Chapter-5

Synthesis of dihydropyridine-2-thione and 3,4-diphenyl thiophene from β-oxodithioesters

5.1 Introduction

Several natural compounds as well as biologically active molecules are found to possess 2-pyridone moiety as a structural unit. The natural compounds with 2-pyridone moiety exhibit antitumour, antiviral, agrochemical and pharmacological properties. This include antibacterial agents, antifungals, cardio tonics and sedatives[1]. 2-Pyridones play a significant role in material science as intermolecular connectors[2].

Tamer *et. al.* synthesised functionalized pyridones having sulphisoxazole moiety[3]. These compounds exhibited better *in vitro* antibacterial, antifungal and antimicrobial activities than the sulphisoxazole. The molecular docking studies showed that these compounds have similar inhibitory properties towards enzymes as shown by sulpha drugs.

Heterocyclic moieties containing nitrogen and oxygen as the ring members are important building blocks for biologically active molecules and so these are regarded as an important class of compounds in medicinal chemistry. Several naturally occurring biologically active compounds possess 2-pyridone moiety. N-amino-2-pyridones exhibit anti-HIV, anticonvulsant, anticoagulant, antimicrobial, antifungal, antimalarial, antibacterial and anticancer properties. The nanoporous metal-organic frameworks (MOFs) exhibit catalytic properties due to porous structure and nano size function as catalysts in organic synthesis. Babaee *et al.* synthesised metal-organic framework MIL-101(Cr)-$N(CH_2PO_3H_2)_2$ that can catalyse the synthesis of N-amino-2-pyridone and pyrano[2,3-c]pyrazole derivatives[4]. In this method, the reaction between ethyl cyanoacetate, hydrazine hydrate, malononitrile and benzaldehyde in

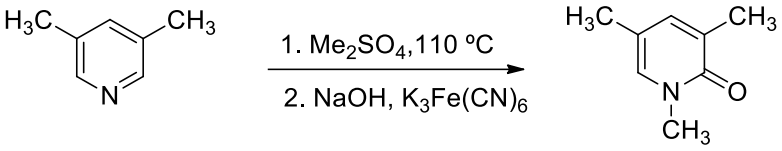
ethanol in the presence of MIL-101(Cr)-N $(CH_2PO_3H_2)_2$ as the catalyst resulted in the synthesis of N-amino-2-pyridone.

2-Pyridone is a diene with some aromatic character. It undergoes Diels-Alder [4+2] cycloaddition reaction with alkenes and alkynes yielding aromatic compounds. N-substituted pyridones undergo cycloaddition reaction with the compounds having activated multiple bonds such as benzyne, maleic anhydride and N-phenyl maleimide yielding Diels-Alder adducts[5]. However, N-unsubstituted 2-pyridones when treated with compounds having activated double bonds undergo nucleophilic addition yielding N-substituted pyridones. Cycloaddition of 2-pyridones with complex molecules is a powerful synthetic method in organic synthesis for obtaining structurally diverse target molecules.

Generally, 2-pyridones act as dienes in the Diels-Alder reaction. But 2-pyridones consisting of electron-withdrawing groups directly attached to the 2-pyridone ring can act as dienophiles. The sulphoxide and sulphone substituent attached to the 2-pyridone ring increases the reactivity of 2-pyridones as dienophiles. These pyridones when treated with electron-rich dienes afforded isoquinoline derivatives. Sulphoxides and sulphone-substituted dihydro-2-pyridones as well as 2-pyridones upon cycloaddition reaction with electron rich dienes afforded bicyclic and tricyclic products[6]. Sulphone-substituted 2-pyridones undergo cross-Diels-Alder reaction with dienes such as cyclopentadiene resulting in isoquinoline derivatives. Thio-substituted 1,3-butadiene undergo Aza-Diels-Alder reaction with aryl sulphonyl isocyanates affording 4-thio-substituted 2-pyridones.

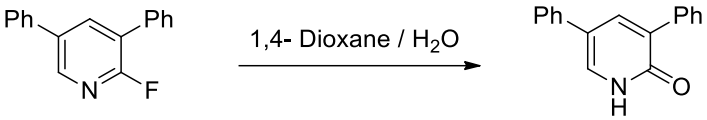
5.2 Synthesis of pyridones- a review

Various synthetic methods have been suggested for 2-pyridones from other aromatic heterocyclic compounds: from acyclic compounds, by miscellaneous cyclisation *etc*.[2]. Simple oxidation of pyridines **1** using dimethyl sulphate and potassium ferricyanide yielded corresponding 2-pyridones **2** (**Scheme 1**). 2-pyridones **4** have been synthesised by the hydrolysis of α-halopyridines **3** (**Scheme 2**).



1 **2**

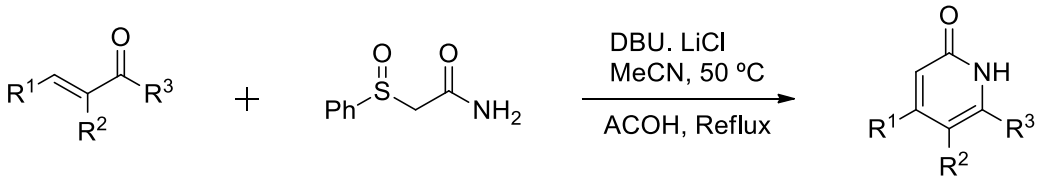
Scheme 1



3 **4**

Scheme 2

Since pyridones have an amide group in the aromatic ring, they exhibit dual properties as aromatic compounds and as amides. Among the various synthetic methods for pyridones, the cyclisation between 2-substituted acetamide and α,β-unsaturated ketone was found to be the most effective. Fuji *et al.* reported the synthesis of 2-pyridones **7** by 1,4-addition reaction between α,β- unsaturated ketones **5** and 2-(phenylsulphinyl)acetamide **6** using the catalyst 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and LiCl in acetonitrile[7] (**Scheme 3**). The 1,4-addition reaction is followed by the addition of acetic acid for cyclisation and subsequent E2 elimination of sulphoxide.

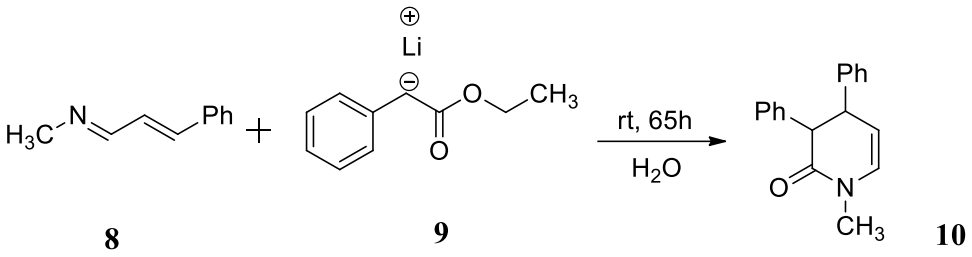


5 **6** **7**

Scheme 3

1-Azabutadienes are regarded as building blocks for derivatives of pyridone since they react with enolates of substituted acetate to provide pyridones or

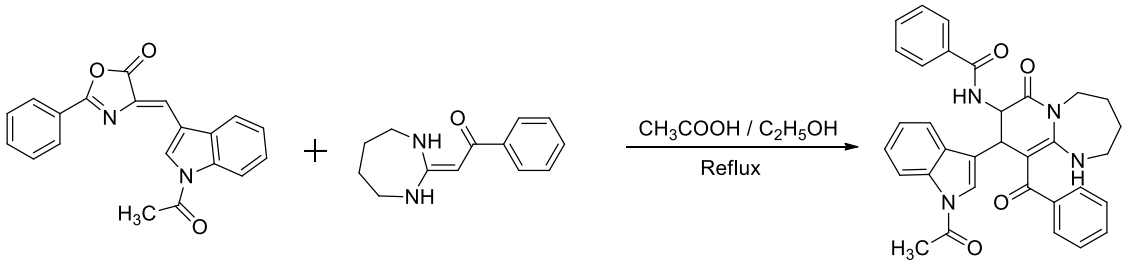
dihydropyridones[8]. One such synthon 1-methyl-4-phenyl-1-azabuta-1,3-diene **8** when allowed to react with a mixture of lithium diisopropylamide **9** and ethyl phenylacetate in THF for 65h at room temperature afforded 3,4-dihydro-2-pyridone **10** as the sole product (**Scheme 4**). The same reaction when carried out at a higher temperature yielded a mixture of four products; 3,4- dihydro-2-pyridone, 3,6-dihydro-2-pyridone, 5,6-dihydro-2-pyridone and 2-pyridone.



8 **9** **10**

Scheme 4

Lin *et al.* devised an economic and environment friendly protocol for functionalized bicyclic pyridone derivatives **13** through cascade reaction between 4-arylmethylene-2-phenyloxazol-5(4H)-ones **11** and heterocyclic ketene aminals **12** [9] (**Scheme 5**).

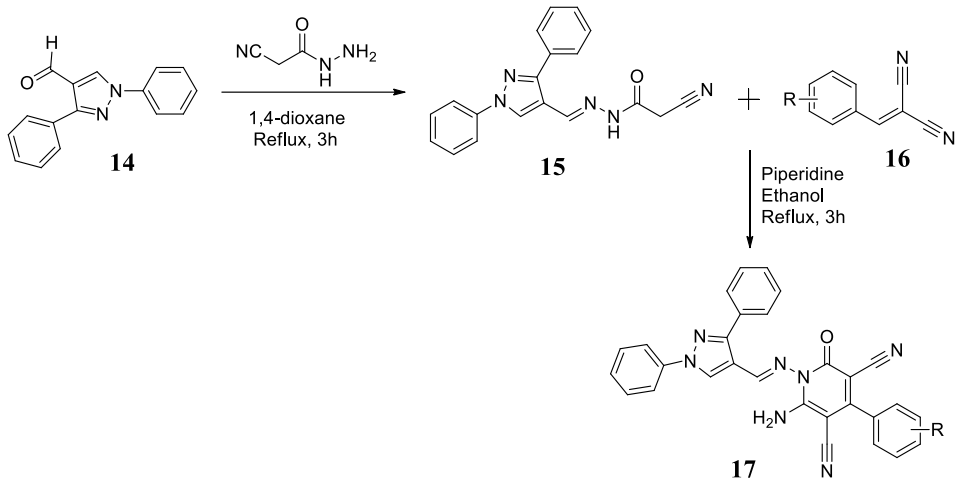


11 **12** **13**

Scheme 5

Pyrazole based 2-pyridones possessing excellent antibacterial activity were synthesised by Desai *et al.* (**Scheme 6**)[1]. In this synthetic method, 1,3-diphenylpyrazole-4-carboxaldehyde **14** was condensed with cyanoacetic acid hydrazide in the presence of 1,4-dioxane as solvent to form 2-cyano-N-((1,3-diphenyl-1H-pyrazol-4-yl) methylene) acetohydrazide **15**. It was then treated with (2-

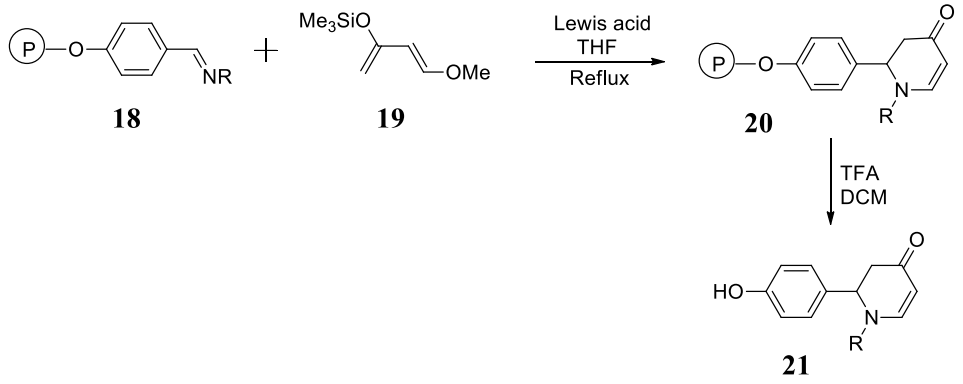
arylidene) malononitriles **16** using piperidine as catalyst and ethanol as solvent to afford 6-amino-1-((1,3-diphenyl-1H-pyrazole-4-yl)methyleneamino)-4-(aryl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitriles **17**.



14 **15** **16** **17**

Scheme 6

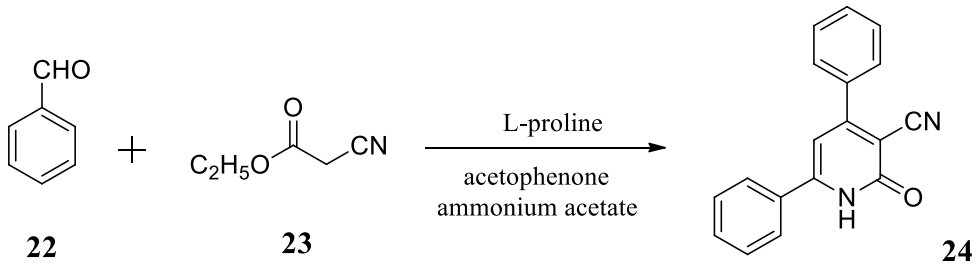
Biologically active compounds often contain heterocyclic structures and it was found that the heterocyclic ring core is responsible for their biological activity. In this regard, several synthetic methods, both in solution-phase and solid phase, have been developed for heterocycles. Wang and Wilson reported the solid-phase synthesis of pyridones[10]. In this Lewis acid catalysed reaction, polymer-bound aldimines **18** and Danishefsky's diene **19** upon tandem Mannich-Michael reaction afforded 2,3-dihydro-4-pyridones **21** (**Scheme 7**).



18 **19** **20** **21**

Scheme 7

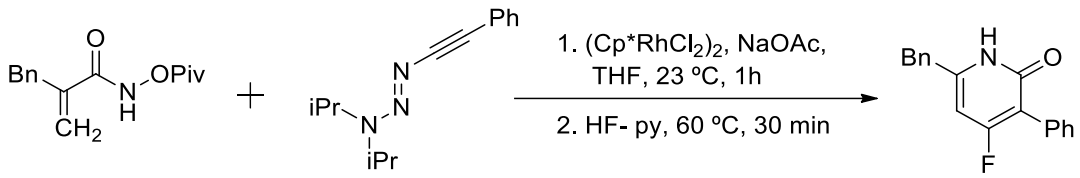
3-Cyano-2-pyridones are important heterocycles present in several pharmaceuticals and various synthetic methods were developed for the synthesis of these compounds. Keshavarz *et al.* developed a one-pot synthetic method for substituted 3-cyano-2-pyridones from aromatic aldehydes[11]. In this method, aromatic aldehydes **22** were refluxed with ethyl cyanoacetate **23**, L-proline in ethanol, acetophenone and ammonium acetate to afford 3-cyano-2-pyridones **24** (**Scheme 8**).



22 **23** **24**

Scheme 8

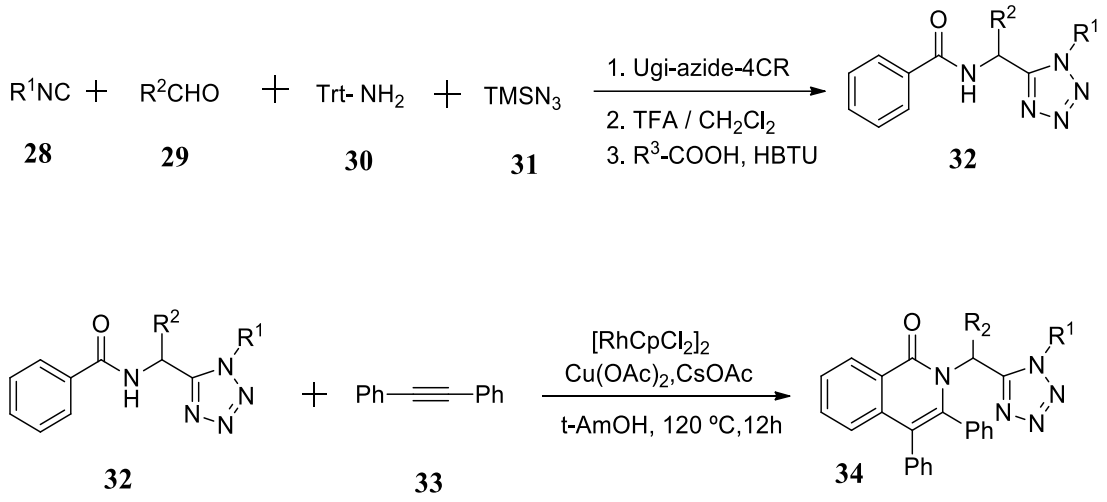
Transition metals such as rhodium, ruthenium and cobalt catalyse C-H annulations for pyridone synthesis. Tan *et al.* reported a highly efficient and regioselective synthetic strategy for fluorinated pyridones *via* alkenyl C-H functionalization using 1-alkyl triazenes and subsequent Wallach reaction using HF-pyridine[12]. In this method, $(Cp^*RhCl_2)_2$ catalysed reaction between N-(pivaloyloxy) acrylamide **25** and alkynyl triazene **26** resulted in the formation of 4-triazenyl pyridone which when treated with HF-pyridine underwent Wallach reaction to afford 4-fluoro-2-pyridones **27** (**Scheme 9**). The solvent THF selectively enable lossen rearrangement that occurs during $(Cp^*RhCl_2)_2$ catalysed alkenyl C-H annulation. NaOAc play a crucial role as the base in providing high yield and regioselectivity for the synthesis.



25 **26** **27**

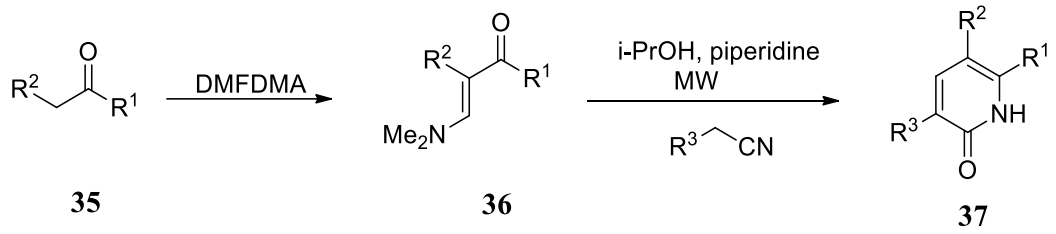
Scheme 9

Ojeda *et al.* reported the rhodium (III) catalysed annulation reaction between aryl acetylenes and N- acyl amino methyl tetrazole affording pyridones. The work included the synthesis of tetrazole-isoquinolone/pyridone hybrids by the combination of Ugi-azide four component reaction (Ugi- azide- 4CR) and rhodium (III) catalysed cyclisation reaction[13] (**Scheme 10**). In this method isocyanide **28**, aldehyde **29**, tritylamine **30** and trimethylsilyl azide **31** were allowed to react by microwave irradiation to afford N-tritylaminomethyltetrazoles. This is followed by the removal of the trityl group to yield N-deprotected amino methyl tetrazole which is then acylated to N-acyl amino methyl tetrazoles **32**. The rhodium (III) catalysed cyclo-addition reaction between N-acyl amino methyl tetrazole **32** and aryl acetylenes **33** afforded the tetrazole-isoquinolone / pyridone hybrids **34**.



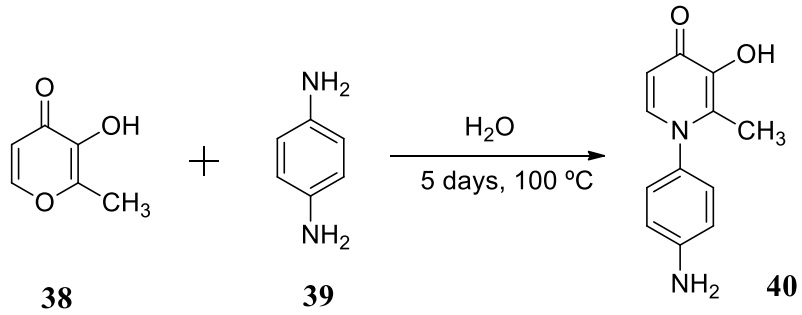
Scheme 10

2-Pyridones were synthesised from various carbonyl compounds by microwave assisted synthetic route[14]. In this method enaminones **36**, obtained by the reaction between carbonyl compounds **35** and N,N-dimethylformamide dimethyl acetal (DMFDMA), were allowed to react with methylene activated nitriles at a temperature of 100 ºC using piperidine as base and 2-propanol as the solvent to yield product 2-pyridone **37** (**Scheme 3**).



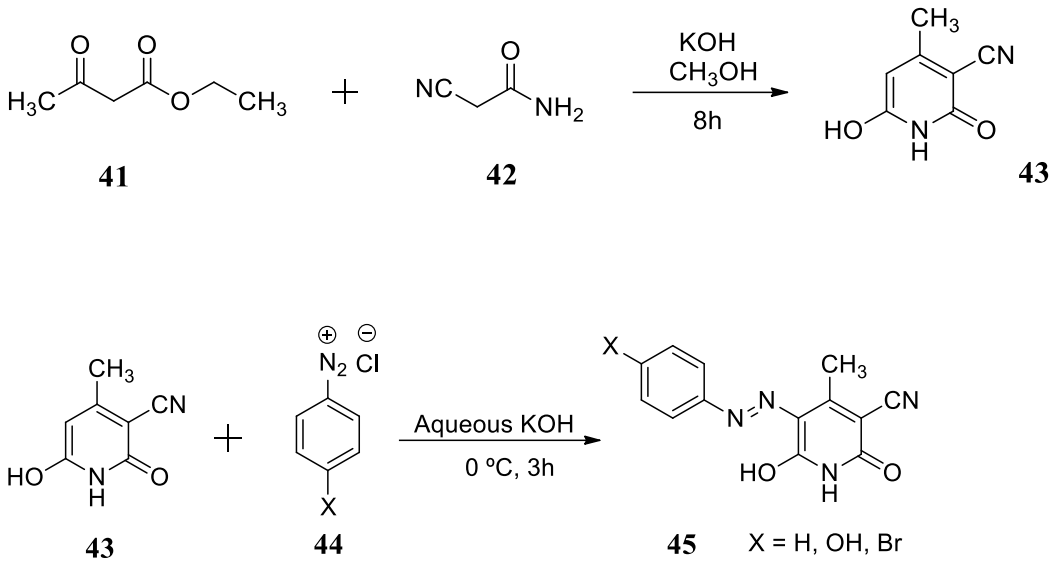
Scheme 11

4-Pyridones are extensively used as complexing agents in spectrophotometry for the estimation of metal ions. Among various derivatives of pyridones, some of them possess potential antibacterial properties and also act as bacterial enzyme inhibitors. For example, 3-hydroxy-2-methyl-1-(ρ-aminophenyl)-4-pyridone **40** was synthesised by the reaction between 3-hydroxy-2-methyl-4-pyrone **38** and p-phenylenediamine **39** in water at 100 ºC (**Scheme 12**)[15].



Scheme 12

Porobic and co-workers succeeded in synthesising some pyridones and aryl azo pyridone dyes from synthesised pyridones[16]. The reported synthesis included the reaction between 2-cyanoacetamide **42**, ethyl acetoacetate **41** and potassium hydroxide in methanol to afford 3-cyano-6-hydroxy-4-methyl-2-pyridone **43**. The synthesised pyridone **43** upon reaction with diazonium salt **44** yielded 5-(4-substitutedphenylazo)-3-cyano-6-hydroxy-4-methyl-2-pyridone dye **45** (**Scheme 13**). These dyes were found to exhibit high thermal stability up to 250 ºC which makes them best suited for photocopying and printing which are high temperature processes[17].

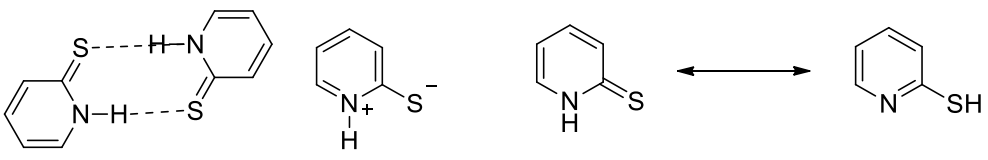


41 **42** **43**

43 **44** **45**

Scheme 13

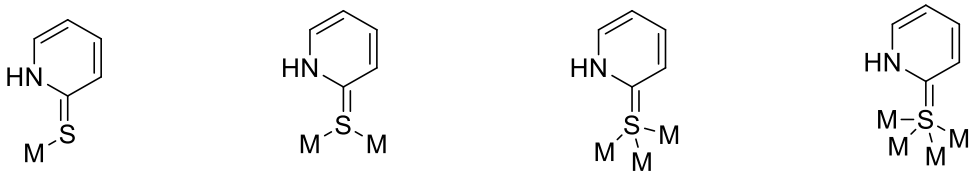
Pyridine-2-thione is the simplest heterocyclic thioamide having monoclinic crystal structure[18]. It acts as a weak acid and exists in hydrogen bonded form **46**, zwitter ionic form **47** and thione **48** - thiole **49** tautomeric forms **(Fig. 1)**. Both thione **48** and thiole **49** tautomers of pyridine-2-thione are observed in dilute cyclohexane solutions. But in acetone and methanol solutions, it exists in thione form. Whereas in chloroform solution, it exists as a hydrogen bonded dimer. Pyridinium-2-thiolate is the zwitter ion of pyridine-2-thione[19]. Self-association as well as protomeric equilibrium were observed for isomeric pairs of 2-mercaptopyridine and 2-thiopyridone. The equilibrium was observed in their dilute solutions in chloroform and cyclohexane[20]. The protomeric equilibrium is highly influenced by the molecular environment that leads to physical interactions like hydrogen bonding, solvation energies, dipole-dipole interactions *etc.*[21].



46 **47** **48** **49**

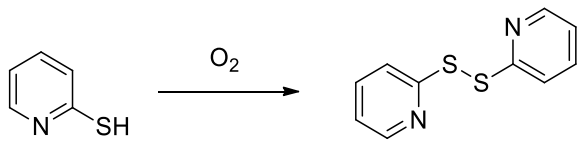
Fig. 1

Pyridine-2-thiones **(Fig. 2)** exhibit diverse bonding properties in neutral and anionic forms. Pyridine-2-thione form sulphur bridged dimers with silver (I) halides. The synthesis involves the reaction between silver(I)halide, PPh_3 in acetonitrile and pyridine-2-thiones to afford complexes having general formula $[Ag_2X_2(\mu\text{-}S\text{-}pySH)_2(PPh_3)_2]$ (pySH = pyridine-2-thione, X=Cl or Br)[22]. Pyridine-2-thiones **54** act as acylating and reducing agents. These are excellent protecting groups for amines and imides. It is susceptible to autocatalytic oxidation to disulphides **55 (Scheme 14)**.



50 **51** **52** **53**

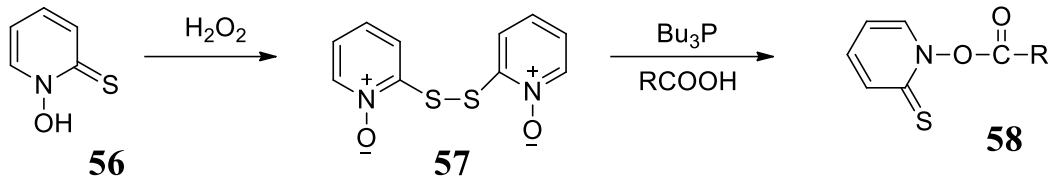
Fig. 2



54 **55**

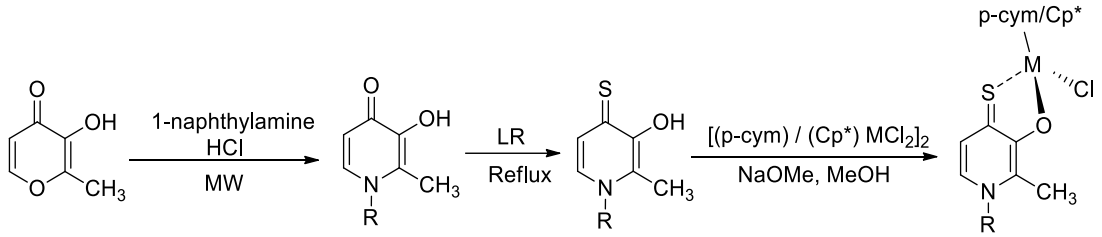
Scheme 14

Barton and Samadi have successfully converted N-hydroxy-2-thio-pyridones to corresponding acyl derivatives *via* treatment with hydrogen peroxide followed by tributylphosphine in the acidic medium[23]. N-hydroxy-2-thio-pyridone **56** when treated with hydrogen peroxide yielded di-N-oxide of 2-thiopyridine disulphide **57** which on further treatment with tributylphosphine in the acidic medium afforded acyl derivatives of N-hydroxy-2-thio-pyridone **58 (Scheme 15)**.



Scheme 15

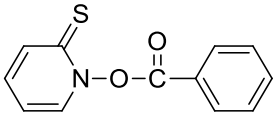
Harringer *et al.* succeeded in synthesising highly stable thiopyridone based water soluble organometallic complexes exhibiting interesting biological properties[24]. The synthesis (**Scheme 16**) started with the conversion of maltol to pyridones. The conversion was accomplished by treating maltol **59** with primary amines at high temperatures using water as solvent. The synthesised pyridones **60** were thionated using Lawesson's reagent to afford thiopyridones **61**. Complexation of thiopyridone was carried out with dimeric metal precursor yielding the corresponding thiopyridone based piano-stool organometallic complexes **62**.



Scheme 16

Oxygen centered species such as thiopyridones are promising candidates in the design of functional molecules. These species are of great interest in the field of drug design, catalysis and synthetic enzymes. N-benzoyloxy-2-thiopyridones **63** (**Fig. 3**) are found to be potential photo-activated nucleic acid cleaving agents[25].

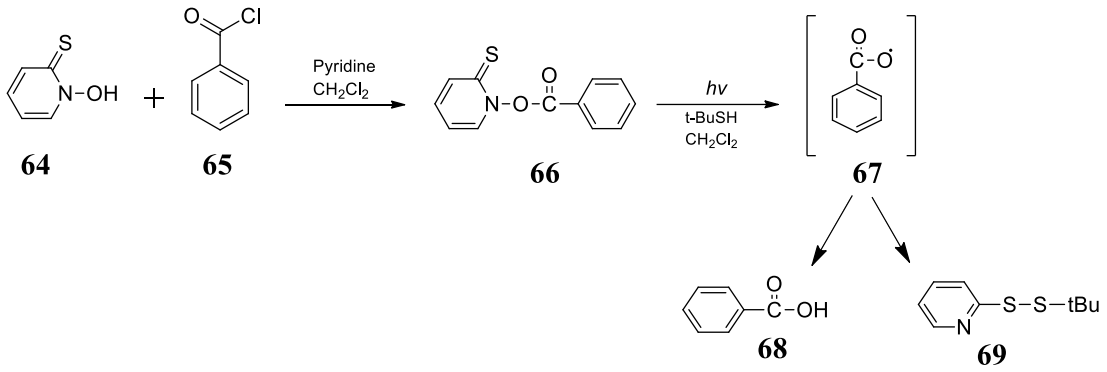
Fig. 3



63

A facile synthesis of nucleic acid cleaving agents such as N-aroyloxy-2-thiopyridones from N-hydroxy-2-thiopyridone has been devised by Blom *et al.* These

compounds are susceptible to photoexcitation in the presence of light giving corresponding free radical. But in the absence of light, it exhibits prolonged stability. N-hydroxy-2-thiopyridone **64** on reaction with benzoyl chloride **65**, pyridine and dichloromethane afforded N-benzoyloxy-2-thiopyridone **66** which undergo photolysis to benzoic acid **68** and disulphide **69** *via* benzoyloxy radical **67**. The DNA photocleavage was found to be time and concentration dependent which can be effectively tuned by structural modification of the thiopyridone core. Thus the N-benzoyloxy-2-thiopyridone **64** acts as a potential synthon for efficient nucleic acid photocleaving reagents of high selectivity[25]. These organic structures act as the progenitor of oxygen centered free radicals **(Scheme 17)**.



Scheme 17

Synthetic applications of pyridine-2-thiones

Organometallic complexes were found to be more effective anticancer agents than pure organic compounds. This is due to the ability of the central metal atom to exhibit variable oxidation states, variable coordination numbers and different geometries. Therefore medicinal chemistry focused on the synthesis of such complexes of metals with organic compounds. Pyridine-2-thiones are important compounds in organometallic chemistry. Since pyridine-2-thiones are good chelating agents, it forms binuclear complexes with various metals. Harringer *et al.* synthesised many piano-stool complexes of pyridine-2-thiones with Ru^{II}, Rh^{III} or Ir^{III} as the metal centre. These complexes exhibited cytotoxic activity against human cancer cell lines[26].

Pyridine-2-thiones form binuclear Cu(I) complexes with the general formula $[Cu(tmtp)(L)I]_2$. These complexes possessing triclinic structure were synthesised by the reaction between $[Cu(tmtp)I]_4$ and pyridine-2-thione[27]. Aslanidis *et al*. synthesised binuclear complexes having the general formula $[Cu(tmtp)(L)Br]_2$ by the reaction between $[Cu(tmtp)Br]_n$ and pyridine-2-thione (tmtp = tri-*m*-tolylphosphine, L = pyridine-2-thione)[28].

5.3 Results and discussion

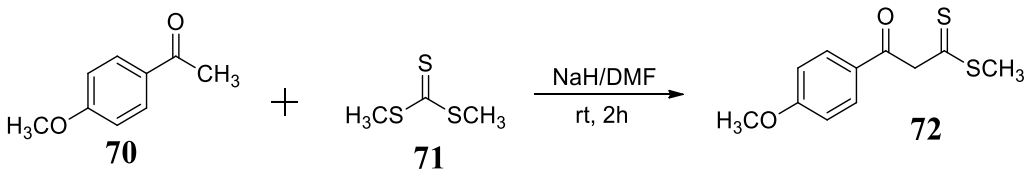
Synthesis of dihydropyridine-2-thione

In the present work, new synthetic protocol for dihydropyridine-2-thione from β-oxodithioester has been developed. The β-oxodithioester was synthesised from ketone which was then converted to dihydropyridine-2-thione.

The synthesis involves the following steps:-

1) Synthesis of β-oxodithioester
2) Synthesis of Thioamide
3) Synthesis of Chalcone
4) Synthesis of dihydropyridine-2-thione

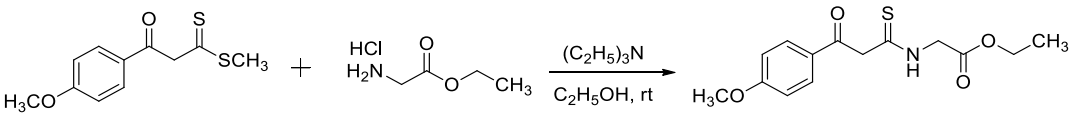
β-Oxo-dithioester has been selected as the synthon for dihydropyridine-2-thione. In a typical procedure, a mixture of 4-methoxy acetophenone **70** (1 eq.), dimethyl trithiocarbonate **71** (1 eq.) and sodium hydride in dimethylformamide was stirred at room temperature for 2h. This is followed by the acidification of the reaction mixture to afford the β-oxodithioester **72** in 90% yield (**Scheme 18**).



Scheme 18

The structure of methyl-3-(4-methoxyphenyl)-3-oxopropanedithioate **72** was confirmed by ^{1}H NMR and ^{13}C NMR spectra. The ^{1}H NMR of compound **72** exhibited three hydrogen singlet at δ 2.6 ppm and δ 3.9 ppm corresponding to proton at -SCH_3 and -OCH_3 respectively. Aromatic protons were denoted by peaks at δ 6.96 ppm to δ 7.9 ppm. Proton at -CH_2 is denoted by a two hydrogen singlet at δ 2.98 ppm. ^{13}C NMR peaks appeared in the aromatic region from δ 114.2-165 ppm. The peak at δ 225.1 ppm and 194.2 ppm were attributed to thiocarbonyl carbon and carbonyl carbon respectively. Carbon at -SCH_3 gave a signal at δ 20.6 ppm and the carbon at -OCH3 gave a signal at δ 55.8 ppm. The data is in agreement with that reported in the literature[29].

Thioamide was synthesised from β-oxodithioester according to the synthetic methodology reported by Mathew and Asokan[30]. A mixture of β-oxodithioester **72** (1 eq.), glycine ethyl ester hydrochloride **73** (1 eq.) and triethylamine (2 eq.) in ethanol was magnetically stirred at room temperature for 20h. After the completion of the reaction, the reaction mixture was acidified to afford ethyl 2-(3-(4-methoxyphenyl)-3-oxopropanethioamido)acetate **74** in good yield **(Scheme 19)**.



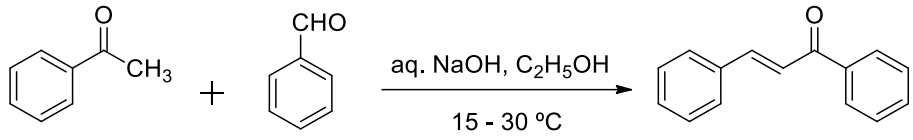
72 **73** **74**

Scheme 19

The structure of thioamide **74** was confirmed by ^{1}H NMR and ^{13}C NMR spectra. The ^{1}H NMR of compound **74** exhibited three proton singlet at δ 3.83 ppm due to hydrogens at the -OCH_3 group. The hydrogen at -CH_2 group between the carbonyl group and thiocarbonyl group was denoted by a two proton singlet at δ 2.98 ppm. A peak at δ 3.92 ppm corresponds to the hydrogen at -CH_2 group between carbonyl and -NH group. A signal at δ 2.0 ppm was attributed to the proton at -NH group. ^{13}C NMR peaks appeared in the aromatic region from δ 114.2-129.8 ppm. The peak at δ 199.3 ppm was attributed to thiocarbonyl carbon. The carbonyl carbons

exhibited peaks at δ 194.2 ppm and δ 169.5 ppm. The data is in agreement with that reported in the literature [30].

The chalcone was synthesised according to the synthetic method reported by Wang and coworkers[31]. Acetophenone **75** (1 eq.) was treated with benzaldehyde **76** (1 eq.) in the basic medium at a temperature of 15-30 ºC for 12h. After the completion of the reaction, the reaction mixture was cooled and filtered to afford (E)-chalcone **77** in good yield **(Scheme 20)**.

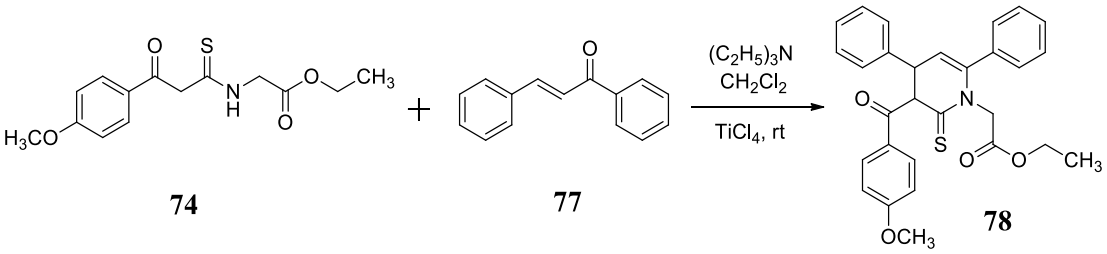


75 **76** **77**

Scheme 20

The structure of the chalcone **77** was confirmed by ^{1}H NMR and ^{13}C NMR spectra. The ^{1}H NMR of compound **77** exhibited signals between δ 7.33-7.89 ppm due to protons in the aromatic region. The ethylenic protons were denoted by peaks at δ 7.59 ppm and δ 8.06 ppm. ^{13}C NMR peaks appeared in the aromatic region from δ 127.9-137.9 ppm. The peaks at δ 145.1 ppm and δ 121.3 ppm were attributed to ethylenic carbons. The carbonyl carbons exhibited peaks at δ 189.7 ppm. The data is in agreement with that reported in the literature [32].

A mixture of thioamide **74** (1 eq.), chalcone **77** (1.2 eq.) and triethylamine (4 eq.) in dichloromethane was treated with the catalytic amount of titanium tetrachloride. It was magnetically stirred at room temperature for 24h to afford dihydropyridine-2-thione **78** in good yield (**Scheme 21**).



74 **77** **78**

Scheme 21

The characterisation of the dihydropyridine-2-thione derivative; Ethyl-2-(3-(4-methoxybenzoyl)-4,6-diphenyl-2-thioxopyridin-1(2H)-yl)acetate (**78**) was carried out using FT-IR, ^{1}H NMR, ^{13}C NMR and single crystal XRD analysis. Based on the data obtained from these analysis, the structure of compound **78** was established as shown in **Fig. 4**.

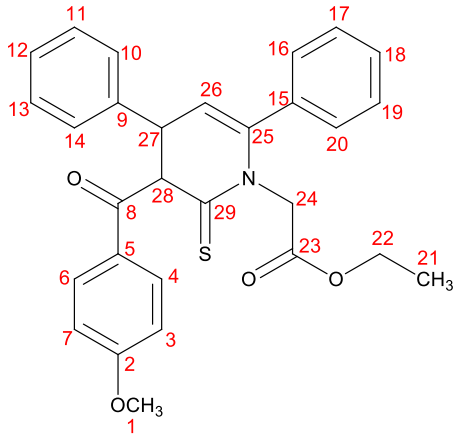


Fig. 4 *Structure of dihydropyridine-2-thione (***78***)*

The FT-IR of dihydropyridine-2-thione (**78**) gave major peaks at 721, 1216, 1376, 1458, 1738, 2852 and 2920 cm^{-1} (**Fig. 5)**. The peak at 721 cm^{-1} was attributed to C-C bending vibration. The absorption at 1216 cm^{-1} is due to C-O stretching vibration in alkyl aryl ether. The signal at 1738 cm^{-1} indicated the presence of the ester group. The absorption at 1458 cm^{-1} was attributed to the presence of the C=S group attached to nitrogen. The signals at 2852 and 2920 cm^{-1} were due to the methylene C-H symmetric stretching and asymmetric stretching vibrations respectively. The absorption at 1376 cm^{-1} was attributed to methyl C-H bending vibration.

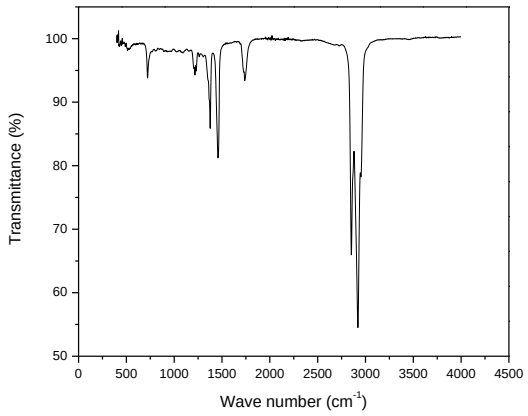


Fig. 5 *FT-IR spectrum of dihydropyridine-2-thione (***78***)*

The structure of dihydropyridine-2-thione (**78**) was confirmed by the ^{1}H NMR spectrum (**Fig. 6)**. One proton doublet at δ 5.38 ppm corresponds to the proton at position C28. The proton at position C27 is denoted by a one proton doublet at δ 5.52 ppm. The proton at C26 is observed as one proton singlet at δ 6.93 ppm. The two proton quartet at δ 4.16-4.14 ppm is attributed to the proton at position C22. Three proton triplet at δ 1.19 ppm corresponds to protons at position C21. Proton at position C1 appeared as three proton singlet at δ 3.86 ppm. Peaks in the range δ 7.99-6.93 ppm denotes aromatic protons.

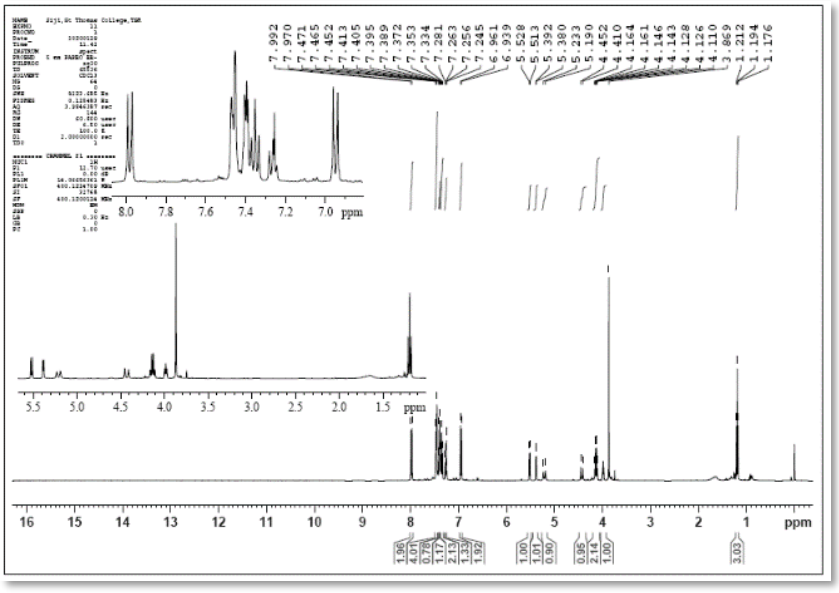


Fig. 6 *^{1}H NMR spectrum of dihydropyridine-2-thione (***78***)*

The ^{13}C NMR spectrum of dihydropyridine-2-thione (**78**) (**Fig. 7**) was in agreement with both FT-IR and ^{1}H NMR data. The downfield at δ 196.5 ppm is due to carbon C29 doubly bonded to sulphur. The carbonyl carbon C8 shows a signal at δ 193.2 ppm. The carbonyl carbon C23 of the ester group appeared at δ 167.2 ppm. The signal at δ 163.7 ppm is attributed to C5 of the phenyl ring attached to the carbonyl group. The carbon atoms at C27 and C28 give signals at δ 41.0 ppm and δ 63.5 ppm respectively. The signal due to methoxy carbon C1 appears at δ 61.2 ppm. The alkyl carbon atoms C21, C22 and C24 present in the side chain attached to nitrogen are indicated by the signals at δ 14.0, 52.4 and 55.5 ppm respectively. The signals at δ 114.0-141.7 ppm are attributed to aromatic and olefinic carbon atoms.

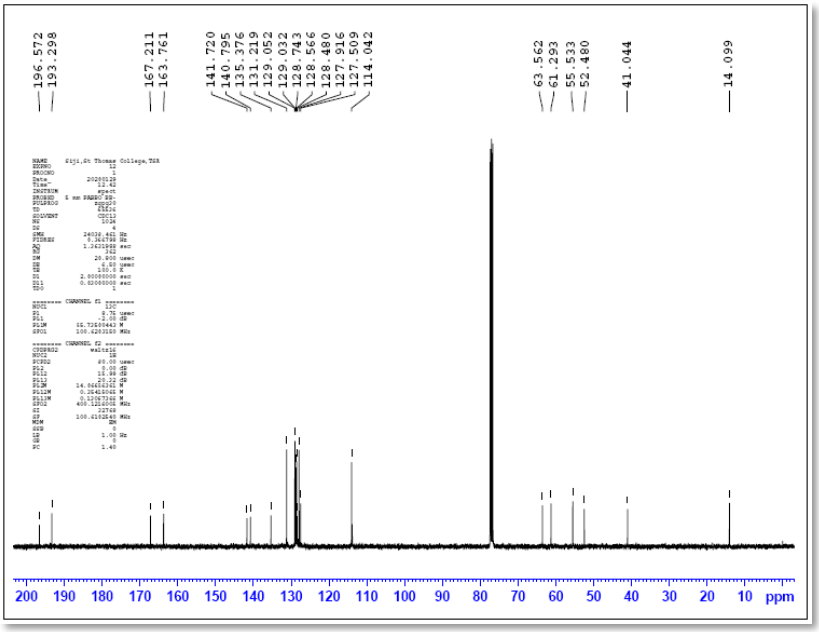


Fig. 7 *^{13}C NMR spectrum of dihydropyridine-2-thione (**78**)*

The proposed structure of dihydropyridine-2-thione (**78**) was verified by single crystal XRD analysis. For this, product **78** was dissolved in dichloromethane and recrystallised by slow evaporation of the solvent. The single crystal of **78** was analysed by X-ray crystallographic experiments. The details are summarised in **Table 1**.

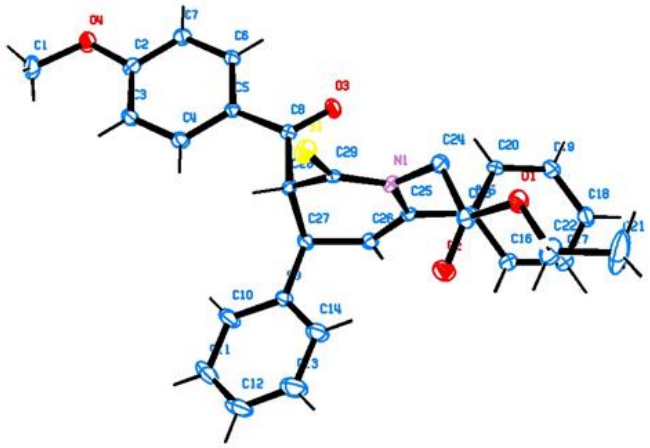


Fig. 8 *Single crystal XRD of dihydropyridine-2-thione (***78***)*

Table.1 Crystallographic data of dihydropyridine-2-thione (**78**)

Parameters	
Empirical formula	$C_{29}H_{27}NO_4S$
Formula weight	485.578
Crystal system	Triclinic
Space group	P -1
a (Å)	8.097(3)
b (Å)	12.576(5)
c (Å)	13.366(6)
α^0	105.264(13)
β^0	90.649(14)
γ^0	106.361(14)
V (A^3)	1254.4(9)
T (K)	296(2)
Z	2
Wavelength (Å)	0.71073
Calculated density (Mg/m^3)	1.283
Absorption coefficient (mm^{-1})	0.164

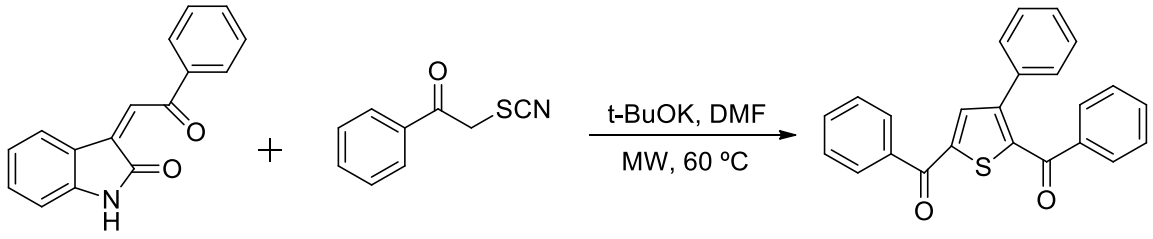
The crystal belongs to the triclinic crystal system with space group P-1. The thiocarbonyl (C_{29}- S_1) bond distance is 1.628 Å. The C_{29}-N, C_{25}-N and C_{24}-N bond distances are 1.33 Å 1.428 Å and 1.462 Å respectively. **Fig. 8** shows the thermal plot of dihydropyridine-2-thione (**78**).

5.4 Synthetic strategies for aryl thiophenes- a review

Thiophene is an electron rich aromatic heterocyclic compound. It is a valuable scaffold in coordination chemistry. Thiophene derivatives exhibit diverse biological and pharmacological properties. Thiophene core is present in many biologically important natural products. Many of the compounds having antifungal, antibacterial, anti-inflammatory, anticonvulsant and anticancer properties are found to possess thiophene nucleus[33].

Several synthetic approaches such as metal-catalysed, metal-free, multi-component, iodine catalysed *etc.* reactions have been developed for thiophenes. Metal-catalysed reactions provide highly regioselective heterocyclisation leading specifically to target compounds[34].

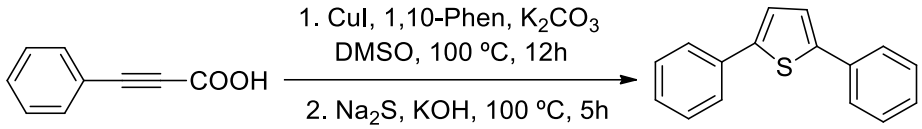
Xu *et al.* introduced a new synthetic approach towards 2,3,5-trisubstituted thiophene derivatives **81**[35]. In this method, a mixture of 3-(2-aryl-2-oxoethylidene)-2-oxindoles **79**, two equivalents of α-thiocyanato ketones **80** and potassium *t*-butoxide were microwave irradiated at 60 ºC (**Scheme 22**). The 3-(2-aryl-2-oxoethylidene)-2-oxindoles undergo [2+2+1] cyclisation with two equivalents of α-thiocyanato ketones providing 2,3,5-trisubstituted thiophene derivatives.



79 **80** **81**

Scheme 22

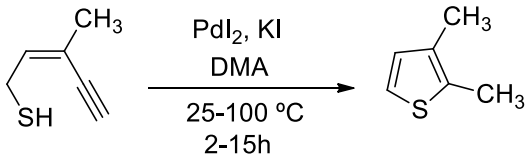
Irudayanathan *et al.* devised a copper-catalysed synthetic method for 2,5-diaryl-substituted thiophenes from aryl propiolic acids[36]. The reaction was carried out in the presence of Na_2S. The alkynyl carboxylic acid **82** when treated with CuI in the basic medium undergoes homocoupling to provide 1,4-diaryldiynes. The 1,4-diaryldiynes thus formed were treated with Na_2S to afford 2,5-diaryl-substituted thiophenes **83** (**Scheme 23**).



82 **83**

Scheme 23

Gabriele & Mancuso devised a metal-catalysed synthesis of thiophenes from alkynes[37]. In this method, the (*Z*)-2-en-4-yne-1-thiols **84** was treated with the catalytic system of PdI_2 - KI in *N*,*N*-dimethylacetamide (DMA) at 25-100 ºC for 2-15h to yield substituted thiophenes **85 (Scheme 24)**. The mechanism of the reaction involves activation of the triple bond by a metal catalyst followed by nucleophilic attack and heterocyclisation to yield heterocyclic compounds.



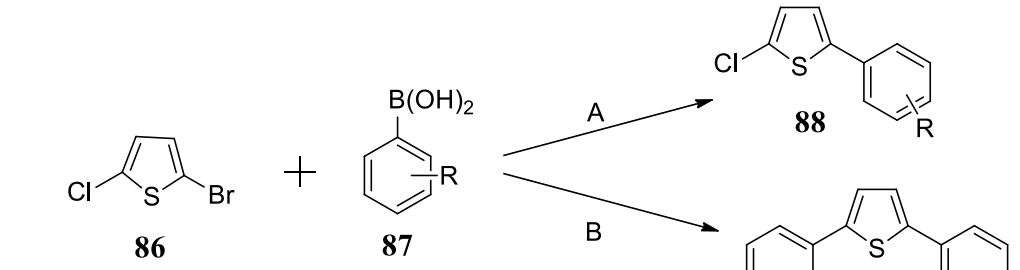
84 **85**

Scheme 24

Synthetic applications of thiophenes

Thiophenes are valuable intermediates in organic synthesis. Thiophene ring when fused with other ring systems give rise to fused heterocyclic compounds with advanced biological activities

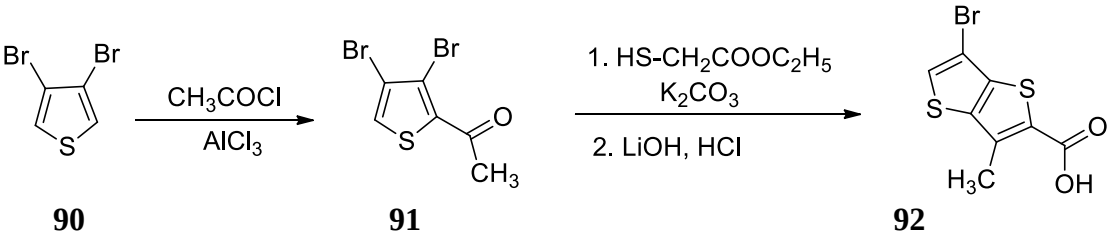
Rasool *et al.* synthesised 2-chloro-5-phenylthiophenes **88** and 2,5-bisarylthiophenes **89** from 2-bromo-5-chlorothiophenes **86** *via* Suzuki cross-coupling reaction[38]. The 2-bromo-5-chlorothiophenes **86** was treated with phenyl boronic acid **87**, K_3PO_4, $Pd(PPh_3)_4$ and $Ar(BOH)_4$ to get the required products (**Scheme 25**). The 2-chloro-5-phenylthiophenes are found to be antibacterial agents whereas 2,5-bisarylthiophenes are antioxidants.



A) K_3PO_4 (2mM), $Pd(PPh_3)_4$ (5M), $Ar(BOH)_2$ (1.1 mmol), 12h, 90 ºC
B) K_3PO_4 (4.7mM), $Pd(PPh_3)_4$ (6M), $Ar(BOH)_2$ (2.6 mmol), 24h, 110 ºC

Scheme 25

Deng *et al.* synthesised thieno[3,2-b]thiophene-2-carboxylic acid derivatives having anticancer properties[39]. The synthesis involves Friedel craft acylation of 3,4-dibromothiophene **90** to get 2-acyl-substituted thiophene **91** which can be transformed into 6-bromo-3-methylthieno[3,2-b]thiophene-2-carboxylic acid **92** (**Scheme 26**).



Scheme 26

5.5 Results and discussion

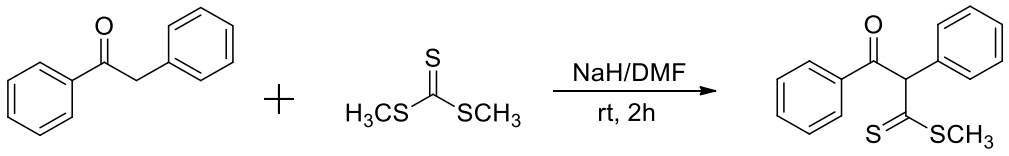
Synthesis of 3,4-diphenyl thiophene

Herein we describe a new strategy for the synthesis of 3,4-diphenyl thiophene from β-oxodithioester. The β-oxodithioester was synthesised from ketone which was then converted to 3,4-diphenyl thiophene in a one step process.

The synthesis involves the following steps.

1) Synthesis of β-oxodithioester from ketone.
2) Synthesis of phenacyl bromide from acetophenone.
3) Synthesis of 3,4-diphenyl thiophene from β-oxodithioester.

β-Oxodithioester has been selected as the synthon for 3,4-diaryl thiophene. A mixture of 1,2-diphenylethanone **93** (1 eq.), dimethyl trithiocarbonate **71** (1 eq.) and sodium hydride in N,N-dimethylformamide was stirred at room temperature for 2h. This is followed by the acidification of the reaction mixture to afford the corresponding β-oxodithioester **94** in good yield (**Scheme 27**).



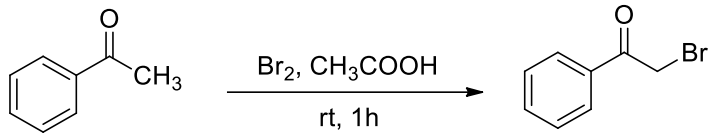
93 **71** **94**

Scheme 27

The structure of methyl-3-oxo-2,3-diphenylpropanedithioate **94** was confirmed by ^{1}H NMR and ^{13}C NMR spectra. The ^{1}H NMR of compound **94** exhibited one hydrogen singlet at δ 5.91 ppm and three hydrogen singlet at δ 2.82 ppm indicating the presence of aliphatic –CH and -SCH_3 protons respectively. Aromatic hydrogens gave signals between δ 7.23-7.94 ppm. ^{13}C NMR peaks appeared at δ 20.9 and 225.1 ppm indicating the presence of two aliphatic carbon atoms. The signals between δ 127.2-140.4 ppm are attributed to aromatic carbons. The spectral data confirmed the

formation of β-oxodithioester from 1,2-diphenylethanone which is in agreement with that reported in the literature[29].

The reaction between acetophenone **95** (1eq.) and bromine (1 eq.) in acetic acid solution furnished phenacyl bromide **96** in quantitative yield (**Scheme 28**)[40].

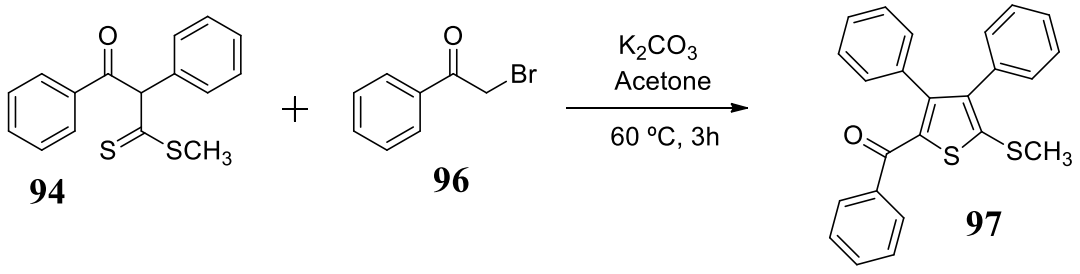


95 **96**

Scheme 28

The structure of phenacyl bromide **96** was confirmed by ^{1}H NMR. The ^{1}H NMR of compound **96** exhibited two hydrogen singlet at δ 4.5 ppm indicating the presence of the –CH_2 group. Aromatic protons gave signals between δ 7.5-7.8 ppm. The data is in agreement with that reported in the literature[41].

The β-oxodithioester **94** (1 eq.) was treated with phenacyl bromide **96** (1 eq.) in the presence of potassium carbonate in acetone at 60 ºC for 3h to afford (5-(methylthio)-3,4-diphenylthiophen-2-yl)(phenyl)methanone **97** (**Scheme 29**).



94 **96** **97**

Scheme 29

The reaction proceeded by enolisation of the dithioester followed by its S-alkylation using phenacyl bromide. The resulting S,S-acetal moiety underwent cyclisation with loss of a water molecule to afford the 3,4-diphenyl thiophene derivative **97**.

The characterisation of the 3,4-diphenyl thiophene (**97**) was confirmed by ^{1}H NMR and ^{13}C NMR spectral analysis. Based on the data obtained from these analyses, the structure of compound **97** was established as given in **Fig. 9**.

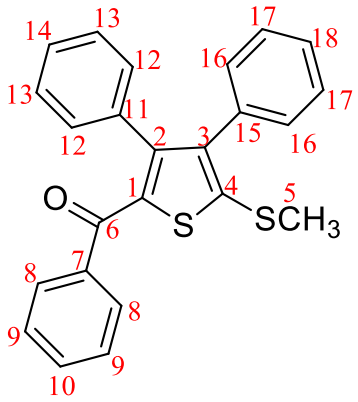


Fig. 9 *Structure of 3,4-diphenyl thiophene (**97**)*

The structure of 3,4-diphenyl thiophene (**97**) is confirmed by ^{1}H NMR spectrum **(Fig. 10)**. Three proton singlet at δ 2.57 ppm is attributed to proton at $-SCH_3$ group. The protons at position C8 is denoted by two proton multiplet at δ 7.27 ppm. One proton multiplet at δ 7.12 ppm corresponds to the proton at position C10. The protons at C12 is observed as two proton multiplet at δ 7.07 ppm. Two proton multiplet at δ 6.90 ppm corresponds to proton at C13. Proton at C14 is observed as one proton multiplet at δ 6.84 ppm.

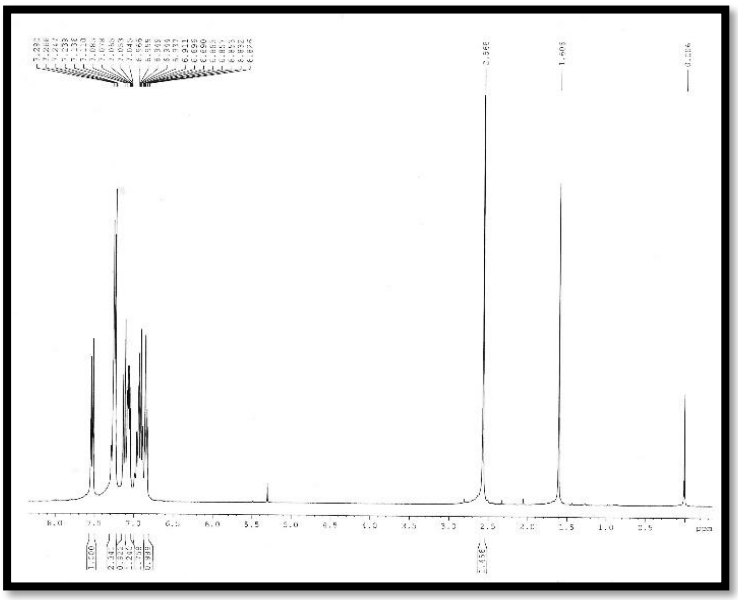


Fig. 10 *^{1}H NMR spectrum of 3,4-diphenyl thiophene (**97**)*

The structure of 3,4-diphenyl thiophene (**97**) is further confirmed ^{13}C NMR spectrum (**Fig. 11**). The carbonyl carbon C6 shows a signal at δ 189.3 ppm. The carbon C5 of -SCH_3 group give a signal at δ 18.8 ppm. The signal due to carbon C4 appears at δ 77.0 ppm. The carbon atoms C1, C2 and C3 showed signals at δ 146.9, 127.4 and 127.2 respectively. The signals between δ 127.6-144.1 ppm are attributed to carbon atoms of the phenyl groups.

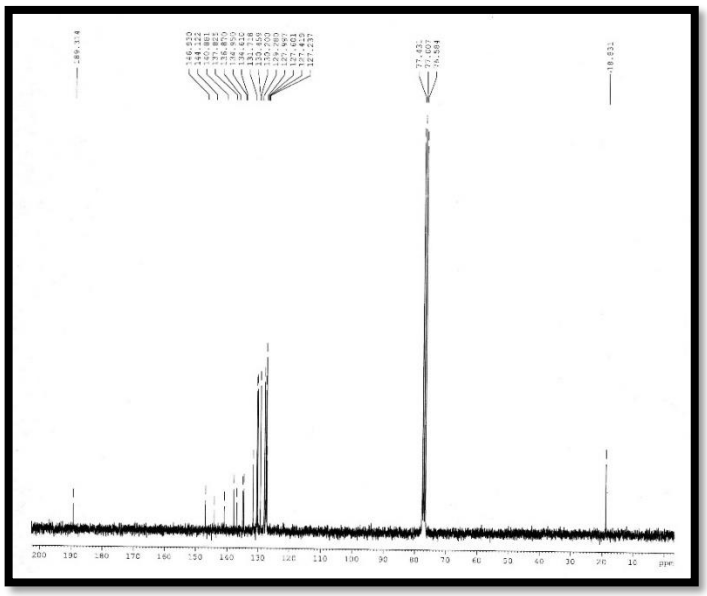


Fig. 11 *^{13}C NMR spectrum of 3,4-diphenyl thiophene (***97***)*

5.6 *In vitro* antibacterial studies

Bacteria inhabit everywhere on our planet. They display a significant role in the environment. Bacteria are responsible for the decomposition of dead organisms and thereby return nutrients present in the body of the dead organisms to the earth. In this concern, the environment benefits from bacteria. But some infectious bacteria are harmful to living organisms[42].

There are substances called antibiotics that can act against bacteria by killing them or by inhibiting their growth[43]. Antibiotics can be categorised into natural,

semisynthetic and synthetic antibiotics. Natural antibiotics such as *penicillin* have been isolated from microorganisms. Semisynthetic antibiotics are the derivatives of natural antibiotics with advanced antimicrobial activity. *Clarithromycin* and *azithromycin* are examples of semisynthetic antibiotics. Synthetic antibiotics include *moxifloxacin, norfloxacin* etc.[44]. Based on the activity, antibiotics have been classified into narrow-spectrum antibiotics and broad-spectrum antibiotics. When narrow-spectrum antibiotics such as *penicillin* and *amoxicillin* are effective against a small number of bacteria, wide-spectrum antibiotics like *ciprofloxacin* kill a large number of bacteria[45].

In the present work, *In vitro* antibacterial activity of dihydropyridine-2-thione **78** was conducted against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The study was carried out using the disc diffusion method. A nutrient agar plate was used for this purpose. *Chloramphenicol* was used as the standard antibiotic for the study.

Disc diffusion method

This method is also known as the agar diffusion test or Kirby-Bauer test[46]. The purpose of this method is to find out effective antibiotics against various pathogenic microorganisms. This method involves the preparation of an agar plate, spreading of bacteria on the agar plate, placing sample discs and incubation at 37 ºC for 24h. The effectiveness of antibiotics against pathogens will be exhibited as the zone of inhibition. This is the area around bacteria where bacterial growth is inhibited. Comparison of the zone of inhibition of the antibiotic with that of a reference compound reveals the efficiency of the antibiotic.

Preparation of agar plate:-

Nutrient agar of pH 7.4 was prepared by dissolving the ingredients- Peptone (5g), NaCl (5g), Beef extract (3g), Yeast extract (2g) and agar (20g) (2%) in1000 mL distilled water. It was poured into a petri dish and allowed to solidify.

Sample disc preparation:-

The sample compound **78** and *chloramphenicol* were dissolved separately in DMSO to get solutions of concentration 30 μgdisc^{-1}. Both solutions were inoculated to sterilised paper discs. The *chloramphenicol* disc served as standard and the disc inoculated with DMSO served as control. The discs were placed in a petri dish and allowed to dry.

The bacterial strain– *Bacillus subtilis*/ *Staphylococcus aureus*/ *Escherichia coli*/ *Pseudomonas aeruginosa*– of 24h culture in the nutrient broth was evenly spread on the surface of agar plates using sterile swab sticks. A sterile paper disc dipped into the sample solution was placed on the agar plate. The paper disc dipped in DMSO was used as negative control and that dipped in *chloramphenicol* served as the positive control. The plates were incubated at 37 ºC for 24h and the zone of inhibition was measured. The antibacterial activity was expressed in terms of the mean diameter of the zone of inhibition in millimetres.

In vitro antibacterial activity of dihydropyridine-2-thione **78** against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was carried out at a concentration of 30 μgdisc^{-1} in DMSO. The activity of the synthesised compound was compared with that of *chloramphenicol* which was selected as the standard antibiotic for the study. From the antibacterial studies, it was revealed that the compound is slightly active against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. But it was ineffective against *Escherichia coli*. The results of the study is shown in **Table 2.** Antibacterial activity of dihydropyridine-2-thione **78** against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* is shown in **Fig. 12**.

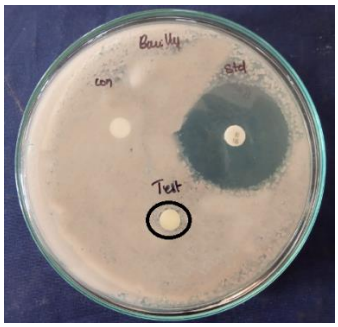


Fig. 12a

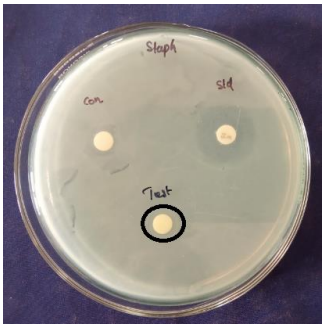


Fig. 12b

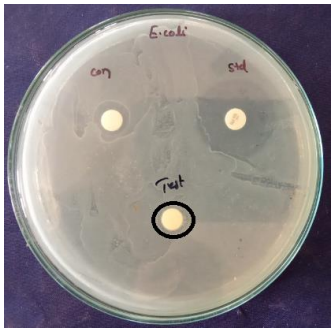


Fig. 12c

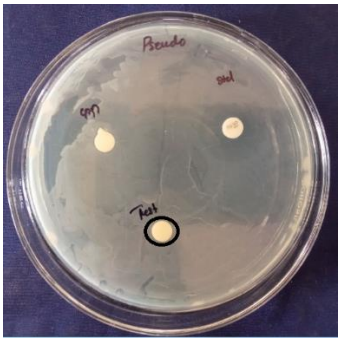


Fig. 12d

Fig. 12 *Antibacterial activity of dihydropyridine-2-thione* **78** *against Bacillus subtilis* ***(12a)****; Staphylococcus aureus* ***(12b);*** *Escherichia coli* ***(12c)*** *and Pseudomonas aeruginosa* ***(12d)***.

Table. 2 Antibacterial activity of dihydropyridine-2-thione (**78**)

Bacteria	Diameter of zone of inhibition for		
	chloramphenicol	DMSO	**Compound 78**
Bacillus subtilis	35 mm	Nil	5 mm
Staphylococcus aureus	17 mm	7 mm	8 mm
Escherichia coli	23 mm	10 mm	8 mm
Pseudomonas aeruginosa	19 mm	Nil	2 mm

The diameter of the zone of inhibition exhibited by compound **78** in *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* are 5 mm, 8 mm, 8 mm and 2 mm respectively. The standard compound (*chloramphenicol*)

showed the zone of inhibition of 35 mm, 17 mm, 23 mm and 19 mm in *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* respectively. The diameter of the zone of inhibition obtained for the control (DMSO) was 0 mm, 7 mm, 10 mm and 0 mm in *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* respectively.

The zone of inhibition exhibited by compound **78** in *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* is larger than that of the control used. This shows that the compound is active against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. But, the zone of inhibition of compound **78** in these bacterial strains is lower than that of the standard used; which showed that the activity of the compound against the selected bacterial strains is very less.

Compound **78** exhibited a zone of inhibition of 8 mm in *Escherichia coli* whereas the standard and control showed zones of inhibition of 23 mm and 10 mm respectively. Since the diameter of the zone of inhibition for the compound is less than that of the control, it can be concluded that the compound is inactive against *Escherichia coli.*

The *in vitro* antibacterial studies proved that dihydropyridine-2-thione **78** is slightly active against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* but inactive against *Escherichia coli*.

5.7 *In silico* molecular docking studies

Most of the thiopyridone and thiophene derivatives were found to exhibit anticancer and antibacterial properties. Therefore we decided to theoretically analyse the anticancer and antibacterial properties of compounds **78** and **97** via *in silico* molecular docking methods. The docking programme Autodock 4.2 was used for analysing anticancer properties and Autodock vina was used for analysing antibacterial properties. Anticancer properties were analysed using human DNA topoisomerase II a (alpha) and II b (beta) as target proteins whereas antibacterial properties were analysed using FtsZ and DNA gyrase present in the bacteria *Bacillus subtilis* as target proteins.

5.7.1 Molecular docking studies on anticancer activity

Dihydropyridine-2-thione

The binding modes of dihydropyridine-2-thione **78** with the target proteins, human DNA topoisomerase II alpha (PDB id: 4fm9) and human DNA topoisomerase II beta (PDB id: 3qx3), has been calculated (**Fig. 13**). The binding energy, inhibition constant and various interactions of the ligands with active site residues (**Fig. 14**) are summarised in **Table 3**.

The binding energy of compound **78** with the protein human DNA topoisomerase II alpha was -7.08 Kcal/mol with an inhibition constant of 6.42 µM. The interactions between compound **78** with the protein human DNA topoisomerase II alpha involve Van der Waals forces, carbon-hydrogen bond, pi- cation, pi-donor hydrogen bond, pi-pi T-shaped, alkyl and pi-alkyl.

The binding energy of compound **78** with the protein human DNA topoisomerase II beta was -7.84 Kcal/mol with an inhibition constant of 1.78 µM. The interactions between compound **78** with the protein human DNA topoisomerase II beta involve Van der Waals forces, pi-sulfur, pi-pi T-shaped, alkyl and pi-alkyl.

The binding energy and the inhibition constant values showed that the dihydropyridine-2-thione derivative **78** is a good inhibitor for the proteins; DNA

topoisomerase II a (alpha) and DNA topoisomerase II b (beta). The highest binding energy and lowest inhibition constant value were obtained for ligand **78** with the protein, human DNA topoisomerase II b (beta) which showed that ligand **78** is a better inhibitor for the protein, DNA topoisomerase II b (beta) than for DNA topoisomerase II a (alpha). The cut-off value for binding energy being -5 Kcal/mol, the values obtained in the docking study clearly established that compound **78** exhibits good anticancer properties.

Table. 3 Binding energies and possible interactions of the compound **78** with proteins:

Ligand	Protein	Binding energy (Kcal/mol)	Inhibition constant (µM)	H-bonds	Other interactions
Compound **(78)**	DNA topoisomerase II alpha (PDB id: 4fm9)	-7.08	6.42	GLN A:544 HIS A:759	Van der Waals C-H bond Pi- cation Pi-donor H-bond Pi-Pi T-shaped Alkyl Pi-alkyl
	DNA topoisomerase II beta (PDB id: 3qx3)	-7.84	1.78	DA F:12 DC F:11 ARG A:820	Van der Waals Pi-sulfur Pi-Pi T-shaped Alkyl Pi-alkyl

Fig. 13a

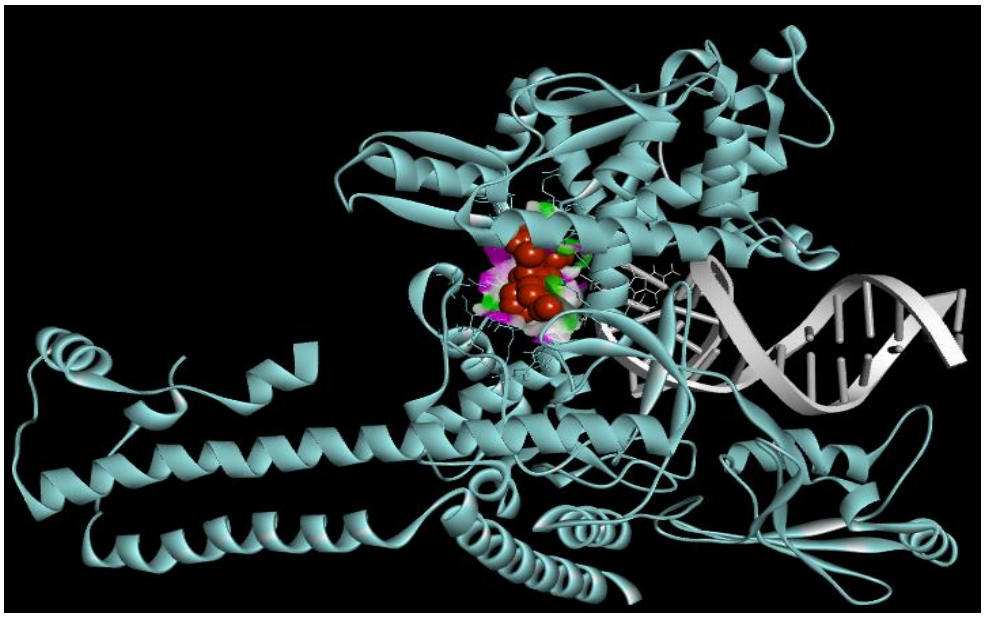


Fig. 13b

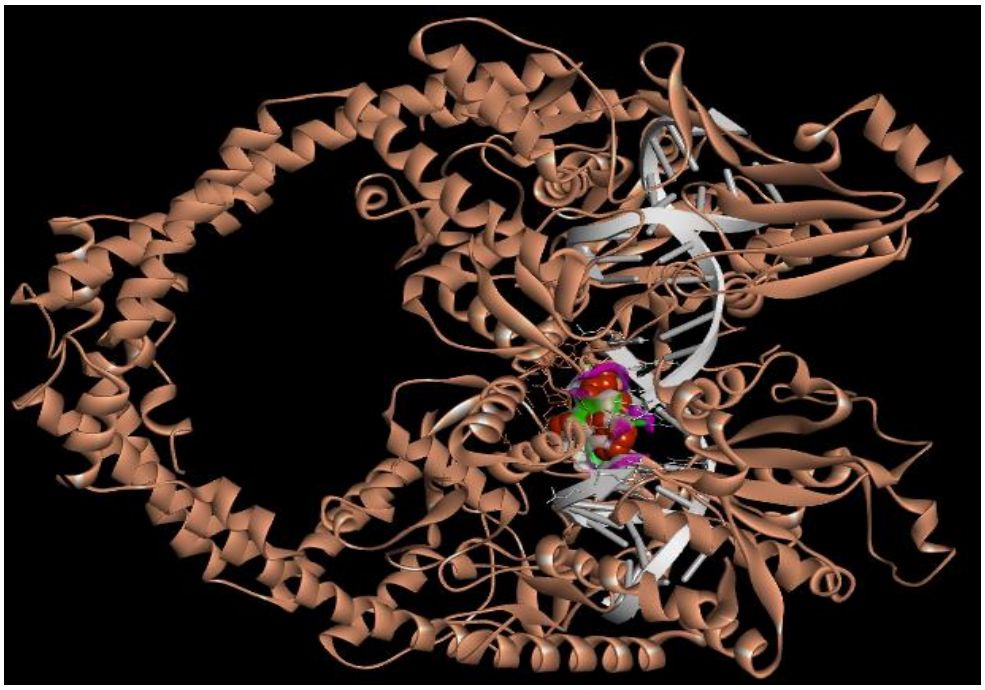


Fig. 13. ***(13a)*** *Bound conformation of human DNA topoisomerase II alpha and ligand* **78** ***(13b)*** *Bound conformation of human DNA topoisomerase II beta and ligand* **78**

Fig. 14a

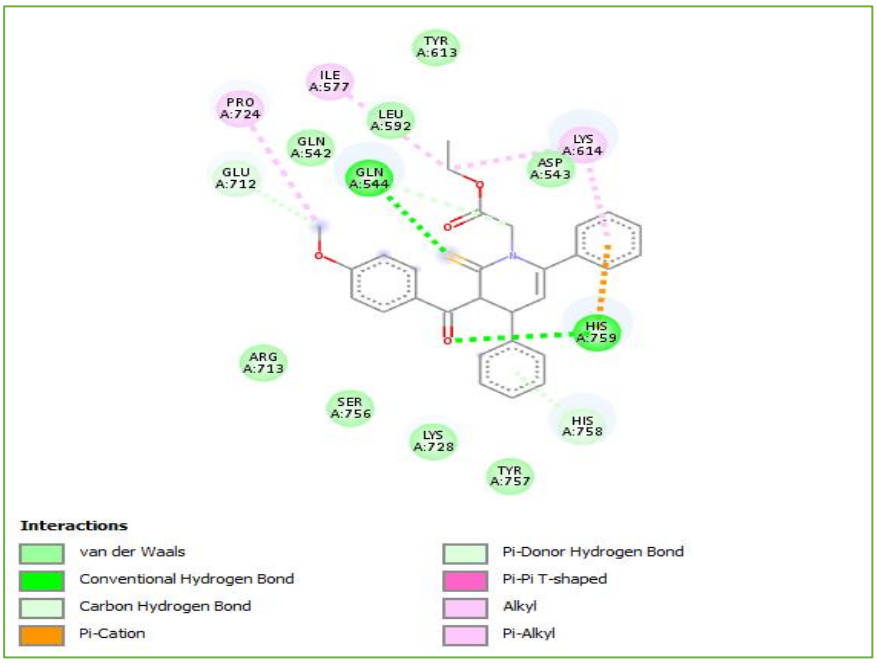


Fig. 14b

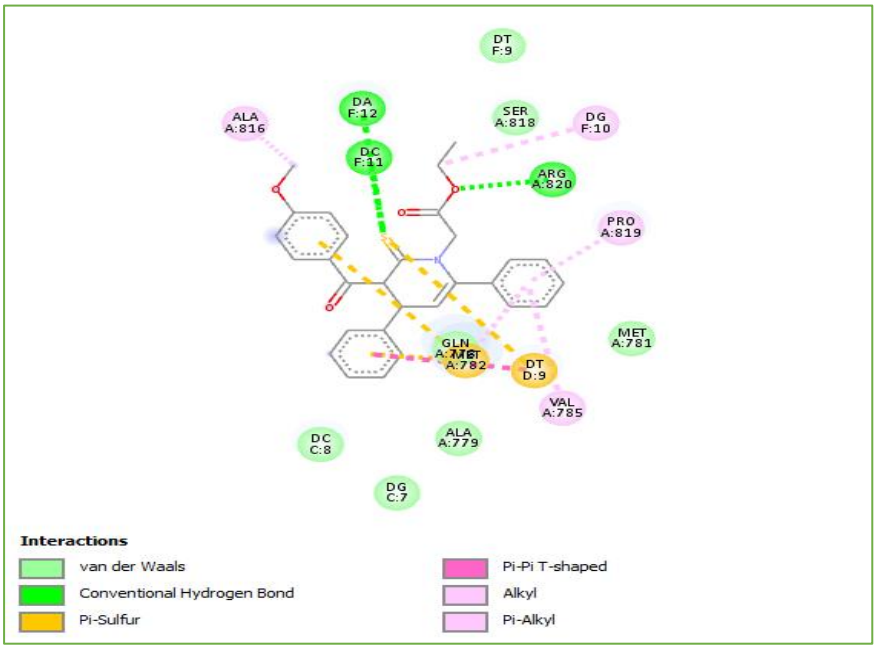


Fig. 14 ***(14a)*** *Interaction between human DNA topoisomerase II alpha and ligand* **78**

(14b) *Interaction between human DNA topoisomerase II beta and ligand* **78**

3,4-Diphenyl thiophene

The binding modes of the compound **97** with the target proteins, human DNA topoisomerase II alpha (PDB id: 4fm9) and human DNA topoisomerase II beta (PDB id: 3qx3), has been calculated (**Fig. 15**). The binding energy, inhibition constant and various interactions of the ligands with active site residues (**Fig. 16**) are summarised in **Table 4**.

The binding energy of compound **97** with the protein human DNA topoisomerase II alpha was -7.1 Kcal/mol with an inhibition constant of 3.13 µM. The interactions between compound **97** with the protein human DNA topoisomerase II alpha involve Van der Waals forces, conventional hydrogen bond, carbon-hydrogen bond, pi- cation, pi-pi T-shaped, alkyl and pi-alkyl.

The binding energy of compound **97** with the protein human DNA topoisomerase II beta was -6.81 Kcal/mol with an inhibition constant of 10.13 µM. The interactions between compound **97** with the protein human DNA topoisomerase II beta involve Van der Waals forces, conventional hydrogen bond, sulfur X, pi-anion, pi-donor hydrogen bond, pi-sigma, pi-pi T-shaped and pi-alkyl.

The binding energy and the inhibition constant values showed that the 3,4-diphenyl thiophene derivative **97** is a good inhibitor for the proteins; human DNA topoisomerase II a (alpha) and human DNA topoisomerase II b (beta). The highest binding energy and lowest inhibition constant value are obtained for ligand **97** with the protein, human DNA topoisomerase II a (alpha) which showed that ligand **97** is a better inhibitor for the protein, human DNA topoisomerase II a (alpha) than for 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 compound **97** exhibits good anticancer properties.

Table. 4

Binding energies and possible interactions of 3,4-diphenyl thiophene **97** with proteins:

Ligand	Protein	Binding energy (Kcal/mol)	Inhibition constant (μM)	H-bonds	Other interactions
Compound **(97)**	DNA topoisomerase II alpha (PDB id: 4fm9)	-7.1	3.13	HIS A:758	Van der Waals C-H bond Pi- cation Pi-Pi T-shaped Alkyl Pi-alkyl
	DNA topoisomerase II beta (PDB id: 3qx3)	-6.81	10.13	GLY A:776 DC C:8	Van der Waals Sulfur X Pi-anion Pi-donor H-bond Pi-sigma Pi-Pi T shaped Pi-alkyl

Fig. 15a

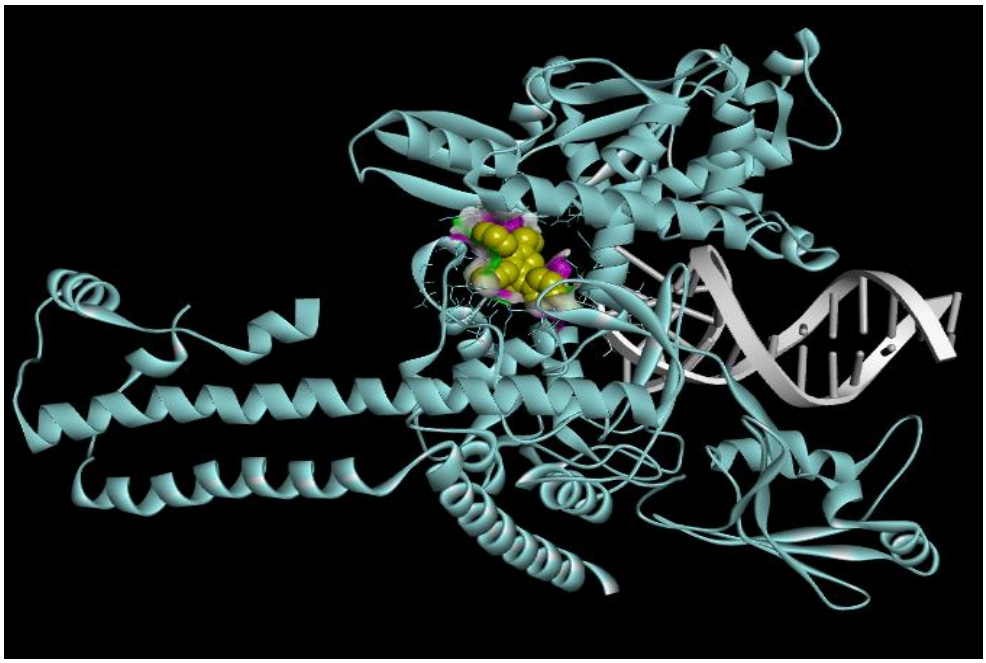


Fig. 15b

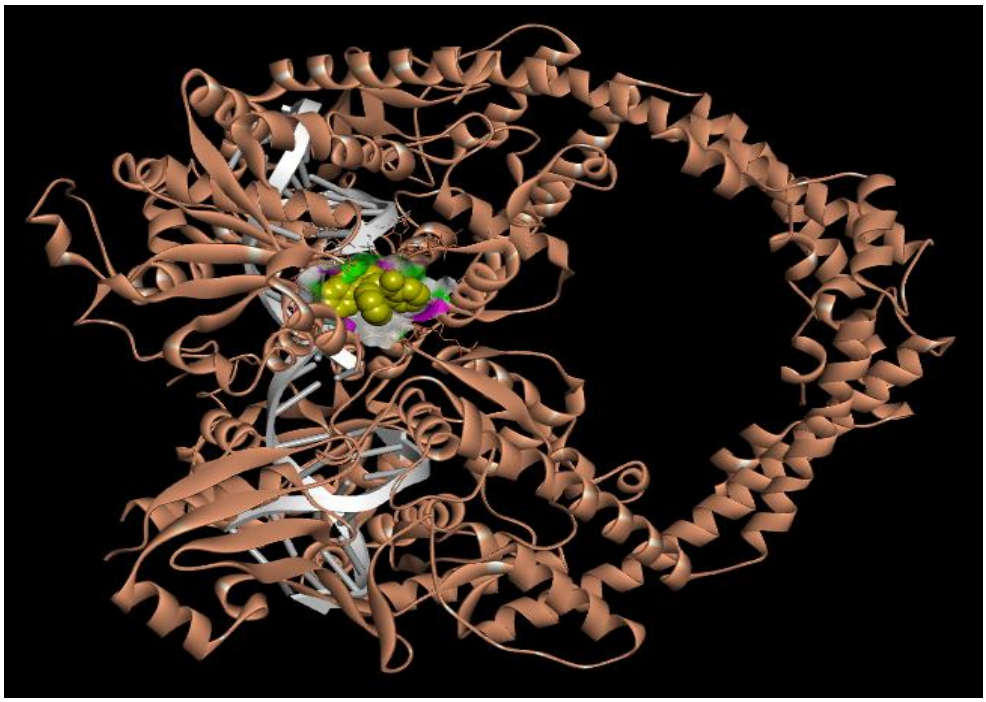


Fig. 15. ***(15a)*** *Bound conformation of human DNA topoisomerase II alpha and ligand* **97**

(15b) *Bound conformation of human DNA topoisomerase II beta and ligand* **97**

Fig. 16a

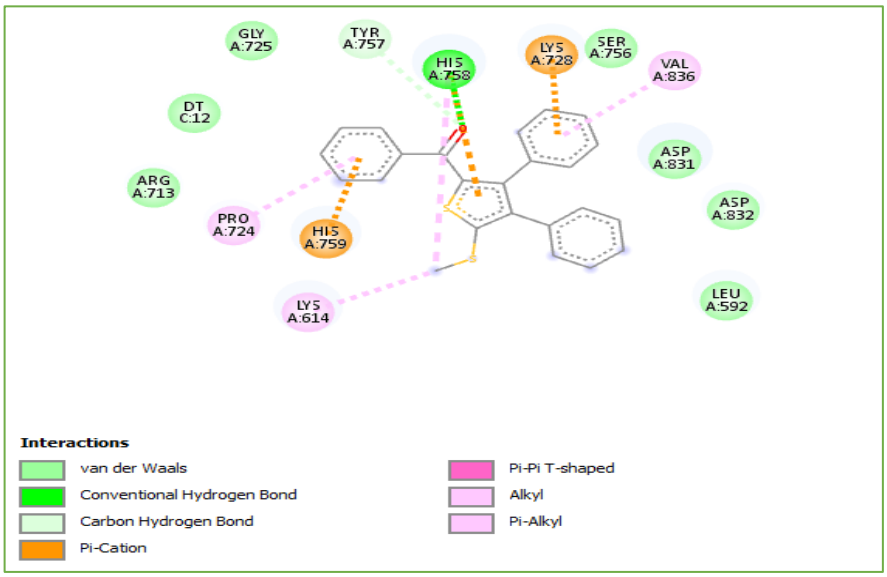


Fig. 16b

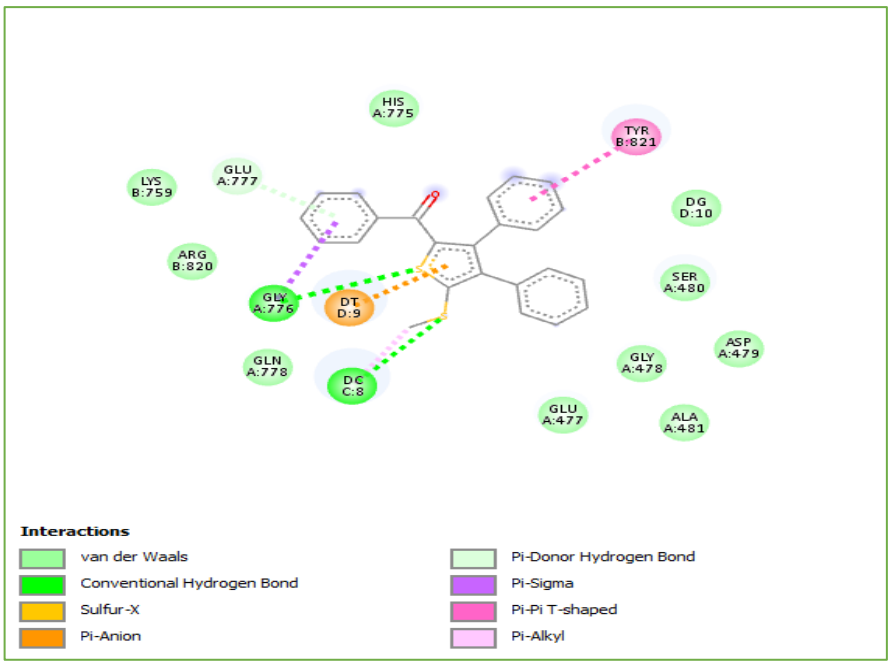


Fig. 16 ***(16a)*** *Interaction between human DNA topoisomerase II alpha and ligand* **97**

(16b) *Interaction between human DNA topoisomerase II beta and ligand* **97**

5.7.2 Molecular docking studies on antibacterial activity

The antibacterial activity of the synthesised dihydropyridine-2-thione **78** and 3,4-diphenyl thiophene **97** against the proteins; FtsZ and DNA gyrase present in *Bacillus subtilis* was theoretically calculated using *in silico* molecular docking methods. The study was performed using the docking programme Autodock vina. The binding modes as well as the binding energy of the dihydropyridine-2-thione **78** and 3,4-diphenyl thiophene **97** with the target proteins have been calculated. The interactions between the proteins and the ligand were checked using the Discovery Studio visualizer.

FtsZ is a cytoskeletal protein present in most bacteria. It plays a crucial role in prokaryotic cell division[47]. DNA gyrase is a topoisomerase type II protein found in every bacterium. It plays a significant role in the relaxation of supercoiled DNA associated with DNA replication and transcription. Inhibition of DNA gyrase inhibits bacterial DNA replication and transcription[48]. Therefore, the molecules that can inhibit the activity of these proteins are said to possess good antibacterial properties. The designing of effective antimicrobials and antibiotics that inhibit FtsZ and DNA gyrase is of special interest in medicinal chemistry.

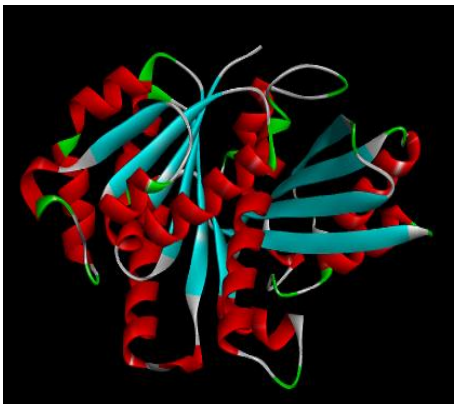


Fig. 17a

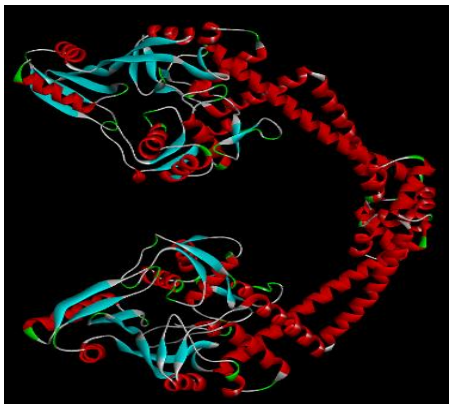


Fig. 17b

Fig. 17. *(**17a**) Structure of Bacillus subtilis FtsZ (PDB id: 2vxy); (**17b**) Structure of Bacillus subtilis DNA gyrase (PDB id: 4ddq)*

The three-dimensional structure of the protein FtsZ and DNA gyrase present in *Bacillus subtilis* was obtained from RCSB PDB (FtsZ - PDB id: 2vxy; DNA gyrase - PDB id: 4ddq) (**Fig. 17**). ACD Chemsketch was used to draw the structure of ligand

molecule. 3D structure in .pdb format was created using the tool CORINA. Before the docking studies, water and other small molecules present in the protein were removed and polar hydrogen atoms were added. This was done using the tool AutoDock 4.2. The final structure of the target and ligand was saved in .pdbqt format. The active site of the target was identified using Castp.

The active site residues present in the protein FtsZ are as follows:

VAL19, GLY20, GLY21, GLY22, ASN44, THR45, ASP46, ALA49, GLY70, ALA71, GLY72, ALA73, GLY104, MET105, GLY106, GLY107, GLY108, THR109, GLY110, THR133, PRO135, GLU139, ARG143, ASN166, PHE183, ALA186, ASP187, LEU190.

The grid was set according to the position of the active site residues and the coordinates are 60 pts in XYZ dimension and centre points are defined at -9.361, 5.194 and 9.167 respectively for x, y and z centres.

The DNA gyrase has 6 chains but as it is a homodimer only chain A and chain B are used for docking. The active site residues in chain A and chain B are as follows:

Chain A:- TYR51, ASN54, ASP55, LEU56, GLY57, THR59, LYS62, PRO63, TYR64, LYS65, LYS66, ARG69, GLU125, THR140, LYS141, TYR368, LYS372, ALA375, ARG376, HIS378, ILE379, LEU383, LYS419, GLN422, ALA423, ASP426, MET427, ARG428, GLN430, ARG431, GLU436, LYS439, ILE440, GLU443.

Chain B:- TYR51, ASN54, ASP55, GLY57, THR59, ASP61, LYS62, PRO63, TYR64, LYS65, LYS66, ARG69, ARG122, TYR123, GLU125, MET133, LEU136, ARG137, ASP138, ILE139, THR140, LYS141, TYR146, TYR368, GLU369, LYS372, ALA373, ARG376, ILE379, LEU383, ALA423, ASP426, MET427, ARG428, GLN430, ARG431, GLU436, LYS439, ILE440, GLU443, LEU447, LEU450.

The grid was set according to the position of the active site residues and the coordinates are 90 pts in XYZ dimension and centre points are defined at 21.86, 0.306 and -0.917 respectively for x, y and z centres.

Dihydropyridine-2-thione

The binding modes of compound **78** with the target proteins*; Bacillus subtilis* FtsZ and DNA gyrase has been calculated (**Fig. 18**). The binding energy and various interactions of the ligand with active site residues of FtsZ and DNA gyrase **(Fig. 19)** are summarised in **Table 5**.

The binding energy of compound **78** with the protein FtsZ was -6.8 kcal/mol. The ligand formed a single hydrogen bond interaction with ARG A:143 of chain A together with Van der Waals, alkyl and pi-alkyl interactions.

The binding energy of compound **78** with the protein DNA gyrase was -6.1 kcal/mol. The ligand formed two H-bond interactions with ARG A:437 of chain A and ARG B:398 of chain B, together with Van der Waals, pi-anion and pi-alkyl interactions.

Table.5 Binding energies and possible interactions of dihydropyridine-2-thione **78** with proteins:

Ligand	Protein	Binding energy (Kcal/mol)	H-bonds	Other interactions
Compound **(78)**	*B. subtilis* FtsZ (PDB id: 2vxy)	-6.8	ARG A:143	Van der Waals alkyl Pi-alkyl
	B. subtilis DNA gyrase (PDB id: 4ddq)	-6.1	ARG A:437 ARG B:398	Van der Waals Pi-anion Pi-alkyl

The binding energy values showed that dihydropyridine-2-thione derivative **78** is a good inhibitor for the proteins; FtsZ and DNA gyrase. The highest binding energy was obtained for ligand **78** with the protein, FtsZ which showed that ligand **78** is a better inhibitor for the protein, FtsZ than for DNA gyrase. The values obtained in the docking study established that compound **78** exhibits good antibacterial properties.

Fig. 18a

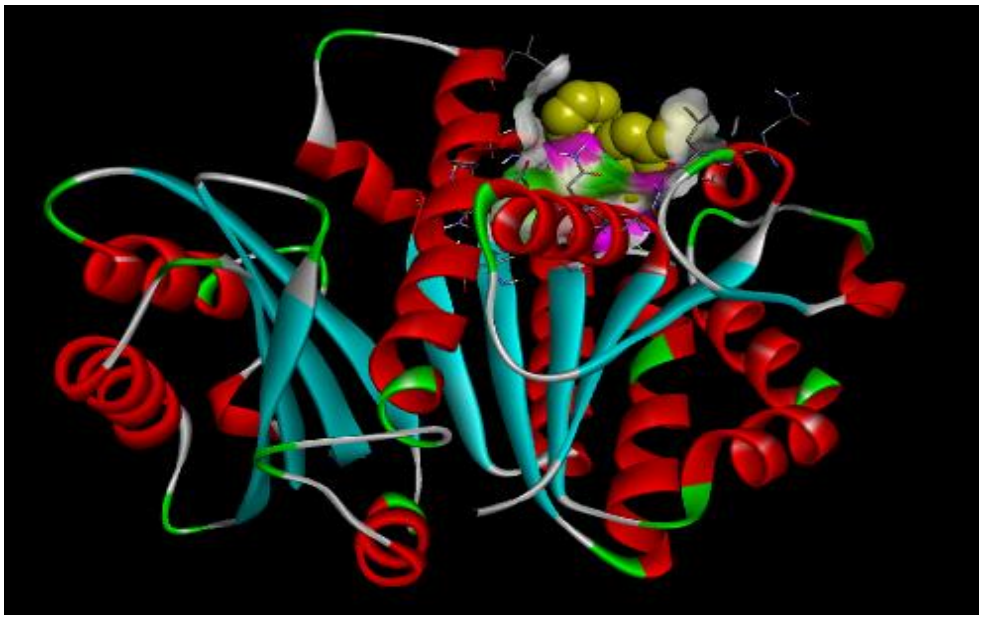


Fig. 18b

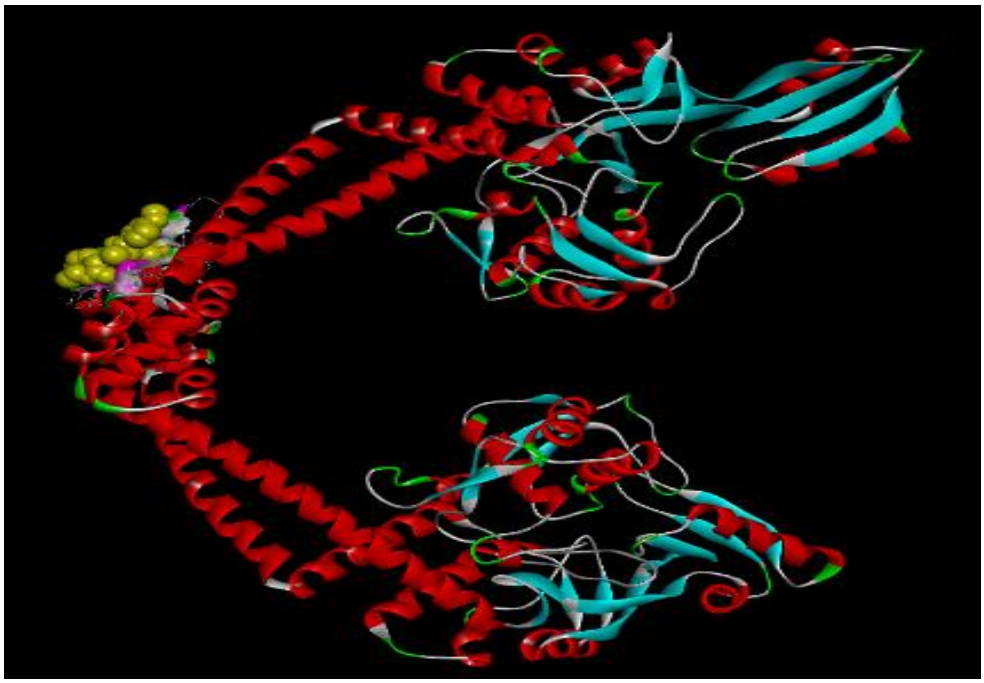


Fig. 18. ***(18a)*** *Bound conformation of Bacillus subtilis FtsZ and ligand* **78**

*(18**b**) Bound conformation of Bacillus subtilis DNA gyrase and ligand* **78**

Fig. 19a

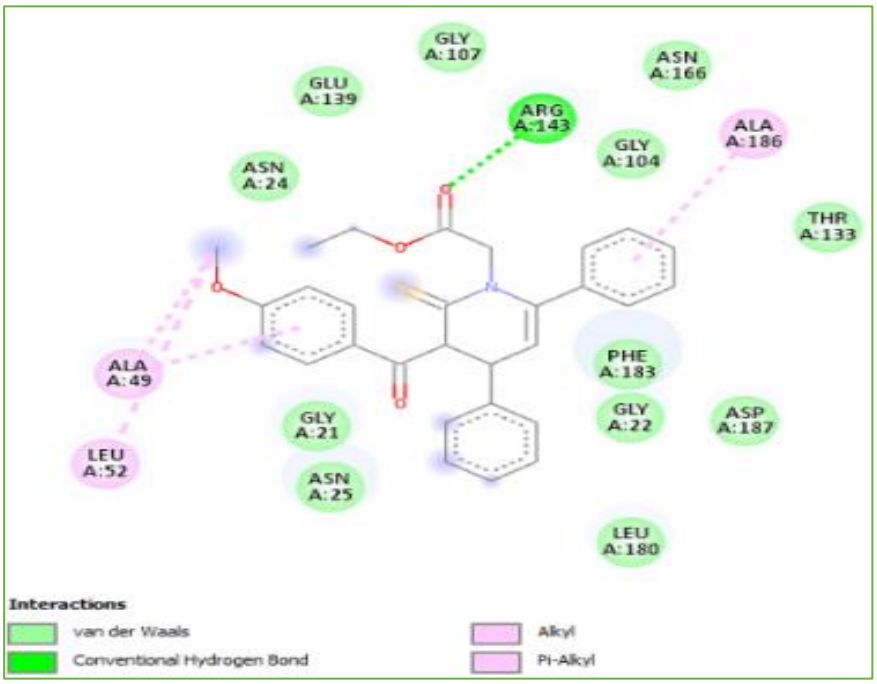


Fig. 19b

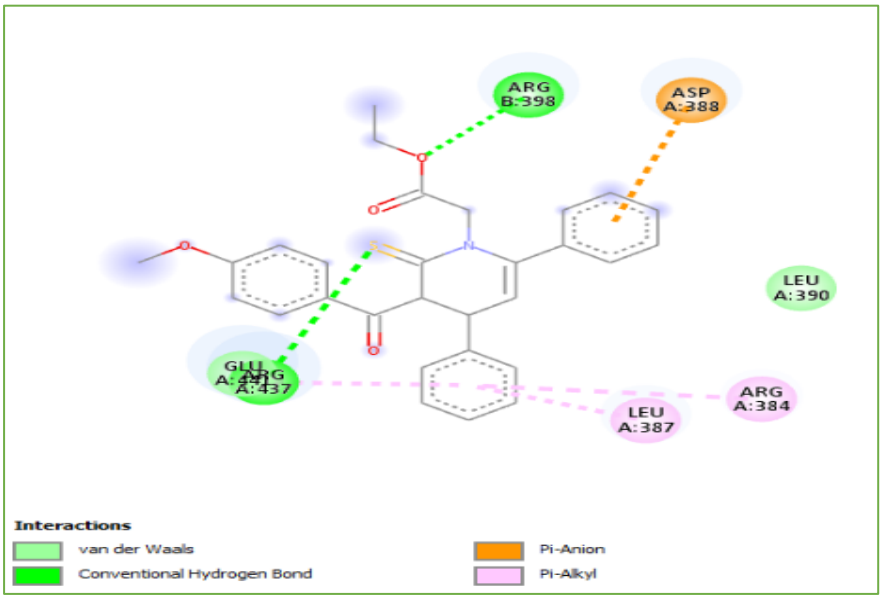


Fig. 19. (19a) *Interaction between Bacillus subtilis FtsZ and ligand* **78**

(19b) *Interaction between Bacillus subtilis DNA gyrase and ligand* **78**

3,4-Diphenyl thiophene

The binding modes of 3,4-diphenyl thiophene **97** with the target proteins; *Bacillus subtilis* FtsZ and DNA gyrase has been calculated (**Fig. 20**). The binding energy and various interactions of the ligand with active site residues of FtsZ and DNA gyrase **(Fig. 21)** are summarised in **Table 6**.

The binding energy of 3,4-diphenyl thiophene **97** with the protein FtsZ was -7.3 kcal/mol. The ligand has two hydrogen bond interactions with GLY A:107 (chain A) and ARG A:143 (chain A) together with Van der Waals, pi-anion, pi-sulfur and pi-pi stacked interactions.

The binding energy of 3,4-diphenyl thiophene **97** with the protein DNA gyrase was -6.1 kcal/mol. The ligand formed one H-bond interaction with LYS A:419 (chain A) together with Van der Waals, pi-cation, alkyl and pi-alkyl interactions.

Table.6 Binding energies and possible interactions of 3,4-diphenyl thiophene **97** with proteins:

Ligand	Protein	Binding energy (Kcal/mol)	H-bonds	Other interactions
Compound **(97)**	*B. subtilis* FtsZ (PDB id: 2vxy)	-7.3	GLY A:107 ARG A:143	Van der Waals Pi-anion Pi-sulfur Pi-pi stacked
	B. subtilis DNA gyrase (PDB id: 4ddq)	-6.1	LYS A:419	Van der Waals Pi-cation Alkyl Pi-alkyl

The binding energy values showed that the 3,4-diphenyl thiophene **97** is a good inhibitor for the proteins; FtsZ and DNA gyrase. The highest binding energy was obtained for ligand **97** with the protein FtsZ which showed that ligand **97** is a better inhibitor for the protein FtsZ than for DNA gyrase. The values obtained in the docking study established that compound **97** exhibits good antibacterial properties.

Fig. 20a

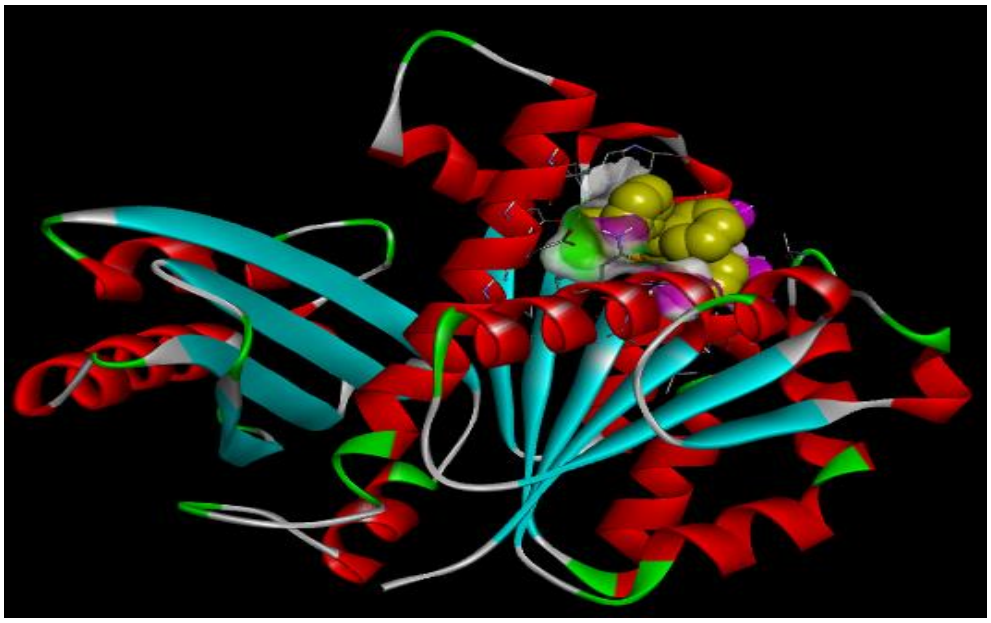


Fig. 20b

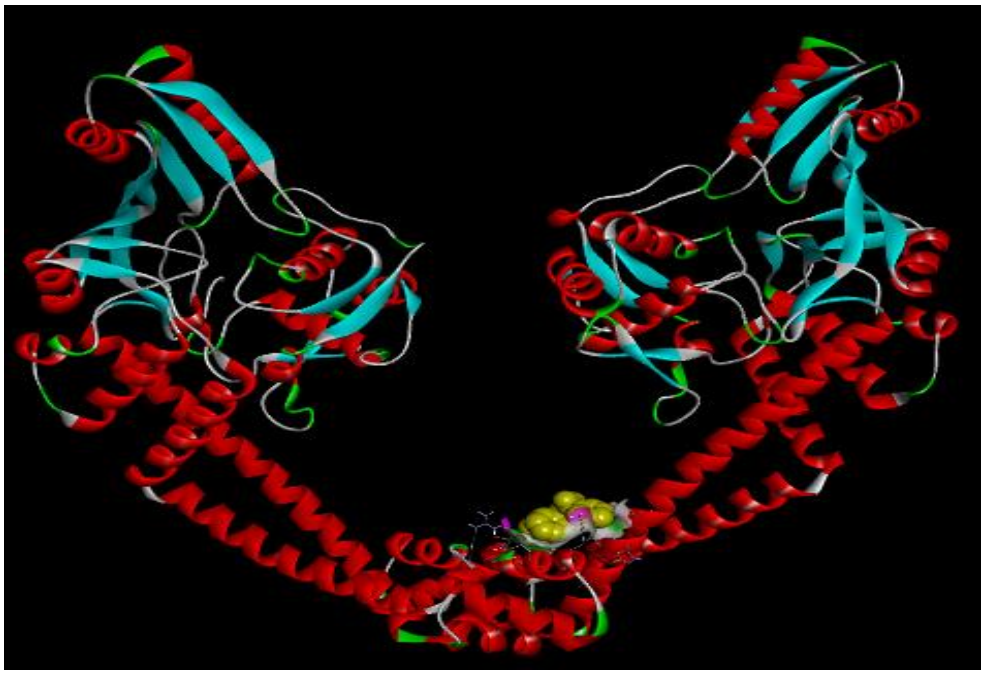


Fig. 20. ***(20a)*** *Bound conformation of Bacillus subtilis FtsZ and ligand* ***97***

(20b) *Bound conformation of Bacillus subtilis DNA gyrase and ligand* ***97***

Fig. 21a

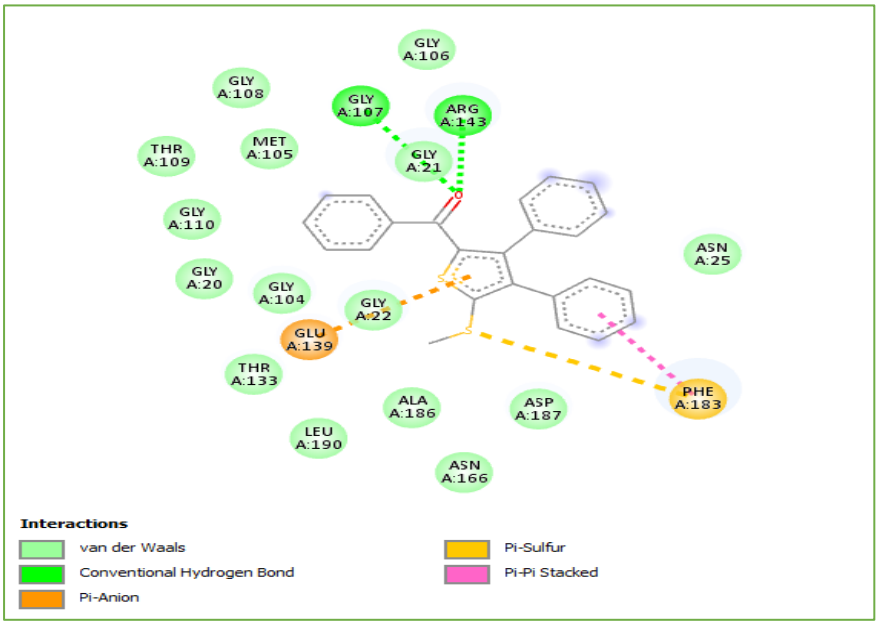


Fig. 21b

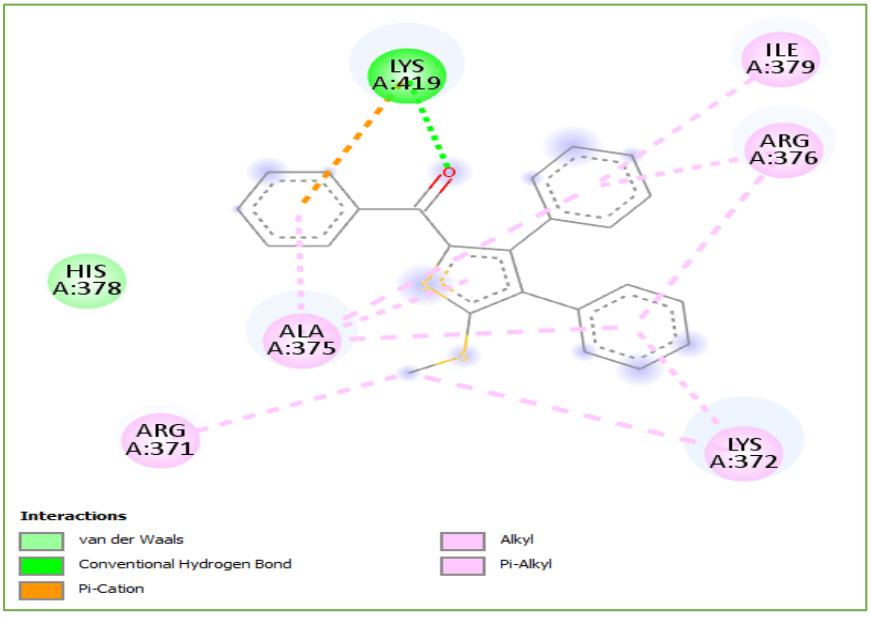


Fig. 21. ***(21a)*** *Interaction between Bacillus subtilis FtsZ and ligand* ***97***

(21b) *Interaction between Bacillus subtilis DNA gyrase and ligand* ***97***

5.8 Experimental details

5.8.1 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 without further purification. FT-IR spectra were recorded on Perkin Elmer Spectrum Two FT-IR with a wavelength range of 8300-350 cm^{-1} and spectral resolution of 0.5 cm^{-1}. ^{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). 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 a UV lamp. Chromatography was carried out using silica gel 60-20 mesh (Merck, India) and different solvents as mobile phases. Molecular docking studies were performed with the help of Green Clones Naturals Pvt. Ltd., Ernakulam.

5.8.2 Synthesis of dihydropyridine-2-thione

Synthesis of β-oxodithioester

β-Oxodithioester **72** was synthesised using the same method as explained in the chapter 3.

Synthesis of thioamide

A 100 mL round bottom (RB) flask fitted with a guard tube and magnetic bead was charged with β-oxodithioester **72** (0.85 mmol, 1 eq.), glycine ethyl ester hydrochloride **73** (1 mmol, 1 eq.) and triethylamine (1.8 mmol, 2 eq.). The reaction mixture was dissolved in ethyl alcohol (10 mL) and magnetically stirred at room temperature for 20h. After the completion of the reaction (monitored by Thin layer chromatography), the reaction mixture was diluted with ice water followed by acidification with dilute hydrochloric acid. The product was extracted with chloroform

(3 x 20 mL). The organic layer was washed with water and dried over anhydrous sodium sulphate. The solvent was removed by evaporation using rotavapor. The residue was purified employing column chromatography where the product was allowed to pass through a column of silica gel packed in hexane and product **74** was eluted with the 1:1 hexane and ethyl acetate mixture.

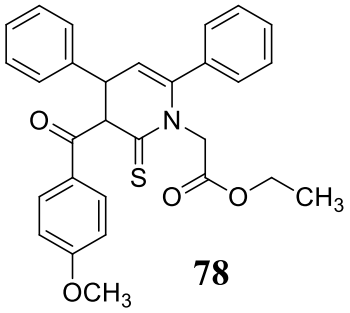
Synthesis of chalcone

Sodium hydroxide (55 mmol) was dissolved in water (30 mL) taken in a 250 mL round bottom (RB) flask provided with a magnetic bead and stopper. Ethyl alcohol (10 mL) was added to the reaction mixture and was cooled in an ice bath. To the ice-cooled reaction mixture, acetophenone **75** (43 mmol, 1 eq.) was added and stirred immediately. Benzaldehyde **76** (44 mmol, 1 eq.) was added to the reaction mixture while stirring and the reaction continued for 12h at 15-30 ºC. After the completion of the reaction (monitored by TLC), the reaction mixture was added to ice water and filtered through a Buchner funnel. The precipitate was dissolved in dichloromethane (DCM) and purified by column chromatography using silica gel as stationary phase and hexane: ethyl acetate (10:1) as eluent to afford chalcone **77** in good yield.

Synthesis of dihydropyridine-2-thione

The thioamide **74** (0.5 mmol, 1 eq.), chalcone **77** (0.6 mmol, 1.2 eq.) and triethylamine (2 mmol, 4 eq.) were taken in a 100 mL round bottom (RB) flask provided with magnetic bead and guard tube. After dissolving the reaction mixture in dichloromethane (10 mL), titanium tetrachloride (2 drops) was added and stirred at room temperature for 24h. After the completion of the reaction, which was monitored by thin layer chromatography (TLC), the reaction mixture was diluted with ice water and the product was extracted with dichloromethane (3 x 20 mL). The organic layer was washed with water and dried over anhydrous sodium sulphate. The solvent was removed by evaporation using rotavapor. The product was purified using column chromatography where it was allowed to pass through a column of silica gel packed in

hexane and eluted with the mixture of 5:1 hexane and ethyl acetate. Evaporation of the solvent yielded pure pale yellow crystals of dihydropyridine-2-thione (**78**).



78

Yield =90%, ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (t, *J*=7.2 Hz, 3H), 3.86 (s, 3H), 4.16-4.14 (m, 2H), 6.93 (s, 1H), 5.38 (d, *J*= 4.8 Hz, 1H), 5.52 (d, *J*= 6 Hz, 1H), 7.99-6.93 (signals of aromatic protons); ^{13}C NMR (100MHz, $CDCl_3$): δ 14.0, 41.0, 52.4, 55.5, 61.2, 63.5, 114.0, 141.7, 163.7, 167.2, 193.2, 196.5. IR (KBr): 721, 1216, 1376, 1458, 1738, 2852 and 2920 cm^{-1}.

5.8.3 Synthesis of 3,4-diphenyl thiophene

Synthesis of β-oxodithioester

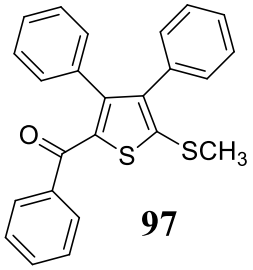
Methyl-3-oxo-2,3-diphenylpropanedithioate was synthesised from 1,2-diphenylethanone using the same method of the synthesis of β-oxodithioester as explained in the chapter 3.

Synthesis of phenacyl bromide

A 100 mL dry round bottom (RB) flask equipped with a magnetic bead and a stopper was charged with acetophenone **95** (25 mmol) and acetic acid. The reaction mixture was heated to 60 ºC to dissolve acetophenone in acetic acid. The solution was cooled in an ice bath and to this ice-cold solution, bromine in acetic acid (8 mL) was added drop by drop while stirring. The reaction mixture was stirred at room temperature for 1h. After that, the product was extracted using diethyl ether. The evaporation of the solvent afforded phenacyl bromide **96** in good yield.

Synthesis of 3,4-diphenyl thiophene

A 100 mL dry round bottom flask equipped with a magnetic bead and a stopper was charged with β-oxodithioester **94** (2 mmol), potassium carbonate (1 mmol) and acetone (10 mL). The reaction mixture was stirred at 60 ºC for 1h. After that, phenacyl bromide **96** (1.7 mmol) was added to the reaction mixture and stirred again at 60 ºC for 2h. The reaction mixture was cooled and the product was extracted using ethyl acetate (2 ×30 mL). The organic layer was washed with water (2 × 25 mL) and dried over anhydrous sodium sulphate. The solvent was removed by evaporation using rotavapor. The product was purified using column chromatography where it was allowed to pass through a column of silica gel packed in hexane and eluted with hexane and ethyl acetate mixture (5:1). Evaporation of the solvent yielded 3,4-diphenyl thiophene **97**.



97

Yield =92%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.56 (s, 3H), 6.85-6.82 (m, 1H), 6.91-6.88 (m, 2H), 7.08-7.04 (m, 2H), 7.13-7.11 (m, 1H), 7.29-7.23 (m, 2H), 7.08-7.04 (m, 2H), 7.54-7.51 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 18.83, 77.00, 127.23, 127.41, 127.60, 127.99, 129.28, 130.20, 130.45, 131.71, 134.61, 134.95, 136.87, 137.82, 140.88, 144.12, 146.93, 189.31.

5.9 Conclusion

New synthetic protocols for dihydropyridine-2-thiones **78** and 3,4-diphenyl thiophene **97** from β-oxodithioesters have been introduced. The structure of pyridine-2-thiones was **78** confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and single crystal X-ray diffraction analysis. The structure of 3,4-diphenyl thiophene **97** was confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy

In vitro antibacterial properties of dihydropyridine-2-thione **78** against bacteria- *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were studied via disc diffusion method. The study showed that the compound exhibit mild activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* but is inactive against *Escherichia coli*.

The antibacterial properties of dihydropyridine-2-thione **78** and 3,4-diphenyl thiophene **97** against bacterial proteins FtsZ and DNA gyrase present in *Bacillus subtilis* have been studied *via in silico* molecular docking methods. Both dihydropyridine-2-thione **78** and 3,4-diphenyl thiophene **97** exhibited the higher binding energy with the protein, FtsZ than with DNA gyrase. These results showed that compounds **78** and **97** are better inhibitors for the protein, FtsZ than for DNA gyrase. The docking study established that compounds **78** and **97** exhibit good antibacterial properties.

The applicability of dihydropyridine-2-thione **78** and 3,4-diphenyl thiophene **97** as anticancer drugs has been theoretically proved *via in silico* molecular docking studies. The molecular docking studies showed that both compounds exhibit various interactions with human DNA topoisomerase II alpha and II beta. These interactions are responsible for the enhanced cytotoxicity of these compounds. In the case of compound **78**, the binding energy and inhibition constant values for ligand-protein interactions showed that dihydropyridine-2-thione **78** is a better inhibitor for DNA topoisomerase II beta than for DNA topoisomerase II alpha. But in the case of compound **97**, the binding energy and inhibition constant values for ligand-protein interactions showed that compound **97** is a better inhibitor for DNA topoisomerase II alpha than for DNA topoisomerase II beta.

The docking studies established that compounds **78** and **97** exhibit good antibacterial and anticancer properties.

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