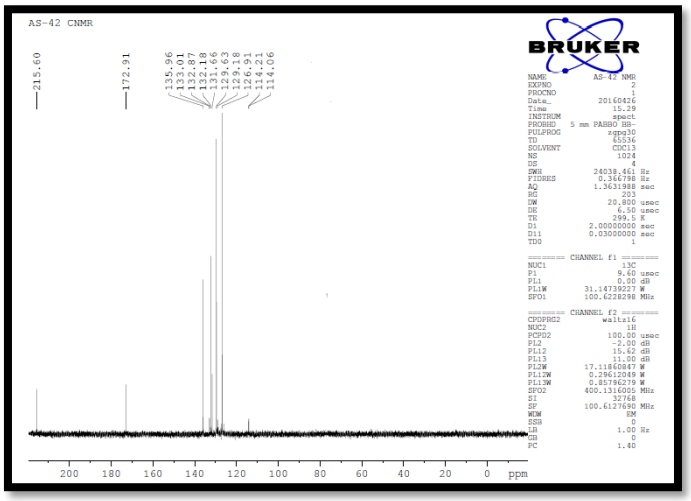


Fig. 5 *^{13}C NMR spectrum of 5-phenyl-3H-1,2-dithiole-3-thione (***74a***)*

4.5 Biological Studies - *In vitro* cytotoxicity

In vitro cytotoxic activity of the synthesised 1,2-dithiole-3-thiones **74** against two cancerous cell lines: DLA (Dalton's Lymphoma Ascites cells) and EAC (Ehrlich Ascites Carcinoma cells) were studied. 5-Fluorouracil was used as the positive control. The tumour cells aspirated from the peritoneal cavity of tumour-bearing mice was used for the study. Cell viability was determined by the trypan blue exclusion method[28].

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 2**. These results indicate that the 1,2-dithiole-3-thiones **74** exhibit significant cytotoxic activity against the two cell lines studied. The activity is higher against EAC than DLA cell lines and found to be better than 5-fluorouracil, the drug used as standard.

The 1,2-dithiole-3-thiones **74** were also screened for their toxicity against normal cell lines (rat spleen cells). The results are shown in **Table 3**.

Table. 2 Cytotoxic activity of 1,2-dithiole-3-thiones against DLA and EAC cell lines:

Entry	1,2-dithiole-3-thiones (**74**)	IC_{50} values (μg/mL)	
		DLA	EAC
1	**74a**	48	54
2	**74b**	>100	>100
3	**74c**	>100	>100
4	**74d**	40	48
5	**74e**	34	44
6	**74f**	44	52
7	5-fluorouracil	27.2	48

Table. 3 Cytotoxic activity of 1,2-dithiole-3-thiones against Normal cell lines:

Drug concentration (μg/mL)	% Cytotoxicity	
	74a	**74e**
10	2.75±0	4.67±0
20	2.75±0	5.74±1
50	3.7±0	8.11±0.9
100	5.1±0.5	13.1±1.2
200	7.72±1.3	17.6±1.3

The *in vitro* cytotoxicity study revealed that the synthesised 1,2-dithiole-3-thiones **74** showed very low lethality of normal cell lines even when a high drug concentration was used. Thus, it was proved that the 1,2-dithiole-3-thiones were selective in killing only cancerous cell lines.

The *in vitro* cytotoxicity studies established the anticancer activity of 1,2-dithiole-3-thiones which was theoretically supported by *in silico* molecular docking studies.

4.6 Molecular docking studies on anticancer activity

The anticancer activity of 1,2-dithiole-3-thione (**74a)** was theoretically studied *via in silico* molecular docking method. Human DNA topoisomerase II a (alpha) and II b (beta) were selected as target proteins. The binding modes of the 1,2-dithiole-3-thione (**74a)** with the target proteins; human DNA topoisomerase II a (alpha) and II b (beta) was calculated (**Fig. 6**). The binding modes of **74a** with human DNA topoisomerase II alpha include conventional hydrogen bond, pi-cation, pi-sulfur and pi-alkyl interactions. The binding modes of **74a** with human DNA topoisomerase II beta include conventional hydrogen bonds, Vander Waals interactions, pi-pi T-shaped and pi-alkyl interactions. The binding energy, inhibition constant and various interactions of the ligands with active site residues are summarised in **Table 4** and **Fig. 7**.

Table. 4 Binding energies and possible interactions of the ligand **74a** with target proteins:

Ligand	Protein	Binding energy (Kcal/mol)	Inhibition constant (μM)	H-bonds	Other interactions
Comp-ound (**74a**)	DNA topoisomerase II alpha (PDB id: 4fm9)	-6.68	12.61	GLY A:855	Pi-cation Pi-sulfur Pi-alkyl
	DNA topoisomerase II beta (PDB id: 3qx3)	-6.14	31.75	SER A:480 DG D:10	Van der Waals Pi-pi T-shaped Pi-alkyl

The compound **74a** showed a binding energy of -6.68 Kcal/mol with an inhibition constant of 12.61 µM with human DNA topoisomerase II alpha whereas the binding energy with human DNA topoisomerase II beta was found to be -6.14 Kcal/mol with an inhibition constant of 31.75 µM. The highest binding energy and lowest inhibition constant value were obtained for the ligand **74a** with the protein, human DNA topoisomerase II a (alpha). The cut-off value for binding energy being −5 Kcal/mol, the values obtained in the docking study establish that the compound exhibit good anticancer properties. The binding energy values and the inhibition constant values show that the 1,2-dithiole-3-thione (**74a)** 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 **74a** is a better inhibitor for the protein, human DNA topoisomerase II a (alpha) than for human DNA topoisomerase II b (beta).

Fig. 6a

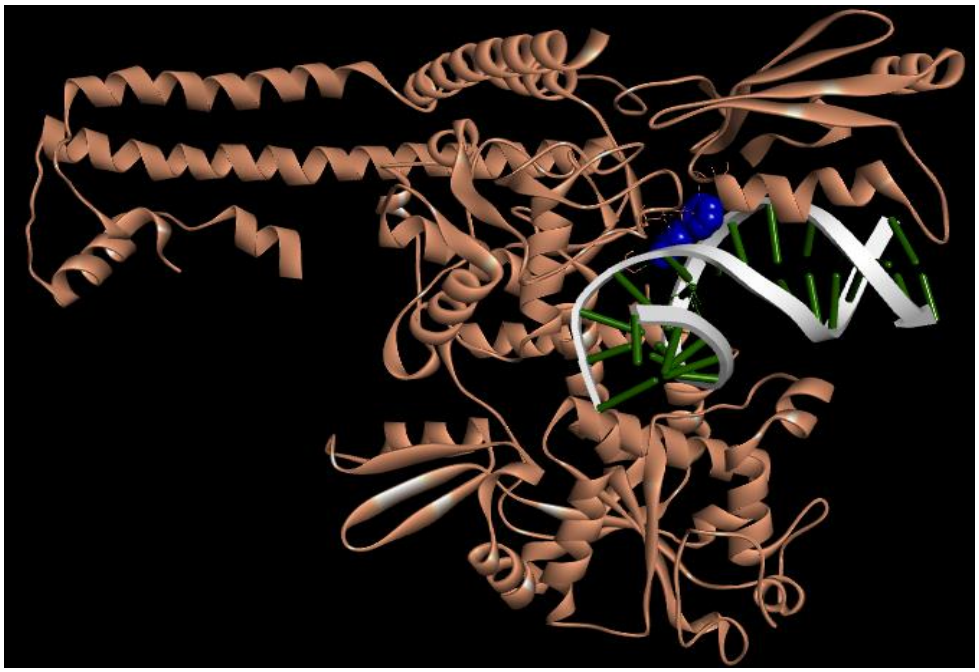


Fig. 6b

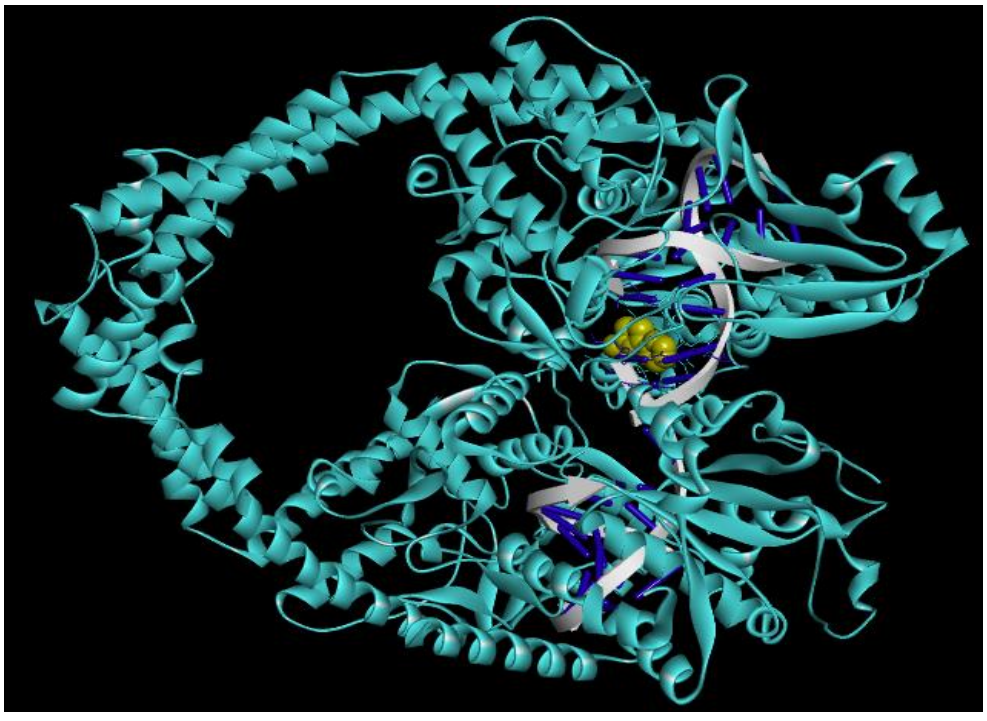


Fig. 6. ***(6a)*** *Bound conformation of human DNA topoisomerase II alpha and ligand* **74a**; ***(6b)*** *Bound conformation of human DNA topoisomerase II beta and ligand* **74a**

Fig. 7a

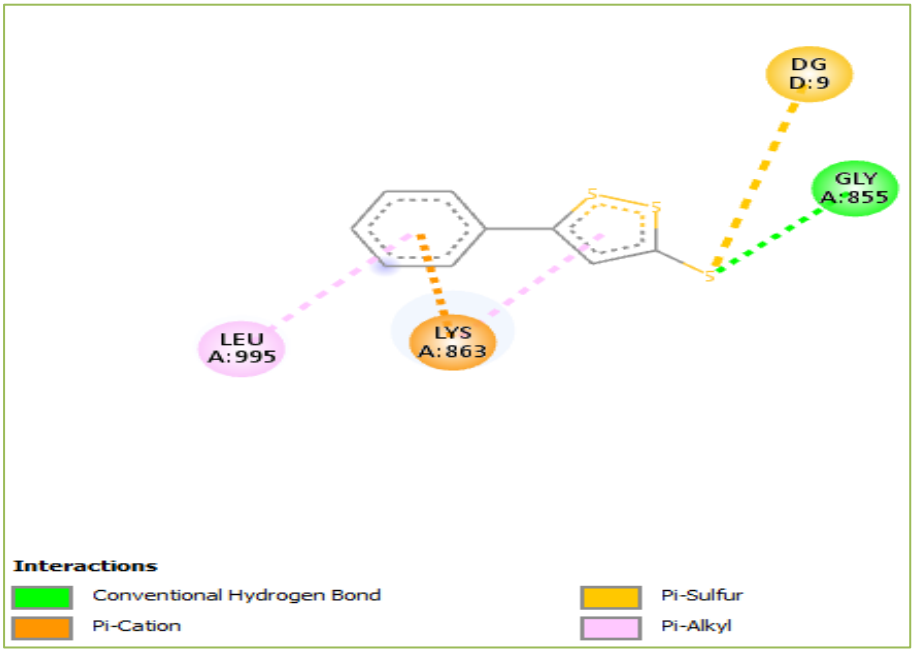


Fig. 7b

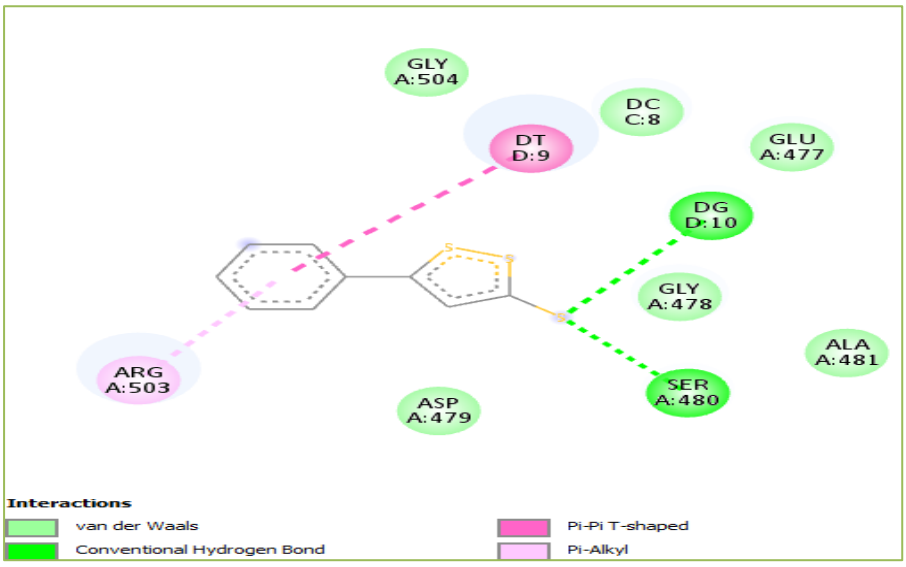


Fig. 7. ***(7a)*** *Interaction between human DNA topoisomerase II a (alpha) and ligand* **74a**; ***(7b)*** *Interaction between human DNA topoisomerase II b (beta) and ligand* **74a**

4.7 Experimental details

4.7.1 Materials and methods

All the chemicals used for the synthesis were purchased from Sigma-Aldrich, Bangalore, India. Organic solvents were purchased from Spectrochem, India. Solvents were used as received. ^{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 shift values 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). 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.

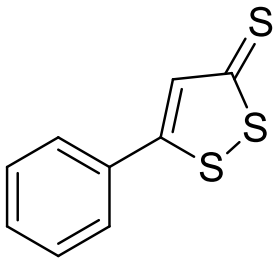
4.7.2 Synthesis of β-oxodithioester

β-oxodithioesters **73** were synthesised using the same method as explained in the chapter 3.

4.7.3 Synthesis of 1,2-dithiole-3-thiones

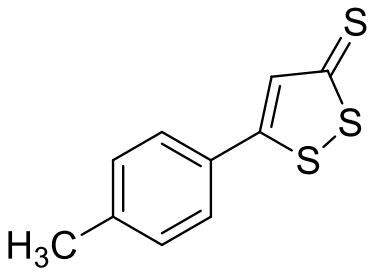
Lawsson's reagent (10 mmol, 1 eq.) was mixed with β-oxodithioester **73** (10 mmol, 1 eq.) in a glass vial and the mixture was irradiated under microwave power of 400 W at 140 °C for 6-10 min. This resulted in the sulphurisation of the β-oxodithioesters followed by its cyclisation affording 1,2-dithiole-3-thiones **74**. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was dissolved in dichloromethane (DCM) and purified by column chromatography using silica gel as stationary phase and hexane: chloroform (8:2) as eluent to afford 5-phenyl-3H-1,2-dithiole-3-thione **74** in 90% yield.

(a) 5-Phenyl-3H-1,2-dithiole-3-thione (**74a**).



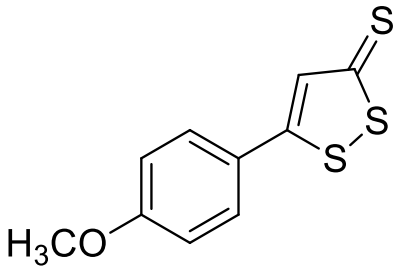
Yield: 92%, orange solid, m.p. 125 −126 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 (s, 1H), 7.43-7.39 (m, 2H), 7.49-7.47 (m, 1H), 7.59-7.57 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 126.9, 129, 132, 133.0, 135.9, 172.9, 215.2; IR (KBr): 1014, 1107, 1174, 1305, 1512, 1589, 3026 cm^{-1}.

(b) 5-(4-Methyl phenyl)-3H-1,2-dithiole-3-thione (**74b**)



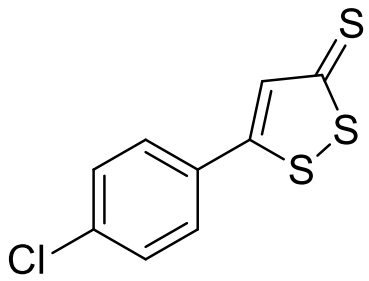
Yield: 94%, orange solid, m.p. 119 −120 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.42 (s, 3H), 7.31 (d, 8 Hz, 2H), 7.44 (s, 1H), 7.52 (d, 8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.4, 126.7, 128.6, 130.2, 135.2, 143.3, 173.2, 215.5; IR (KBr): 1016, 1182, 1228, 1278, 1311, 1475, 1595, 3018 cm^{-1}.

(c) 5-(4-Methoxyphenyl)-3H-1,2-dithiole-3-thione (**74c**)



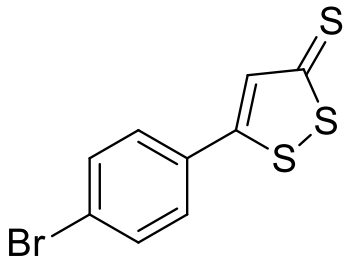
Yield: 93%, orange solid, mp 110 −111 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.88 (s, 3H), 6.99 (d, 8.4 Hz, 2H), 7.38 (s, 1H), 7.62 (d, 8.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 55.6, 114.9, 124.1, 128.5, 134.6, 162.8, 173.0, 215.0; IR (KBr): 1086, 1252, 1464, 1615, 2918, 3021 cm^{-1}.

(d) 5-(4-Chlorophenyl)-3H-1,2-dithiole-3-thione (**74d**)



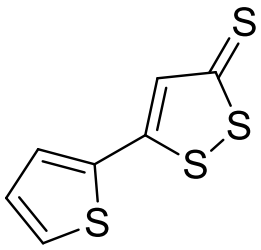
Yield: 85%, orange solid, mp 136 −137 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.16 (s, 1H), 7.36 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = 127.6, 129.5, 129.8, 135.6, 138.0, 171.2, 215.4; IR (KBr): 1092, 1254, 1432, 1612, 2934, 3026 cm^{-1}.

(e) 5-(4-Bromophenyl)-3H-1,2-dithiole-3-thione (**74e**)



Yield: 84%, orange solid, mp 127 −128 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31 (s, 1H), 7.45 (d, 8.7 Hz, 2H), 7.48 (m, 2H), 7.55 (d, 8.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 126.8, 128.1, 130.4, 132.8, 136.0, 171.1, 214.6; IR (KBr): 1062, 1178, 1234, 1473, 1579, 2924, 3043 cm^{-1}.

(f) 5-(Thiophen-2-yl)-3H-1,2-dithiole-3-thione (**74f**).



Yield: 84%, orange solid, mp 128 −129 ºC. Yield: 86%, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.12 (t, J = 2.1 Hz, 1H), 7.34 (s, 1H), 7.54 (d, J = 3.6 Hz, 1H), 7.55 (d, J = 5.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 128.8, 129.3, 131.2, 133.6, 134.2, 165.4, 214.3; IR (KBr): 1065, 1268, 1475, 1642, 2916, 3024 cm^{-1}.

4.8 Conclusion

A new solvent-free synthetic protocol for 1,2-dithiole-3-thiones from β-oxodithioesters has been developed. The ease of preparing the starting materials and simple and mild reaction conditions are the characteristics of this method. The reaction takes place in an environmentally benign condition using solvent less microwave heating. The structure of the 1,2-dithiole-3-thiones was confirmed by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

The synthesised 1,2-dithiole-3-thiones were screened for *in vitro* cytotoxicity against two cancerous cell lines DLA and EAC. The *in vitro* cytotoxic analysis proved that some of the 1,2-dithiole-3-thiones were 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 (rat spleen cells). The *in vitro* cytotoxic analysis of 1,2-dithiole-3-thiones against normal cell lines revealed that they are selective in killing only the cancerous cell lines.

The use of 1,2-dithiole-3-thiones as anticancer drugs have been theoretically proved *via in silico* molecular docking studies. The molecular docking studies showed that the 1,2-dithiole-3-thiones exhibited various interactions with human DNA

topoisomerase II a and II b. These interactions are responsible for the enhanced cytotoxicity of these compounds. The binding energy and inhibition constant for ligand-protein interaction being lowest for human DNA topoisomerase II a (alpha), it can be concluded that 1,2-dithiole-3-thiones are better inhibitors for human DNA topoisomerase II a (alpha) than human DNA topoisomerase II b (beta).

References

(1) Prochaska, H. J.; Fernandes, C. L.; Pantoja, R. M.; Chavan, S. J. Inhibition of Human Immunodeficiency Virus Type 1 Long Terminal Repeat-Driven Transcription by an in Vivo Metabolite of Oltipraz: Implications for Antiretroviral Therapy. *Biochem. Biophys. Res. Commun.* **1996**, *221*, 548–553. https://doi.org/10.1006/bbrc.1996.0633.

(2) Munday, R.; Zhang, Y.; Munday, C. M.; Li, J. Structure-Activity Relationships in the Induction of Phase II Enzymes by Derivatives of 3H-1,2-Dithiole-3-Thione in Rats. *Chem. Biol. Interact.* **2006**, *160*, 115–122. https://doi.org/10.1016/j.cbi.2005.12.011.

(3) Doherty, G. P.; Leith, M. K.; Wang, X.; Curphey, T. J.; Begleiter, A. Induction of DT-Diaphorase by 1,2-Dithiole-3-Thiones in Human Tumour and Normal Cells and Effect on Anti Tumour Activity of Bioreductive Agents. *Br. J. Cancer* **1998**, *77*, 1241–1252. https://doi.org/10.1038/bjc.1998.209.

(4) Otieno, M. A.; Kensler, T. W.; Guyton, K. Z. Chemoprotective 3H-1,2-Dithiole-3-Thione Induces Antioxidant Genes in Vivo. *Free Radic. Biol. Med.* **2000**, *28*, 944–952. https://doi.org/10.1016/S0891-5849(00)00175-1.

(5) Zhang, J.; Zhang, L.; Tao, G. A Novel and High-Efficiency Inhibitor of 5-(4-Methoxyphenyl)-3h-1,2-Dithiole-3-Thione for Copper Corrosion Inhibition in Sulfuric Acid at Different Temperatures. *J. Mol. Liq.* **2018**, *272*, 369–379. https://doi.org/10.1016/j.molliq.2018.09.095.

(6) Munday, R.; Zhang, Y.; Paonessa, J. D.; Munday, C. M.; Wilkins, A. L.; Babu, J. Synthesis, Biological Evaluation, and Structure-Activity Relationships of Dithiolethiones as Inducers of Cytoprotective Phase 2 Enzymes. *J. Med. Chem.* **2010**, *53*, 4761–4767. https://doi.org/10.1021/jm100425v.

(7) Giannini, F. A.; Aimar, M. L.; Sortino, M.; Gomez, R.; Sturniollo, A.; Juarez, A.; Zacchino, S.; De Rossi, R. H.; Enriz, R. D. In Vitro-in Vivo Antifungal Evaluation and Structure-Activity Relationships of 3H-1,2-Dithiole-3-Thione Derivatives. *Farm.* **2004**, *59*, 245–254. https://doi.org/10.1016/j.farmac.2003.08.004.

(8) Maxuitenko, Y. Y.; Libby, A. H.; Joyner, H. H.; Curphey, T. J.; MacMillan, D. L.; Kensler, T. W.; Roebuck, B. D. Identification of Dithiolethiones with Better Chemopreventive Properties than Oltipraz. *Carcinogenesis* **1998**, *19*, 1609–1615. https://doi.org/10.1093/carcin/19.9.1609.

(9) Aimar, M. L.; Rossi, R. H. One-Pot Synthesis of 5-Alkylthio-3H-1,2-Dithiole-3-Thiones. *Tetrahedron Lett.* **1996**, *37*, 2137–2140. https://doi.org/10.1016/0040-4039(96)00209-2.

(10) Aimar, M. L.; Kreiker, J.; De Rossi, R. H. One-Pot Synthesis of 3H-1,2-Dithiole-3-Thione Derivatives from Dithiolmalonic Esters. *Tetrahedron Lett.* **2002**, *43*, 1947–

1949. https://doi.org/10.1016/S0040-4039(02)00180-6.

(11) Curphey, T. J. Thionation of Esters and Lactones with the Reagent Combination of Phosphorus Pentasulfide and Hexamethyldisiloxane. *J. Org. Chem.* **2002**, *67*, 6461–6473. https://doi.org/10.1016/S0040-4039(01)02144-X.

(12) Koley, S.; Chanda, T.; Samai, S.; Singh, M. S. Switching Selectivity of α-Enolic Dithioesters: One Pot Access to Functionalized 1,2- and 1,3-Dithioles. *J. Org. Chem.* **2016**, *81*, 11594–11602. https://doi.org/10.1021/acs.joc.6b01802.

(13) Brown, D. A.; Betharia, S.; Yen, J. H.; Tran, Q.; Mistry, H.; Smith, K. Synthesis and Structure-Activity Relationships Study of Dithiolethiones as Inducers of Glutathione in the SH-SY5Y Neuroblastoma Cell Line. *Bioorganic Med. Chem. Lett.* **2014**, *24*, 5829–5831. https://doi.org/10.1016/j.bmcl.2014.10.005.

(14) McKinnon, D. .; Buchshriber, J. M. The Reaction of 5-Unsubstituted-1,2-Dithiole-3-Thiones with Some Activated Acetylenes. *Can. J. Chem.* **1971**, *49*, 3299–3304.

(15) Lu, F. L.; Keshavarz-K, M.; Srdanov, G.; Jacobson, R. H.; Wudl, F. A New Preparation of 5-(Alkylthio)-l,2-Dithiole-3-Thiones and a Highly Functionalized 1,3-Dithiole-2-Thione. *J. Org. Chem.* **1989**, *54*, 2165–2169. https://doi.org/10.1021/jo00270a028.

(16) Manahelohe, G. M.; Shikhaliev, K. S.; Potapov, A. Y. Synthesis of 1H-1 , 2-Dithiol-1-Thiones and Thioamides Containing Hydroquinoline Group. *Eur. Chem. Bull* **2015**, *4*, 350–355.

(17) Adams, H.; Chung, L. M.; Morris, M. J.; Wright, P. J. A Convenient Route to 4-Mercapto-1,2-Dithiole-3-Thiones from Terminal Alkynes. *Tetrahedron Lett.* **2004**, *45*, 7671–7674. https://doi.org/10.1016/j.tetlet.2004.08.091.

(18) Curphey, T. J.; Libby, A. H. Dianions of 3-Oxodithioic Acids: Preparation and Conversion to 3H-1,2-Dithiole-3-Thiones. *Tetrahedron Lett.* **2000**, *41*, 6977–6980. https://doi.org/10.1016/S0040-4039(00)01191-6.

(19) Kartsev, V.; Shikhaliev, K. S.; Geronikaki, A.; Medvedeva, S. M.; Ledenyova, I. V.; Krysin, M. Y.; Petrou, A.; Ciric, A.; Glamoclija, J.; Sokovic, M. Appendix A. Dithioloquinolinethiones as New Potential Multitargeted Antibacterial and Antifungal Agents: Synthesis, Biological Evaluation and Molecular Docking Studies. *Eur. J. Med. Chem.* **2019**, *175*, 201–214. https://doi.org/10.1016/j.ejmech.2019.04.046.

(20) Hammers, M. D.; Singh, L.; Montoya, L. A.; Moghaddam, A. D.; Pluth, M. D. Synthesis of Amino-ADT Provides Access to Hydrolytically Stable Amide-Coupled Hydrogen Sulfide Releasing Drug Targets. *Synlett* **2016**, *27*, 1349–1353. https://doi.org/10.1055/s-0035-1560603.

(21) Jin, H.; Jiang, D.; Gao, J.; Qiang, G.; Gong, Y. A Facile Synthesis of 3H-Benzo[1,2]Dithiole-3-Thiones and Their Condensation with Active Methylene Compounds. *Phosphorus, Sulfur Silicon Relat. Elem.* **2011**, *186*, 2341–2349. https://doi.org/10.1080/10426507.2011.600743.

(22) Hafez, H. N.; Hussein, H. A. R.; El-Gazzar, A. R. B. A. Synthesis of Substituted Thieno[2,3-d]Pyrimidine-2,4-Dithiones and Their S-Glycoside Analogues as Potential Antiviral and Antibacterial Agents. *Eur. J. Med. Chem.* **2010**, *45*, 4026–4034. https://doi.org/10.1016/j.ejmech.2010.05.060.

(23) Timoshenko, V. M.; Bouillon, J. P.; Shermolovich, Y. G.; Portella, C. Fluorinated

Ketene Dithioacetals. Part 9: Synthesis and Some Chemical Properties of New Fluorinated 3H-1,2-Dithiole-3-Thiones. *Tetrahedron Lett.* **2002**, *43*, 5809–5812. https://doi.org/10.1016/S0040-4039(02)01179-6.

(24) Fluery, M. .; Largeron, M. Studies of the Reaction f 1,2-Dithiole-3-Thiones with Nucleophiles. *Tetrahedron Lett.* **1985**, *41*, 3705–3715.

(25) Barsy, M. A.; Latif, F. M. A. El; Ahmed, S. M.; El-maghraby, M. A. Ring-Opening Ringn-Closure of 4-Phenyl-1,2-Dithiole-3-Thione. Novel Synthesis of Thio-Pyrano[2,3-b]Pyrane Derivatives. *Phosphorus. Sulfur. Silicon Relat. Elem.* **2000**, *165*, 1–6. https://doi.org/10.1080/10426500008076320.

(26) Couto, M.; Cabrera, M.; Echeverría, G. A.; Piro, O. E.; González, M.; Cerecetto, H. A Serendipitous One-Step Conversion of 3H-1,2-Dithiole-3-Thione to (E)-3-[1-(Alkylthio)Alkylidene]-3H-1,2-Dithiole: An Experimental and Theoretical Study. *Mol. Divers.* **2014**, *18*, 285–294. https://doi.org/10.1007/s11030-013-9499-x.

(27) Fuertes, P.; García-Valverde, M.; Cuevas, J. V.; Díaz De Greñu, B.; Rodríguez, T.; Rojo, J.; Torroba, T. Synthesis of Pyrrolidine-Fused 1,3-Dithiolane Oligomers by the Cycloaddition of Polycyclic Dithiolethiones to Maleimides and Evaluation as Mercury(II) Indicators. *J. Org. Chem.* **2014**, *79*, 2213–2225. https://doi.org/10.1021/jo500076c.

(28) Srtober, W. Trypan Blue Exclusion Test of Cell Viability, Appendix 3B. *Curr. Protoc. Immunol.* **1997**.