Chapter 1

Synthesis, Type II Diabetes Inhibitory Activity and Docking Studies of Novel Thiazole Molecules

1.1 Introduction

1,3-thiazoles, which include sulphur and nitrogen, are a significant class of bioactive molecules and are well recognized for their various activities. The composition of vitamin B includes the thiazole core. Thiazoles are utilised to create complexes of transition metals with free carbene molecules and their salts are used as catalysts in the Stetter and benzoin condensation reactions.[1] Hantzsch and Weber initially described thiazole's significant biological uses in 1887.[2] While certain of their variants act identically to thiophene and furan, it exhibits similar chemical and physical properties to pyridine and pyrimidine. The molecular electrostatic potential points out that nitrogen is the most negative atom compared to carbon and sulphur, which are neutral.[3] Heterocycles having sulphur and nitrogen atom like thiazole showed a key role in medicinal research (I-V, **Fig 1**).

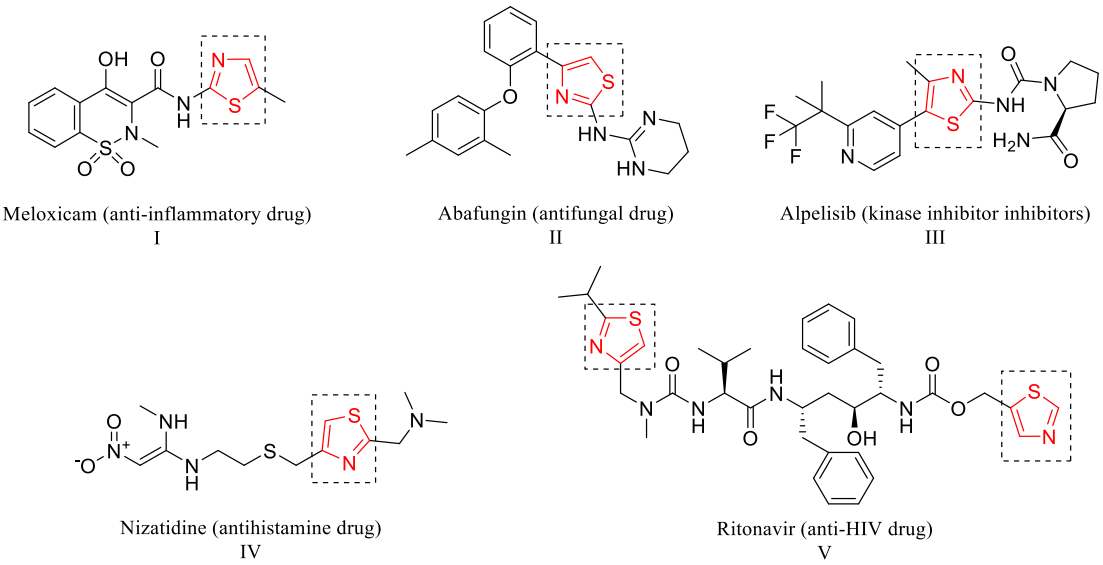


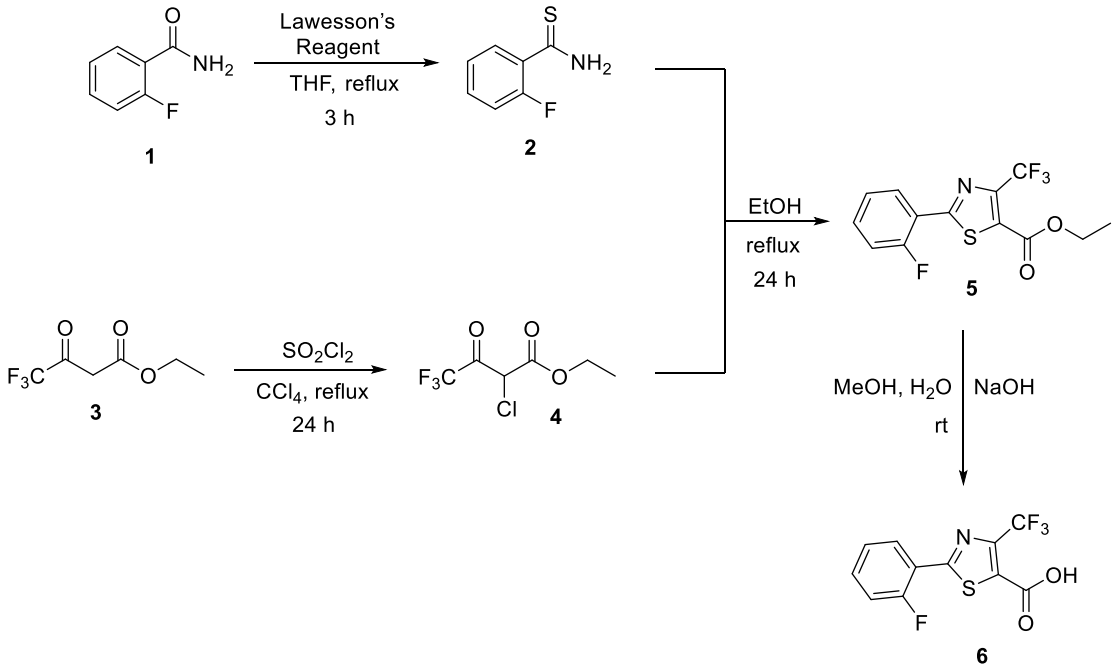
Fig 1. Several bioactive thiazoles I-V.

The thiazole molecule has fascinated good interest of researchers due to their ready accessibility, good chemical and biological activities such as anticancer,[4-6]

antidiabetic,[7] antimicrobial,[8] antiviral,[9] antifungal,[10] antitubercular,[11] antihypertensive[12] and antialzheimer's agent.[13]

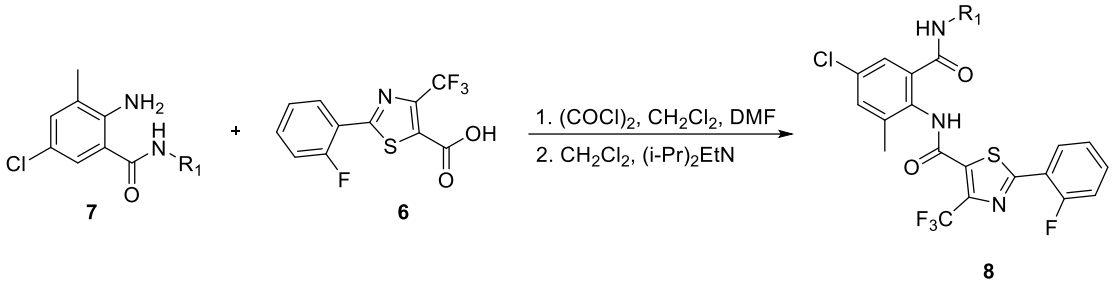
1.1.1 Synthetic approaches for the substituted thiazole scaffold and its biological importance

W. Cai *et al*[14] reported thiazole derivatives synthesis starting from 2-fluoro benzamide **1** converted to thioamide **2** via reaction with lawesson's reagent in tetrahydrofuran at reflux for 3 hr. The reaction of thioamide **2** with ethyl 2-chloro trifluoro acetoacetate **4** gave cyclized thiazole ring **5** with trifluoro substitution **(Scheme 1.1)**.



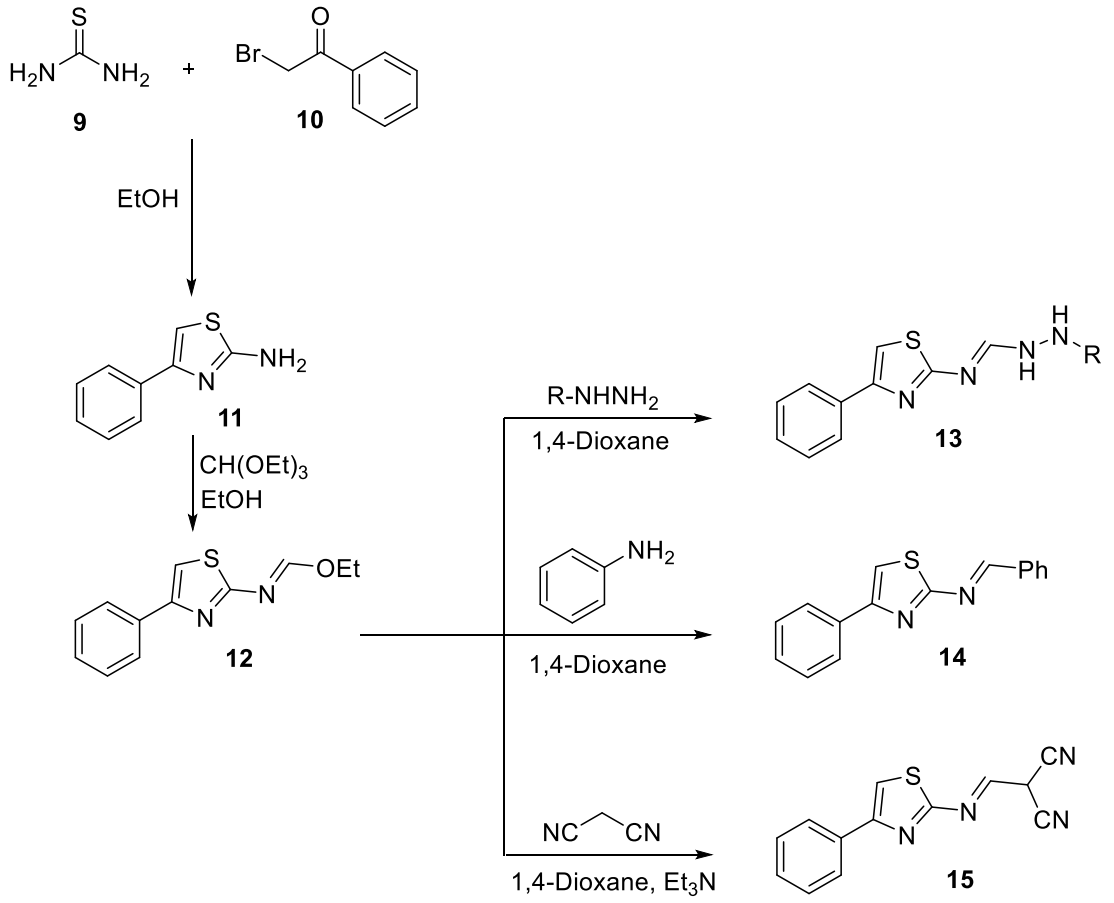
Scheme 1.1

This thiazole ester was reacted with sodium hydroxide, methanol and water to convert it to acid **6**. Furthermore, reaction of thiazole acid **6** molecule with various amino benzamide **7** in DMF with oxalyl chloride, chloroform and DIPEA gave series of thiazole derivatives **8 (Scheme 1.2)**. The synthesized molecules were screened for their anticancer activity in which showed moderate to good inhibitory activity. It was also found that chloro group was responsible for the increase in inhibitory activity.



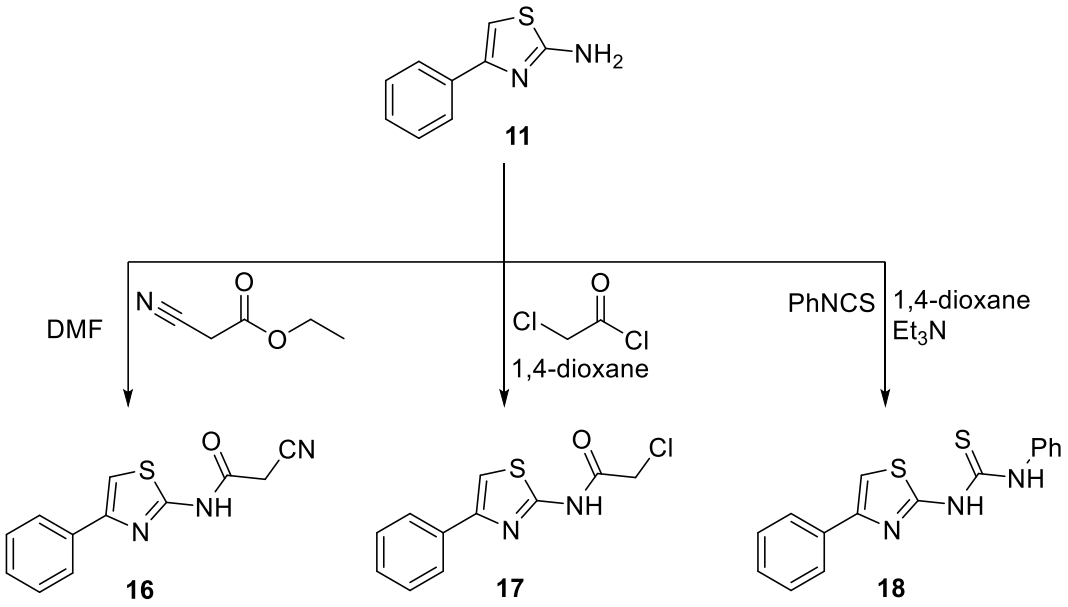
Scheme 1.2

A. Abdallah *et al*[15] reported synthesis of thiazole derivatives starting from thiourea **9** was reacted with derivatives of phenacyl bromide **10** to get amino phenyl pyrazole **11**. Moreover, reaction of **11** with triethyl orthoformate gave molecule **12**, which was reacted with various substitutions like phenyl hydrazine, aniline and malononitrile to give derivatives of thiazoles **13**, **14** and **15 (Scheme 1.3)**.



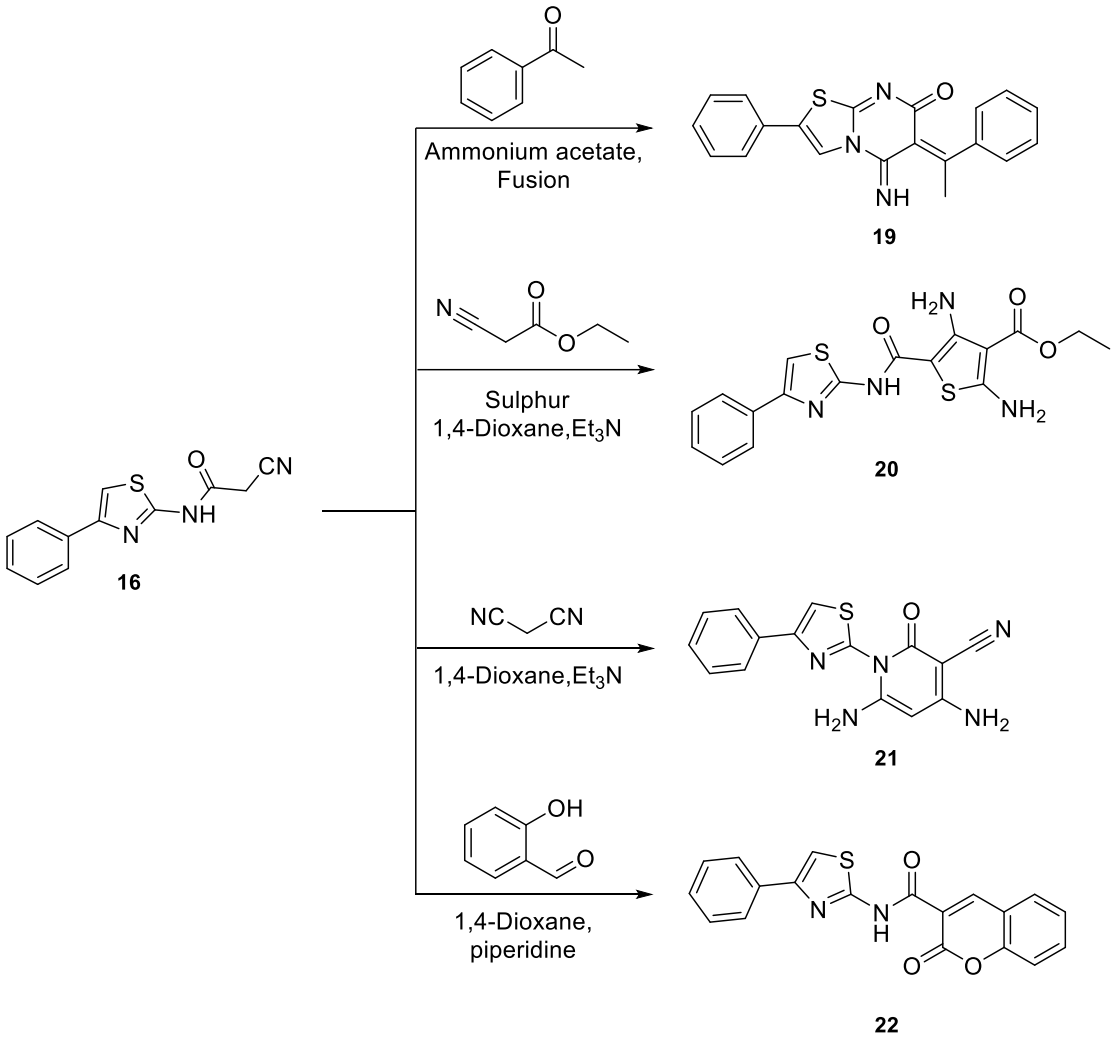
Scheme 1.3

The reaction of molecule **11** with different compounds like ethyl cyanoacetate in DMF, chloroacetyl chloride in 1,4-dioxane and phenyl isothiocyanate and triethyl amine in 1,4-dioxane gave different substituted derivatives **16**, **17** and **18 (Scheme 1.4)**.



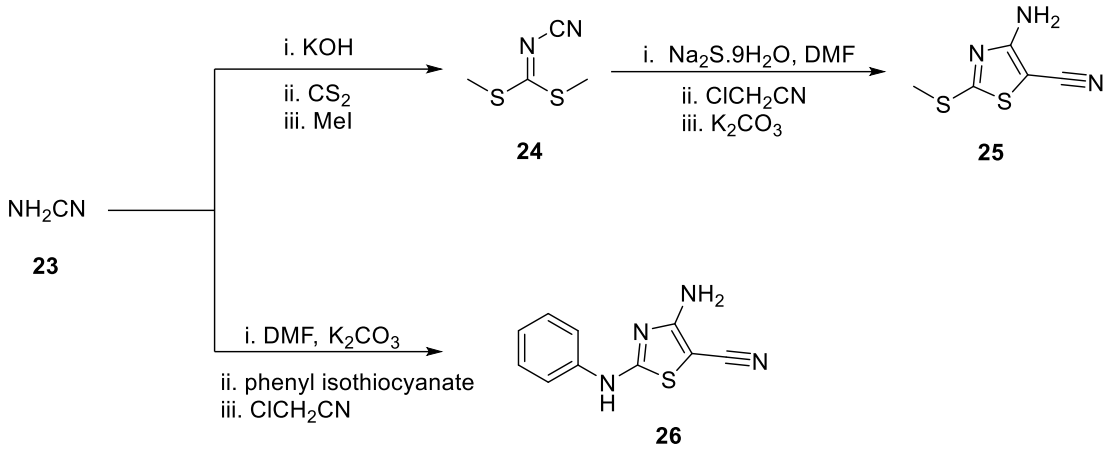
Scheme 1.4

The reaction of cyanoacetamide **16** with acetophenones, ethyl cyanoacetate, sulphur, malononitrile and salicylaldehyde gave a series of molecules **19**, **20**, **21** and **22 (Scheme 1.5)**. The synthesized molecules were screened for anticancer evaluation, in which it was found that the molecule thiazole-thiophene **20** showed a higher inhibition zone compared to standard doxorubicin against various cell lines MCF-7, NCI-H460 and SF-268.



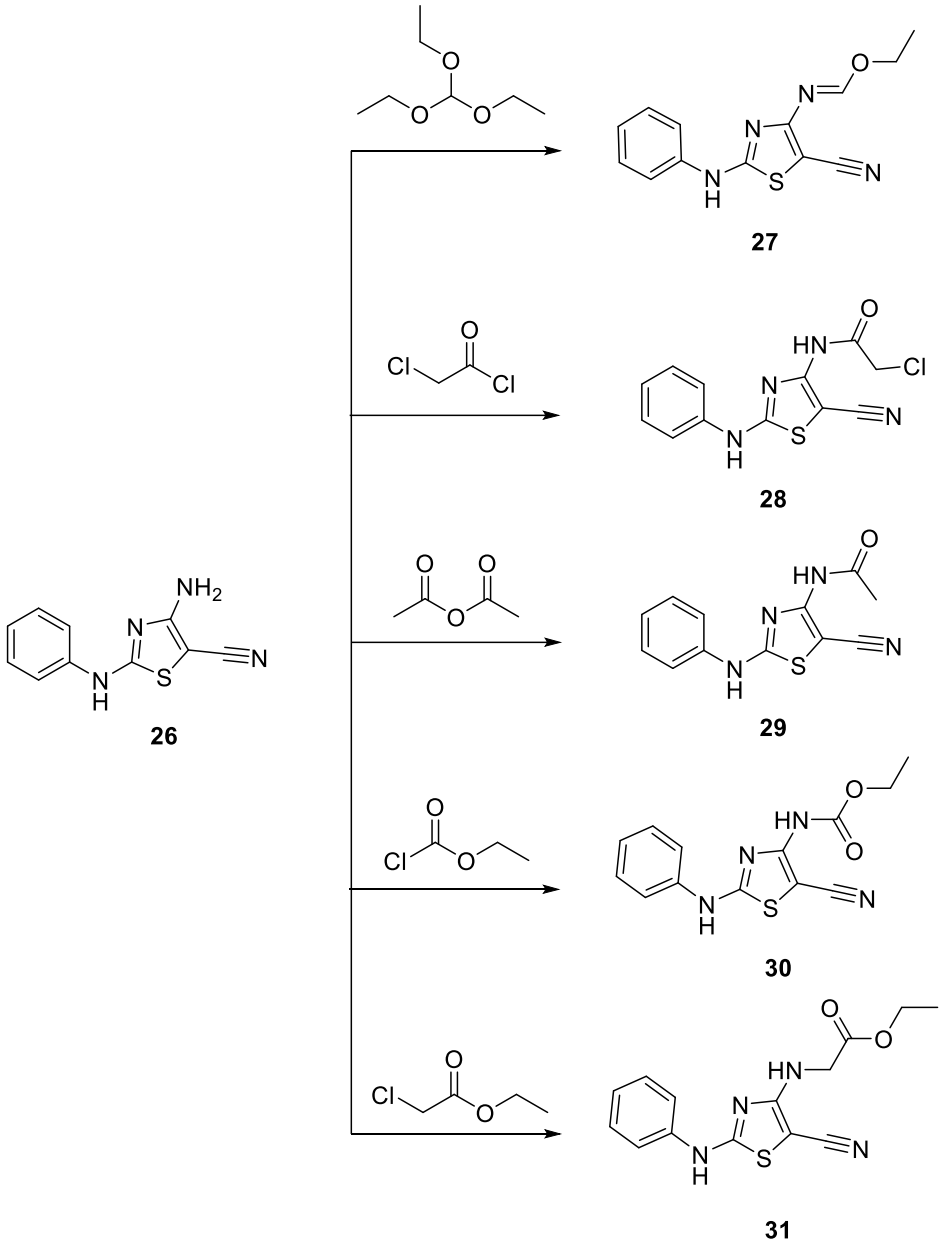
Scheme 1.5

D. Thomae *et al*[16] reported new thiazole derivatives synthesis starting from cyanamide **23** reacted with potassium hydroxide, carbon disulphide followed by methylation with methyl iodide gave ketene dithioacetal derivative **24**, which was further cyclized via reacting with sodium sulphide and chloro acetonitrile to give new thiazole derivative **25**. Similarly, reacting cyanamide **23** with phenyl isothiocyanate and chloroacetonitrile gave the phenyl thiazole derivative **26 (Scheme 1.6)**.



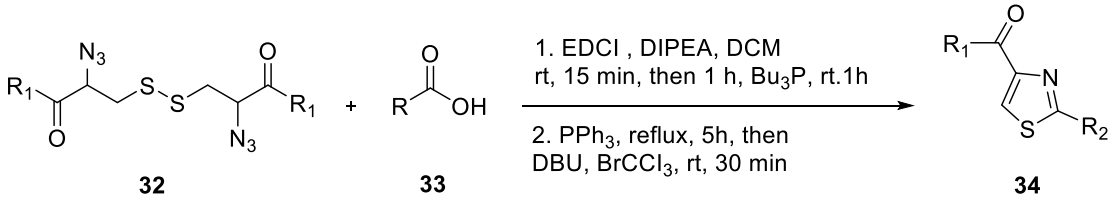
Scheme 1.6

A. Elhameed *et al*[17] reported synthesis of thiazole derivatives starting from phenyl thiazole **26** was reacted with various molecules like triethyl orthoformate, chloroacetyl chloride, acetic anhydride, ethyl chloroformate and ethyl chloroacetate to give range of molecules **27**, **28**, **29**, **30** and **31**. The synthesized molecules were screened for antimicrobial, antiquorum sensing and antitumor activity **(Scheme 1.7)**.



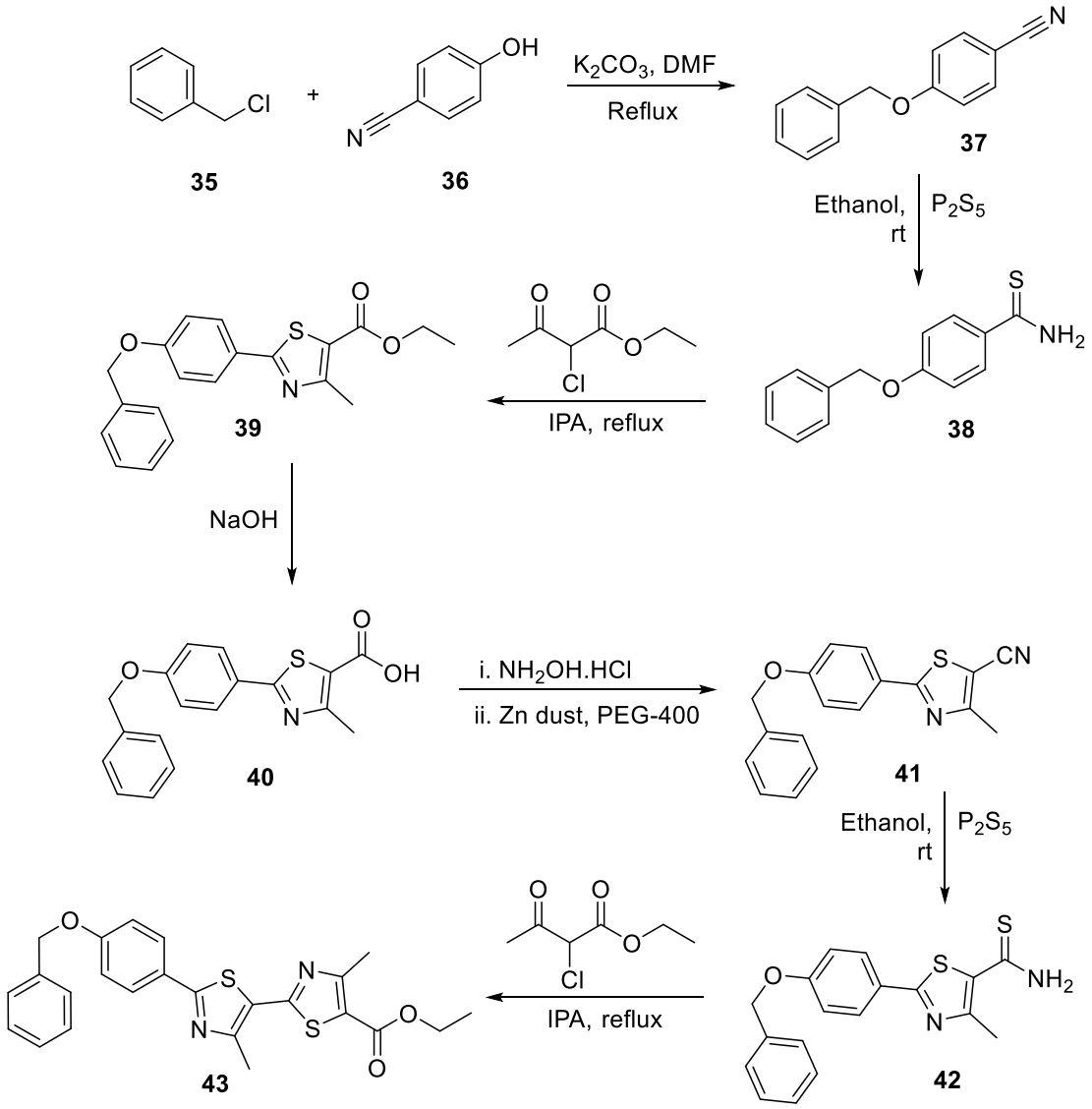
Scheme 1.7

Y. Liu *et al*[18] reported an impressive one-pot thiazole synthesis, starting from β-azido disulphide ester reacted with carboxylic acid derivatives in presence of bromo trichloro methane and DBU gave novel thiazole derivatives **(Scheme 1.8)**.



Scheme 1.8

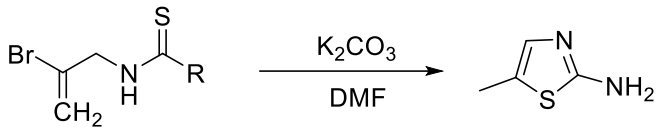
R. Borde *et al*[19] reported the synthesis of bis-thiazole derivatives, starting from benzyl chloride **35** and 4-hydroxybenzonitrile **36**, which was reacted in DMF to form molecule **37,** which was reacted with potassium pentasulphide to form a thioamide molecule, which was cyclized by reaction with ethyl 2-chloro acetoacetate to form a thiazole molecule **39**. Furthermore, ester of thiazole molecule was hydrolyzed **40** and converted into cyanide **41**, which was again reacted with potassium pentasulphide **42** and ethyl 2-chloro acetoacetate to form bis-thiazole molecule **43**. The synthesized molecules showed moderate to good antimicrobial activity **(Scheme 1.9)**.



Scheme 1.9

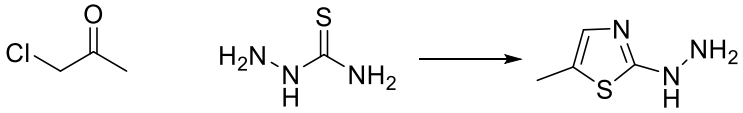
L. Rahem *et al*[20] showed a variety of reactions to synthesize novel thiazole derivatives. Compound **44** was cyclized by refluxing in DMF with potassium hydroxide

and gave amino thiazole **45 (Scheme 1.10)**. α-halo acetone **46** was reacted with thiosemicarbazide **47** to obtain hydrazinyl thiazole molecule **48 (Scheme 1.11)**. Furthermore, the reaction of acetophenone **49**, NBS **50** and thiourea derivatives **51** afforded cyclized phenyl thiazole derivatives **52 (Scheme 1.12)**.



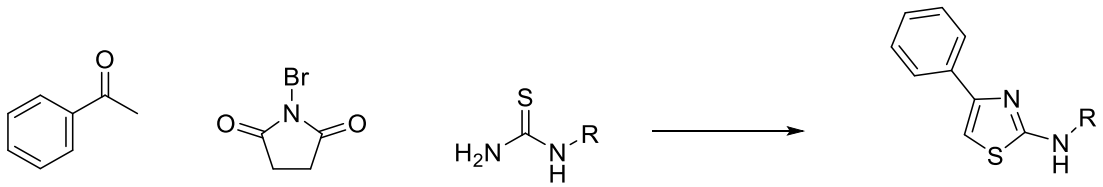
44 **45**

Scheme 1.10



46 **47** **48**

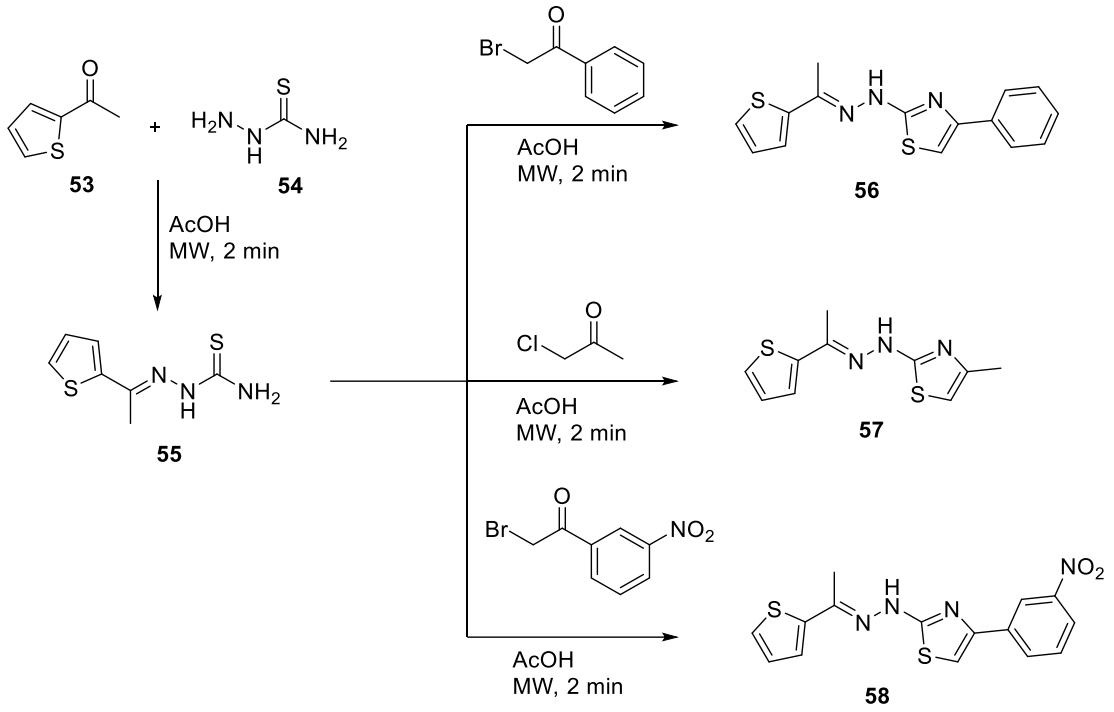
Scheme 1.11



49 **50** **51** **52**

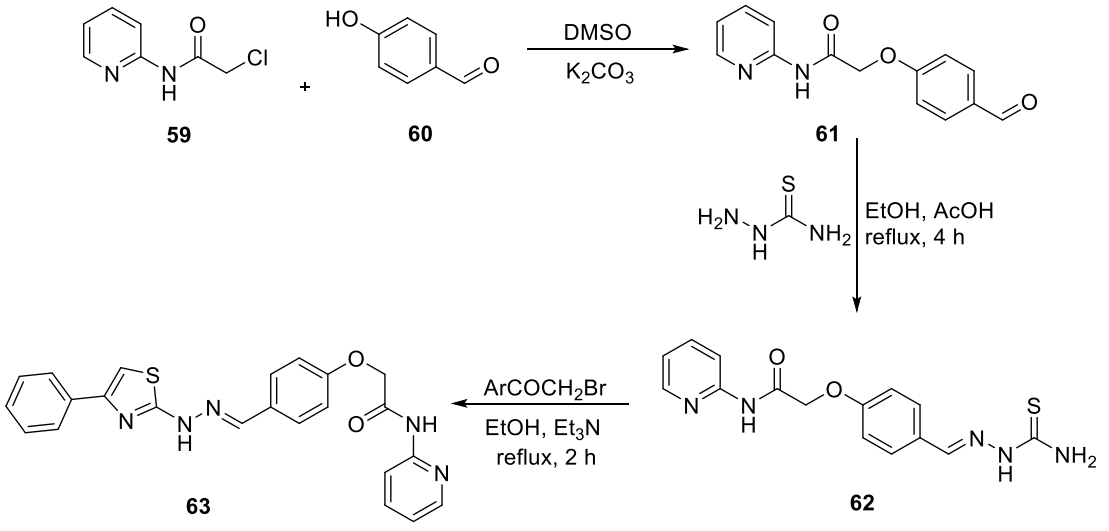
Scheme 1.12

A. Naggar *et al*[21] reported the environment-friendly sequential one-pot synthesis of novel thiazole derivatives, starting from acetyl thiophene **53** which was reacted with thiosemicarbazide to form the intermediate molecule **55**. Furthermore, molecule **55** was reacted with phenacyl bromide, chloro acetone and 3-nitro phenacyl bromide to form novel thiazole molecules **56**, **57** and **58**. Synthesized molecules were screened for anticancer activity, in which it was discovered that methoxy substitution on the phenyl ring gave good inhibitory activity against various cancerous cell lines **(Scheme 1.13)**.



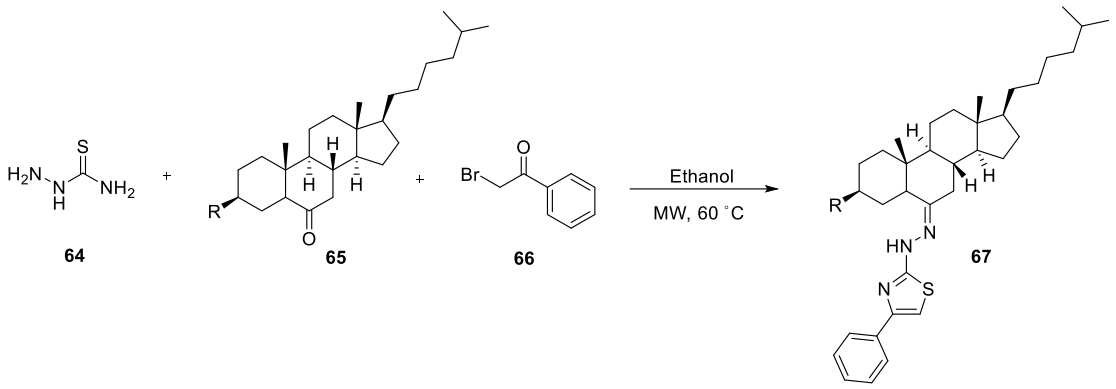
Scheme 1.13

A. Alqahtani *et al*[22] reported the synthesis of phenyl thiazole derivatives. Molecule **61** was reacted with thiosemicarbazide in ethanol containing catalytic amount of glacial acetic acid gave intermediate **62**, which was cyclized via reaction with phenacyl bromide in ethanol containing catalytic amount of triethyl amine afforded novel phenyl thiazole **63** derivatives. The synthesized molecules were screened for antiproliferative activity, in which some molecules showed promising activity against the MCF-7 and HepG2 cell lines **(Scheme 1.14)**.



Scheme 1.14

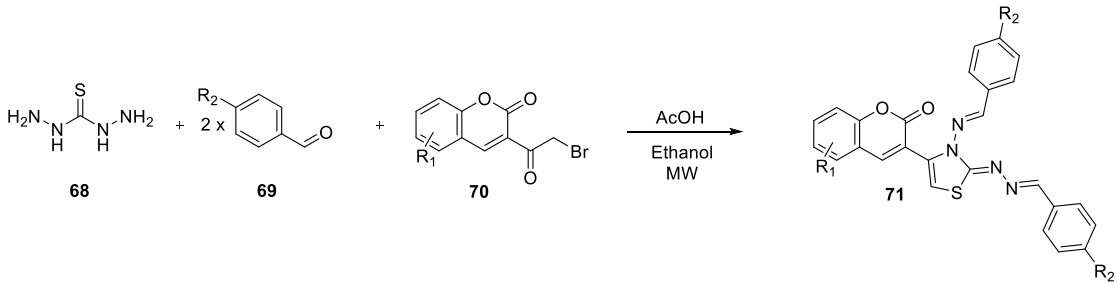
M. Asif *et al*[23] reported the synthesis of one-pot of steroidal thiazole molecules, starting from thiosemicarbazide **64,** steroidal molecule **65** and phenacyl bromide **66** in EtOH, microwaved for 35 to 40 min to obtain the steroidal thiazole molecules **67**. The synthesized molecules were screened for their antioxidant activity, in which it was found that some molecules exhibited moderate to good activity **(Scheme 1.15)**.



64 65 66 67

Scheme 1.15

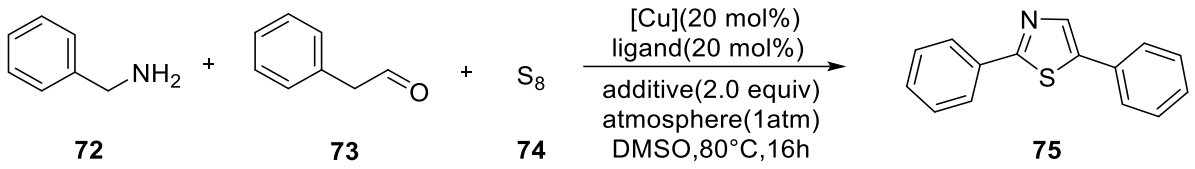
S. Mamidala *et al*[24] reported coumarin incorporated thiazole microwave synthesis, starting from thiocarbohydrazide **68**, 2 equivalents of aldehyde **69** and α-halo carbonyl coumarin **70** in ethanol containing a catalytical amount of acetic acid gave a novel coumarin-thiazole molecule **71**. The synthesized molecules were screened for antibacterial activity and found that some molecules showed good activity comparable to the standard drug novobiocin **(Scheme 1.16)**.



68 69 70 71

Scheme 1.16

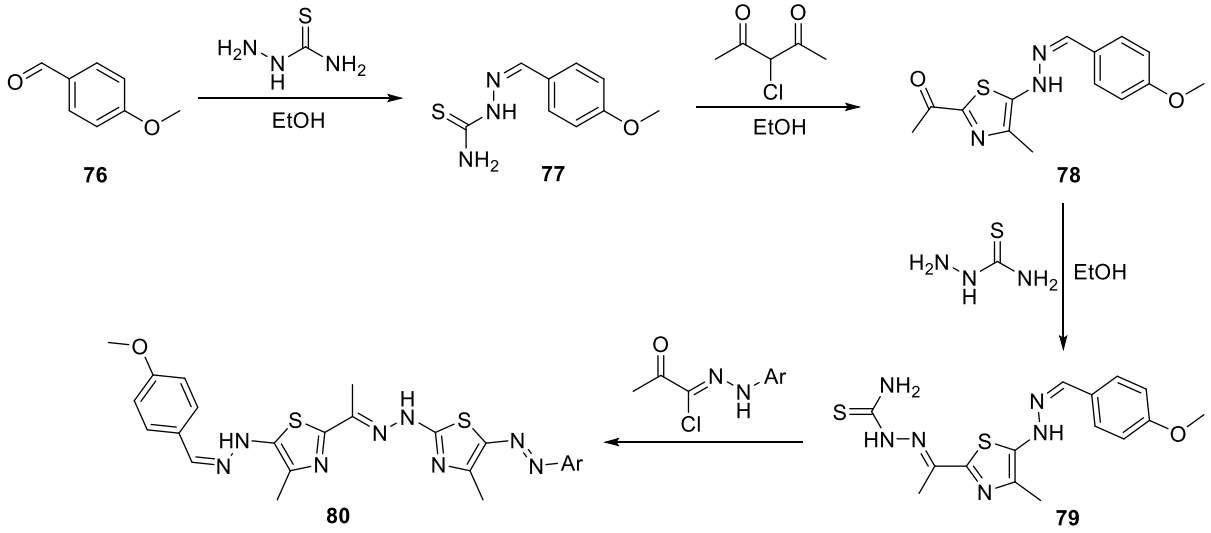
X. Wand *et al*[25] reported a new copper catalyzed synthesis of thiazole derivatives by aerobic oxidative sulfuration. The reaction of benzyl amine **72**, 2-phenylacetaldehyde **73** and sulphur **74** in the presence of copper bromide as a catalyst, 1,10-Phenanthroline and DBU gave a new bis-phenyl thiazole molecule **75 (Scheme 1.17)**.



72 73 74 75

Scheme 1.17

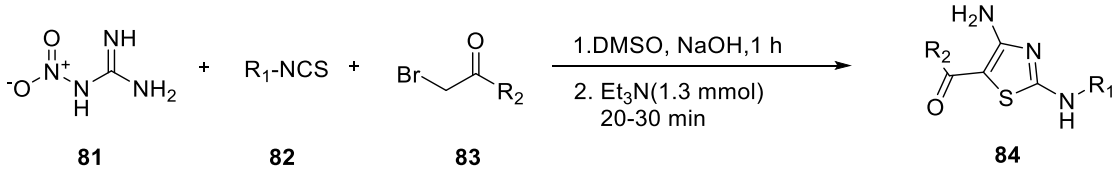
L. Mutabagani *et al*[26] reported that bis-thiazole synthesis starting from anisaldehyde **76** was reacted with thiosemicarbazide to get molecule **77**, followed by cyclization with 2-chloro acetylacetone to obtain thiazole molecule **78**. Furthermore, again the thiazole molecule was reacted with thiosemicarbazide to obtain the intermediate **79** compound and cyclized to get product **80 (Scheme 1.18)**.



76 77 78 79 80

Scheme 1.18

S. Kumaran *et al*[27] reported sequential one-pot thiazole synthesis starting from nitro guanidine **81**, isothiocyanate **82** and α-haloketone **83** derivatives reacted to form 2,4-diamino thiazole derivative **84**. These newly synthesized derivatives are expected to be pharmacologically active, specifically as an anticancer agent **(Scheme 1.19)**.

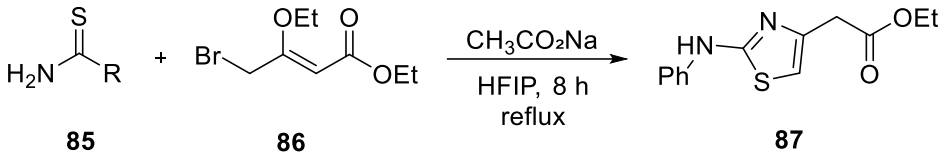


81 82 83 84

Scheme 1.19

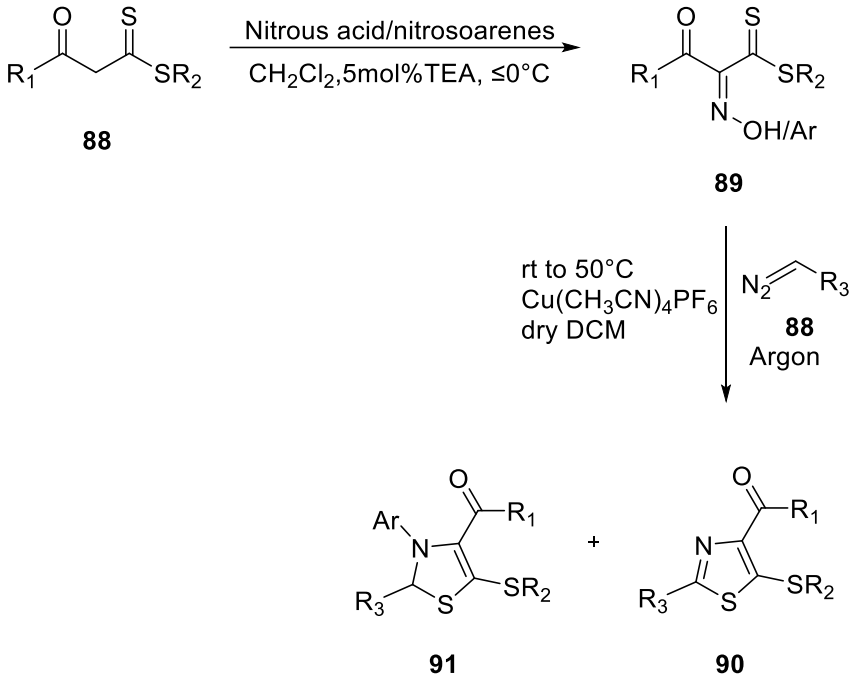
Z. Alsharif *et al*[28] reported the synthesis of thiazole derivatives, starting from the thiourea derivative **85** reacted with 4-bromo-3-ethoxycrotonate **86** in the presence

of sodium acetate in hexafluoro isopropanol as a solvent refluxed for 8 hr to form an ester containing thiazole molecules **87 (Scheme 1.20)**.



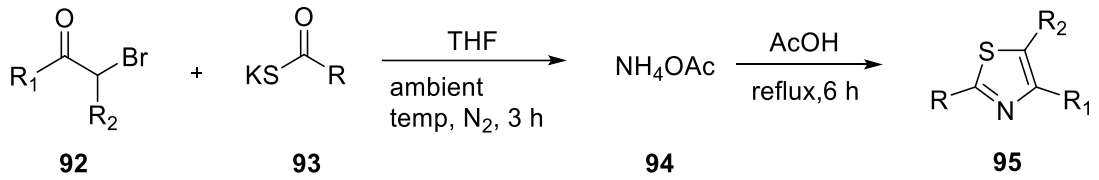
Scheme 1.20

A. Srivastava *et al*[29] reported copper catalyzed highly substituted thiazole synthesis, imino β-oxodithio ester **88** and diazo carbonyl compound form thiazole derivatives **90** and **91**, molecule **90** is 75-96% and molecule **91** is 72-93% yield was formed **(Scheme 1.21)**.



Scheme 1.21

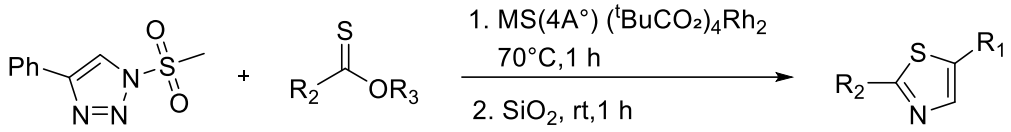
E. Venkateswararao *et al*[30] reported the thiazole derivatives synthesis from the reaction of α-bromo ketone **92** and β-keto thioester **93** reacted in dry THF as a solvent and in nitrogen atmosphere at ambient temperature for 3 hr. Followed by the addition of ammonium acetate **94** and acetic acid and the reaction mass was refluxed for 6 hr to form highly functionalized thiazole derivatives **95 (Scheme 1.22)**.



92 **93** **94** **95**

Scheme 1.22

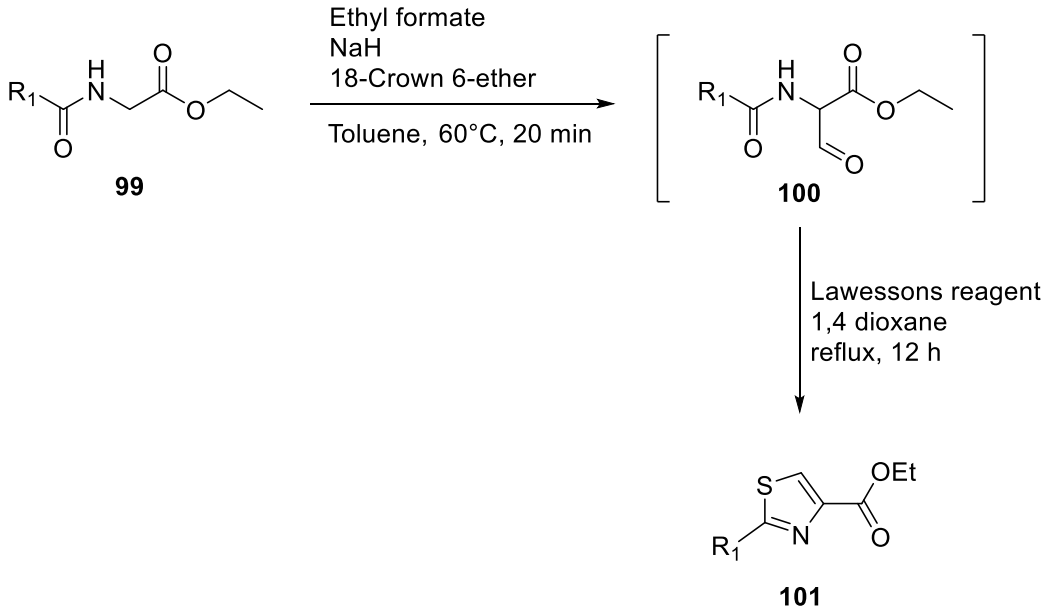
M. Tomoya *et al*[31] reported a facile synthesis of thiazole derivatives via reacting sulfonyl triazole **96** derivatives with thiono ester **97** in the presence of 4 angstrom molecular sieve and rhodium catalyst, followed by silica gel formed thiazole derivatives in good yields **(Scheme 1.23)**.



96 **97** **98**

Scheme 1.23

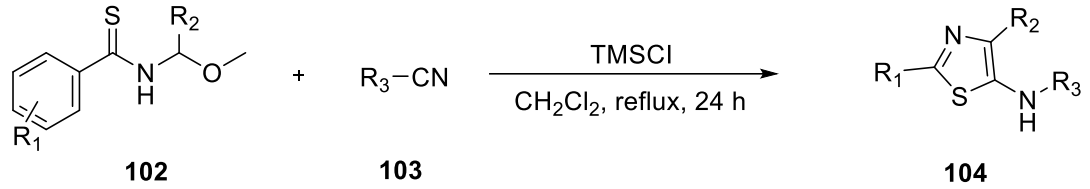
G. Khose *et al*[32] reported synthesis of thiazole derivatives via reacting glycine ethyl ester derivative **99** with ethyl formate, sodium hydride and crown-18 to generate aldehyde molecule **100**, this molecule was cyclized via reaction with lawesson's reagent in 1,4-dioxane to form thiazole ring **101 (Scheme 1.24)**.



99 **100** **101**

Scheme 1.24

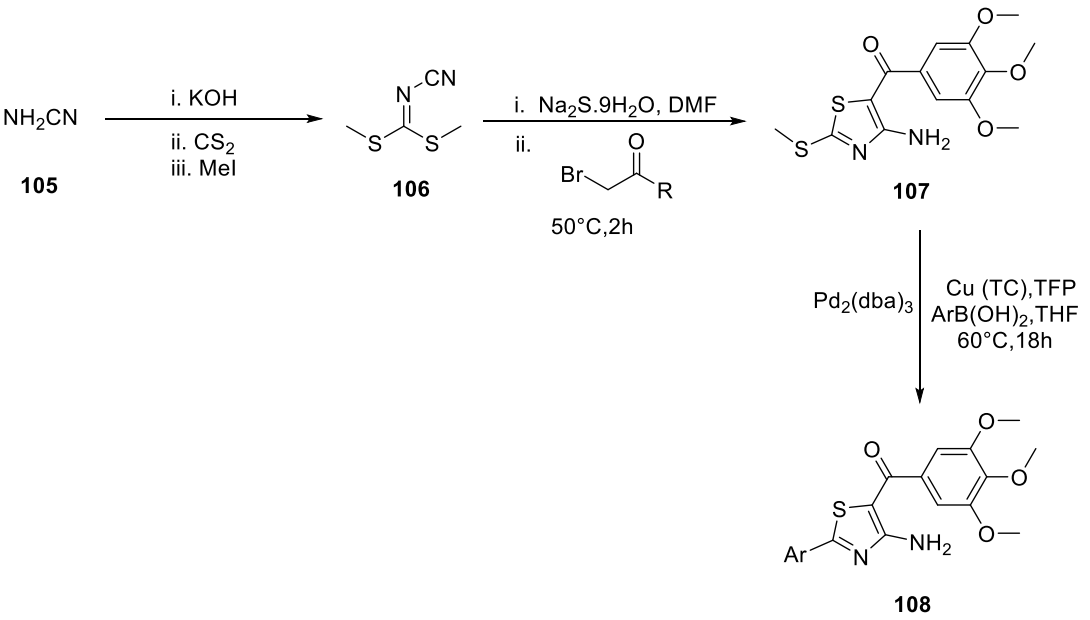
T. Soeta and co-workers[33] reported synthesis of thiazole derivatives using 4+1 cycloaddition amid acylimine **102** and isocyanides **103** in dichloromethane containing tetramethyl silane and refluxed for 24 hr to form cyclized thiazole derivative **104 (Scheme 1.25)**.



102 **103** **104**

Scheme 1.25

R. Romagnoli *et al*[34] reported thiazole containing novel anticancer agents having trimethoxy group **108** attached with it. The synthesis starts from cyanamide **105** converted to diketene thioacetal **106** and then cyclized via reaction with sodium sulphide and phenacyl bromide derivatives to form molecule **107**. Furthermore, various boronic acids were attached in the presence of tris(dibenzylideneacetone)dipalladium and copper catalyst to form molecule **108**. Some of the molecules screened for anticancer activity showed pointedly inhibited development of the HT-29 xenograft in a nude mouse model **(Scheme 1.26)**.

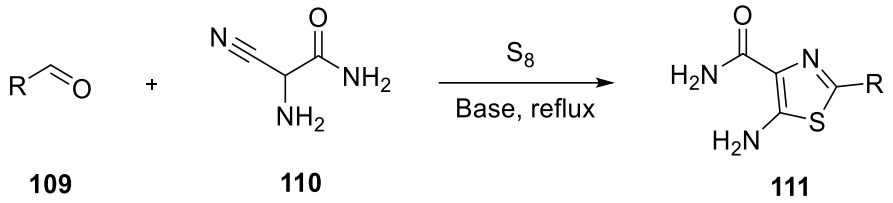


105 **106** **107** **108**

Scheme 1.26

K. Childers *et al*[35] reported easy synthesis of amino carboxamide thiazoles starting from various aldehydes **109** that reacted with sulphur and amino cyan

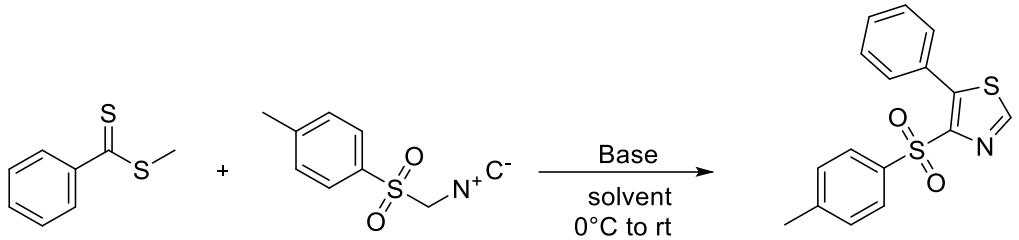
acetamide **110** in the presence of base and refluxed in alcohol to form amino carboxamide thiazole derivatives **111 (Scheme 1.27)**.



109 **110** **111**

Scheme 1.27

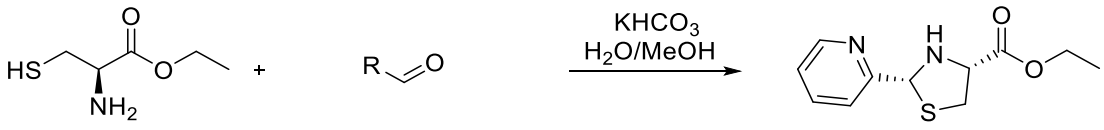
G. Lingaraju *et al*[36] reported easy disubstituted thiazole synthesis by reacting methyl benzodithioate **111** with tosyl methyl isocyanide **112** in the presence of sodium hydride and DMF to form thiazole derivatives **113 (Scheme 1.28)**.



111 **112** **113**

Scheme 1.28

B. Credico *et al*[37] reported the thiazole derivatives synthesis from cysteine amino acids. The l-cysteine ethyl ester **114** and various substituted aldehydes **115** were reacted in the presence of potassium bicarbonate in water and the methanol mixture formed thiazole ester molecule **116 (Scheme 1.29)**.

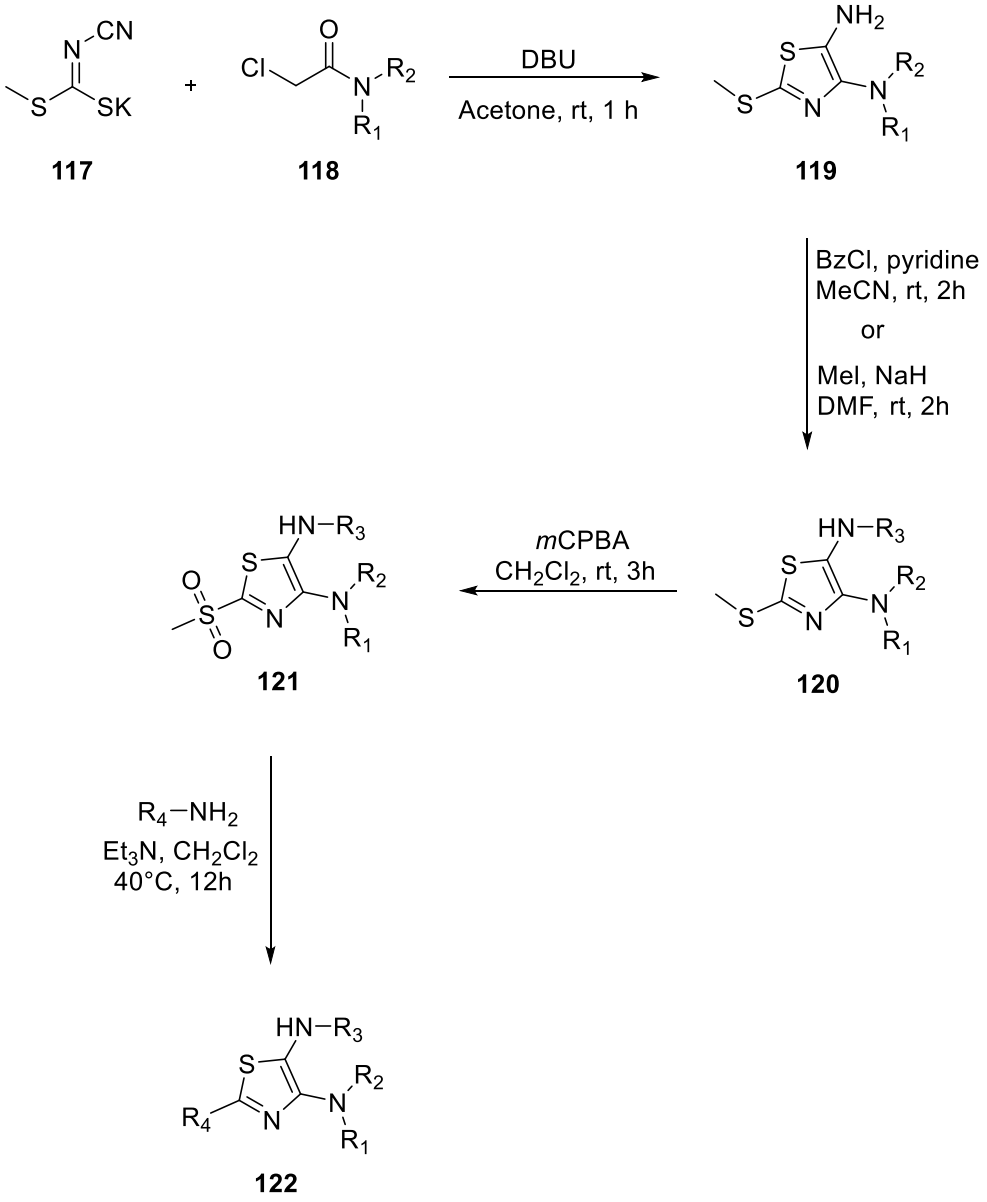


114 **115** **116**

Scheme 1.29

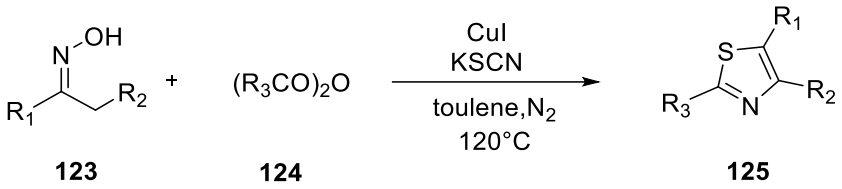
D. Kim and co-workers[38] reported synthesis of highly functionalized thiazoles, starting from potassium methyl cyano carbonimidodithioate **117** was reacted with chloro acetamide **118** derivatives to form cyclized thiazole molecule **119**. Furthermore, molecule **119** was reacted with chloro substituted derivative or iodo derivative to form

molecule **120**, the thio methyl group of molecule **120** was reacted with *meta*-chloro peroxy benzoic acid to form methyl sulfonyl molecule **121**, to which various substituted amines were attached to form highly functionalized thiazole molecules **122 (Scheme 1.30)**.



Scheme 1.30

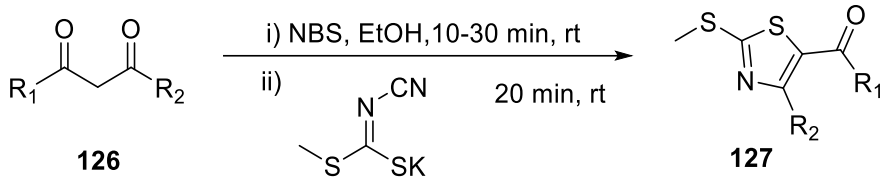
X. Tang *et al*[39] reported a copper-catalyzed (3+1+1) type condensation reaction between oxime **123**, anhydride **124** and potassium thiocyanate in toluene and an inert atmosphere that formed a functionalized thiazole molecules **125 (Scheme 1.31)**.



123 124 125

Scheme 1.31

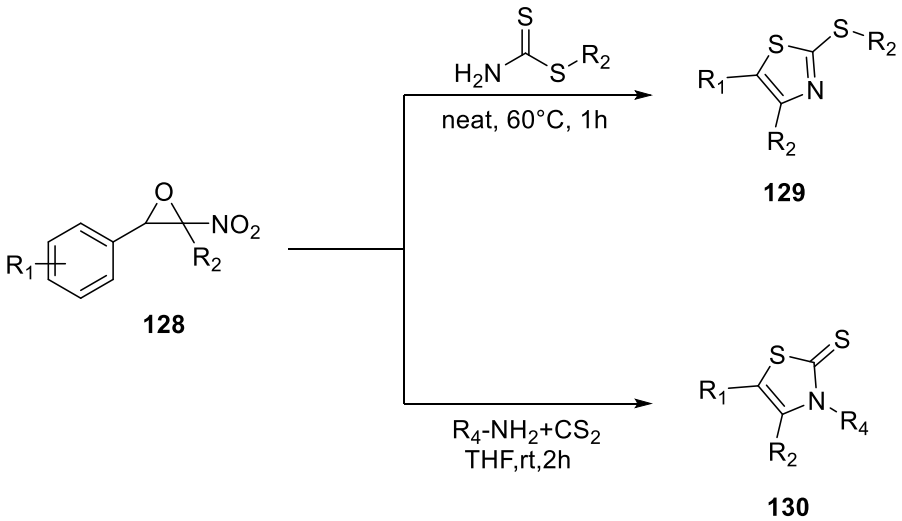
L. Luo *et al*[40] reported that the synthesis of thiazole molecules starting from the diketone molecule **126**, which was brominated using NBS in ethanol, followed by cyclization to form the thiazole molecule **127 (Scheme 1.32)**.



126 127

Scheme 1.32

A. Halimehjani *et al*[41] reported synthesis of thiazoles starting from nitro epoxide **128**, which was reacted solvent free with s-alkyl dithiocarbamate to form thiazole molecule **129**. The reaction of nitro epoxide **128** with amine and carbon disulphide in tetrahydrofuran as a solvent formed substituted thiazole molecules **130 (Scheme 1.33)**.

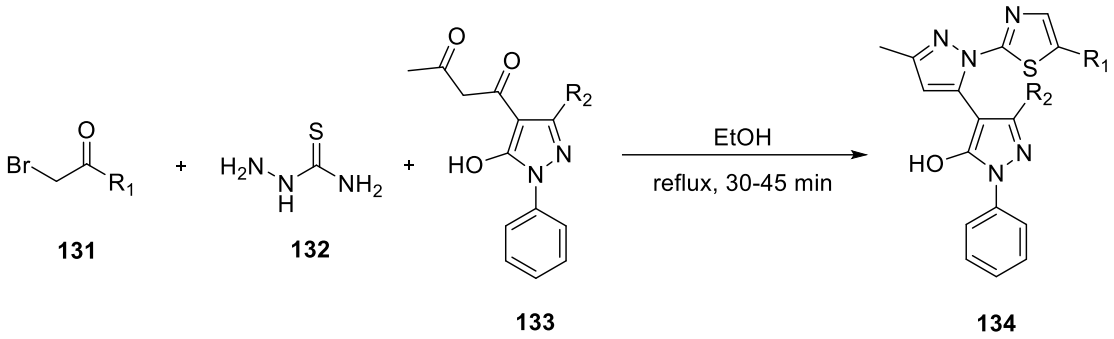


128 129 130

Scheme 1.33

A. Saidoun *et al*[42] reported one-pot synthesis of thiazole pyrazole molecules by reacting phenacyl bromide derivative **131** with thiosemicarbazide **132** and the

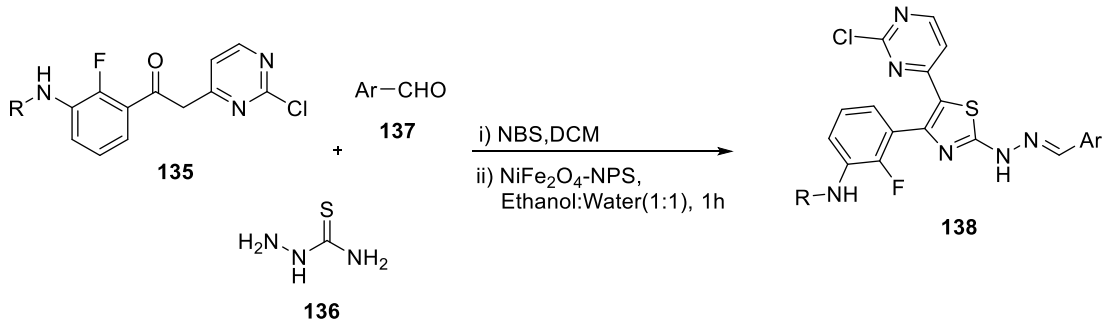
derivatives of diketo phenylpyrazole **133** which were refluxed in ethanol for 35 to 40 min to form the bis-pyrazole thiazole molecule **134 (Scheme 1.34)**.



131 132 133 134

Scheme 1.34

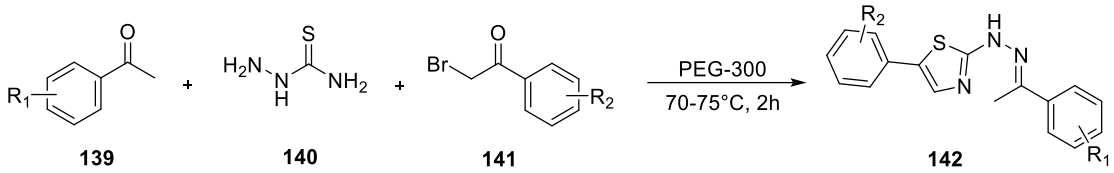
A. Sharma and co-workers[43] reported $NiFe_2O_4$ nano particles catalyzed pyrimidine thiazole molecules, the one-pot reaction of molecule **135** with thiosemicarbazide **136** and various substituted benzaldehydes with NBS in DCM followed by catalyst reaction formed thiazole pyrimidine molecule **138**. The synthesized molecules were screened for their anticancer activity against the A375, HeLa and MCF-7 cell lines, in which one of the molecules having a dibromo and difluoro substitution showed good anticancer activity against all screened cell lines. The molecular docking studies revealed that the synthesized molecules showed a good binding energy against Hsp90 protein. ADME profile also showed good pharmacological values **(Scheme 1.35)**.



135 137 136 138

Scheme 1.35

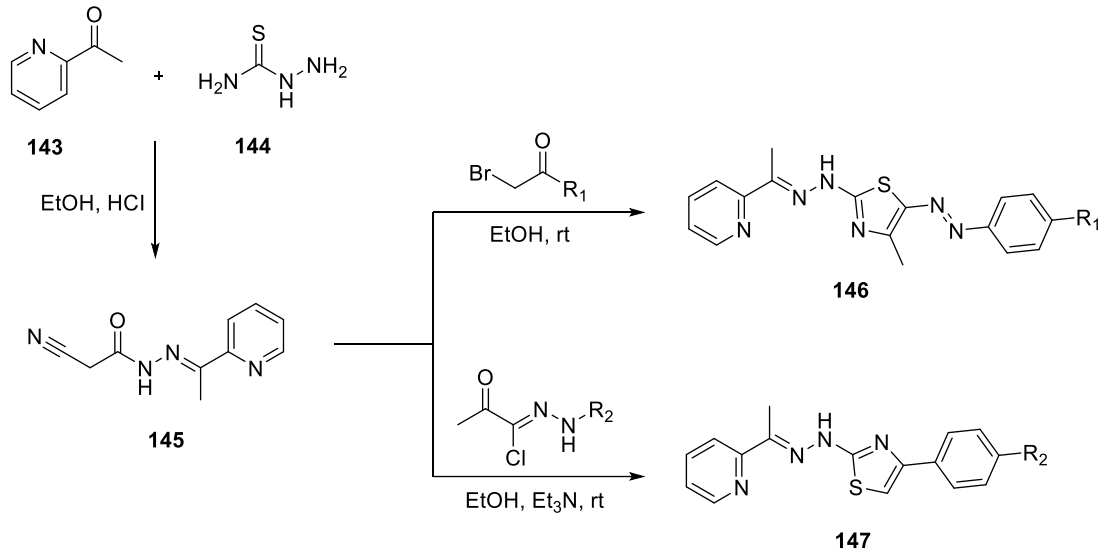
D. Raut and R. Bhosale[44] reported one-pot PEG mediated synthesis of thiazole molecules. The one-pot reaction between acetophenone derivative **139**, thiosemicarbazide **140** and the phenacyl bromide derivatives **141** in polyethylene glycol 300 which acts as a solvent and catalyst formed novel hydrazinyl thiazole molecules **142 (Scheme 1.36)**.



139 140 141 142

Scheme 1.36

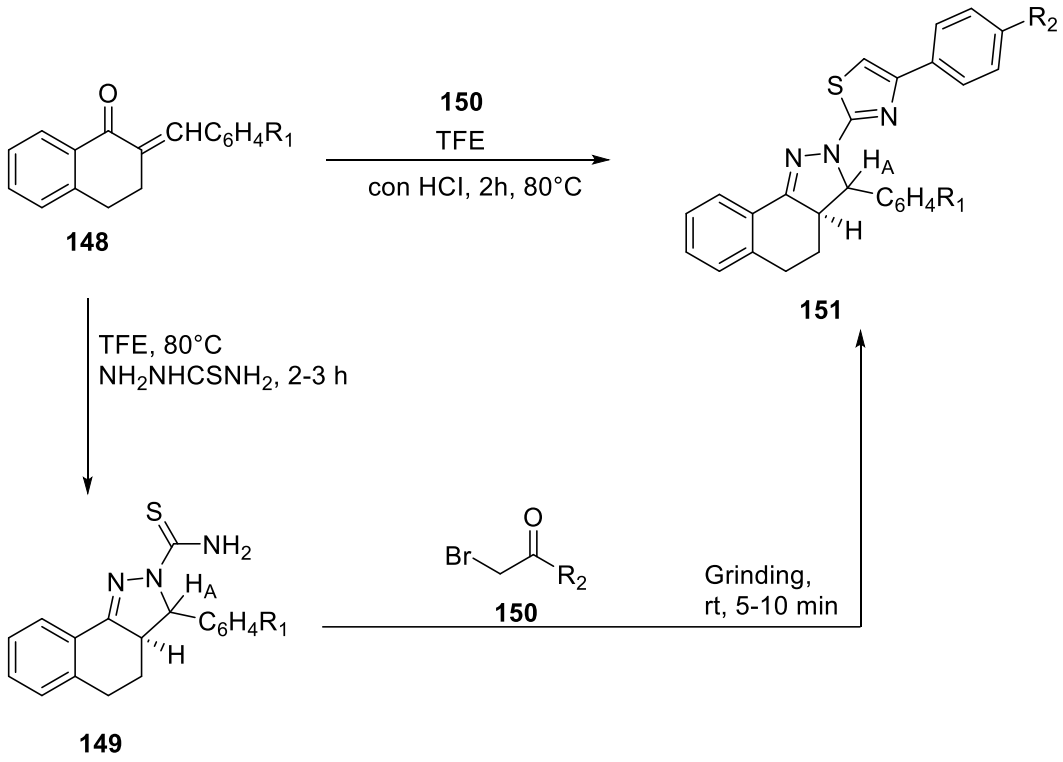
R. Khidre *et al*[45] reported the synthesis of pyridine incorporated thiazole derivatives by reacting pyridine derivative **143** with thiosemicarbazide **144** in ethanol to form intermediate molecule **145**, which was further reacted with phenacyl bromide derivative to form the thiazole molecule **146** and hydrazonoyl chloride derivative to form molecule **147**. The newly pyridine thiazole molecules were screened for their antimicrobial activity **(Scheme 1.37)**.



143 144 145 146 147

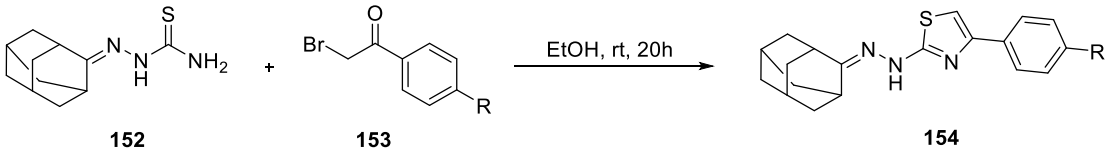
Scheme 1.37

P. Gautam and co-workers[46] reported one-pot indazolyl thiazole derivative synthesis via reacting benzylidene tetralone **148** molecule with thiosemicarbazide and phenacyl bromide **150** derivatives in trifluoroethanol and concentrated hydrochloric acid formed indazolyl thiazole molecules **151**. The synthesized molecules were screened for their antifungal and antibacterial activity in which some of the screened molecules displayed promising antimicrobial activity **(Scheme 1.38)**.



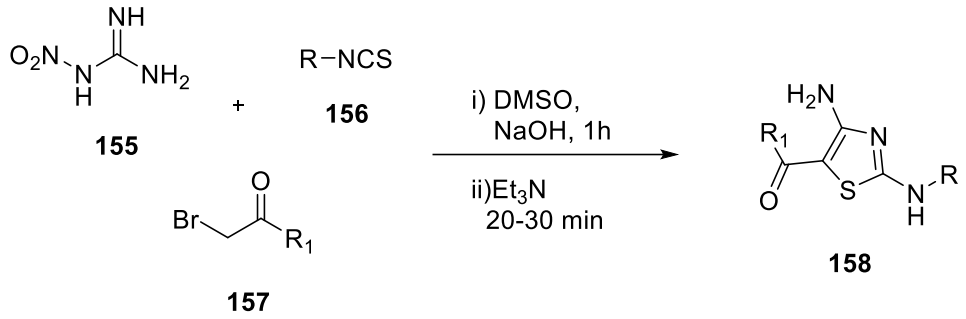
Scheme 1.38

K. Laczkowski *et al*[47] reported the synthesis of adamantane thiazole molecules by reacting adamantane thiosemicarbazone **152** and phenacyl bromide derivative **153** was stirred in ethanol at room temperature to form thiazole molecules **154**. The synthesized molecules were subjected to antimicrobial screening in which it was discovered that some of the screened molecules showed promising antifungal and antibacterial activity **(Scheme 1.39)**.



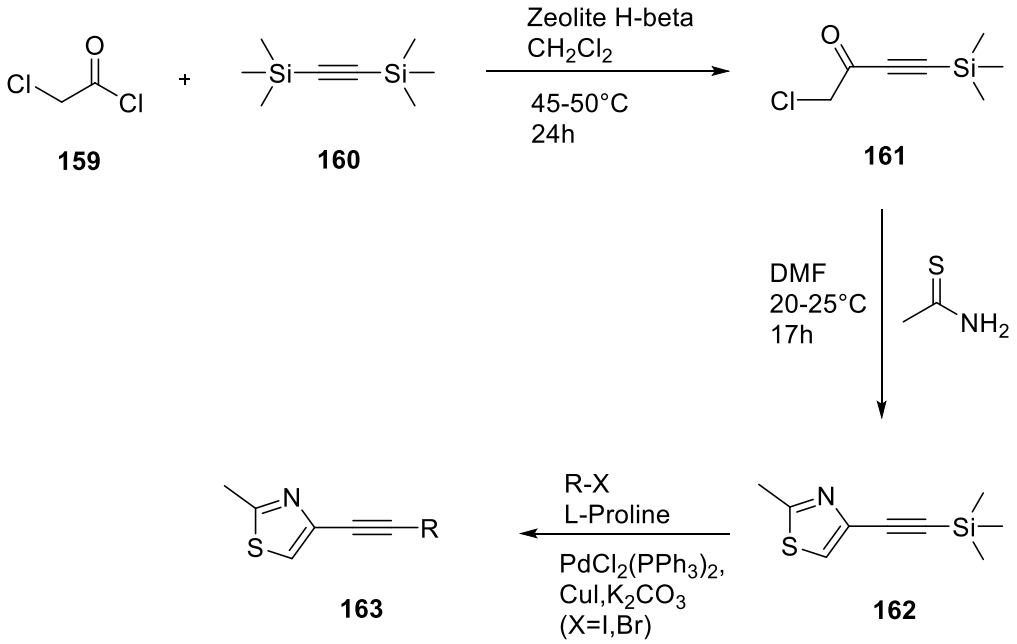
Scheme 1.39

H. Kumaran *et al*[48] reported the synthesis of thiazoles from three component reaction between nitroguanidine **155**, isothiocyanate derivative **156** and α-haloketones to form novel amino thiazole molecules **158 (Scheme 1.40)**.



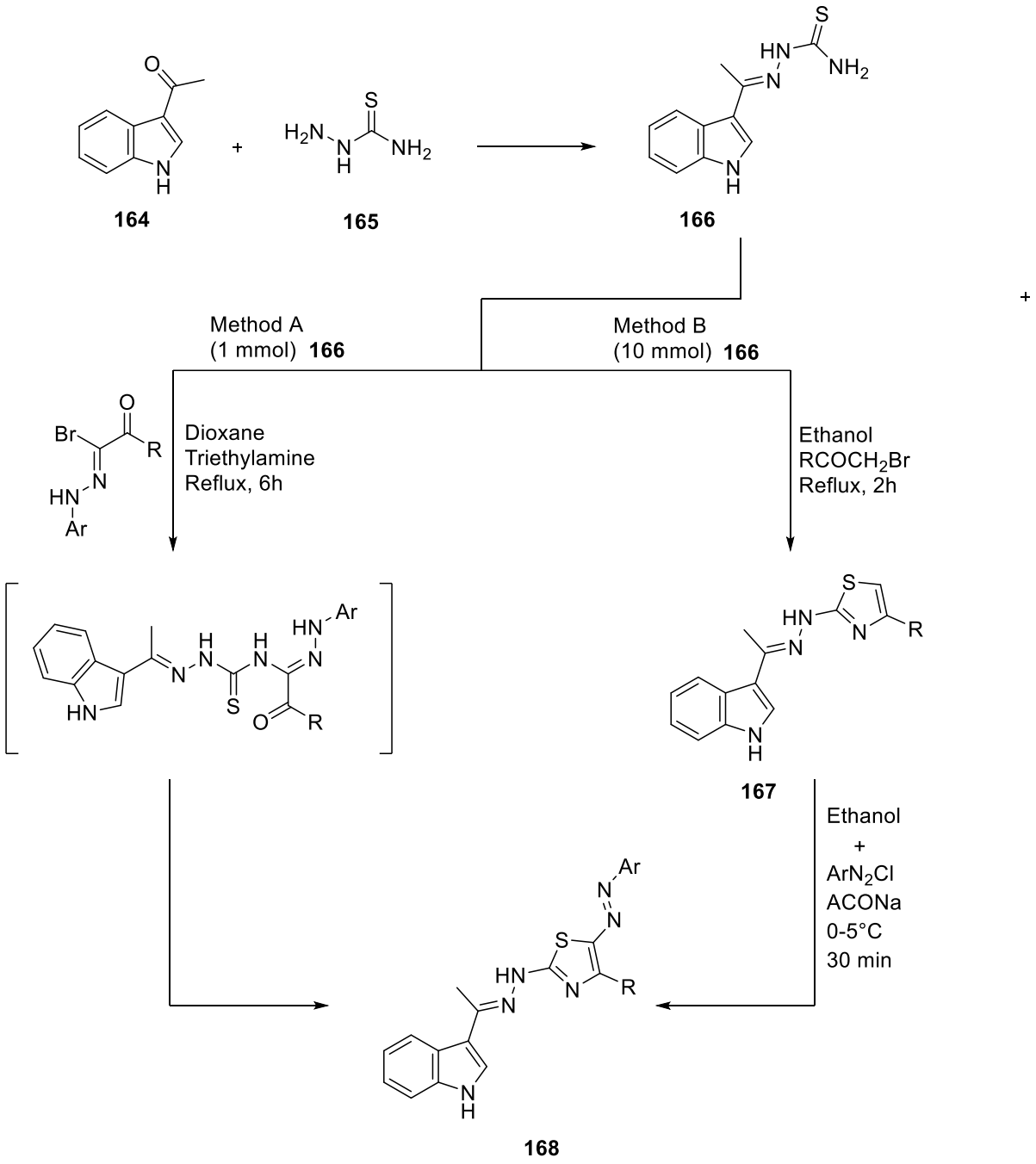
Scheme 1.40

K. Arunkumar *et al*[49] reported the synthesis of thiazoles catalyzed by zeolite via the reaction of chloroacetyl chloride **159** and 1,2-bis-trimethyl silyl acetylene **160** to form the intermediate molecule **161**, which was cyclized to the thiazole molecule via reaction with thioacetamide **162**. Then a modified sonogashira reaction was done by attaching various halides to form molecule **163 (Scheme 1.41)**.



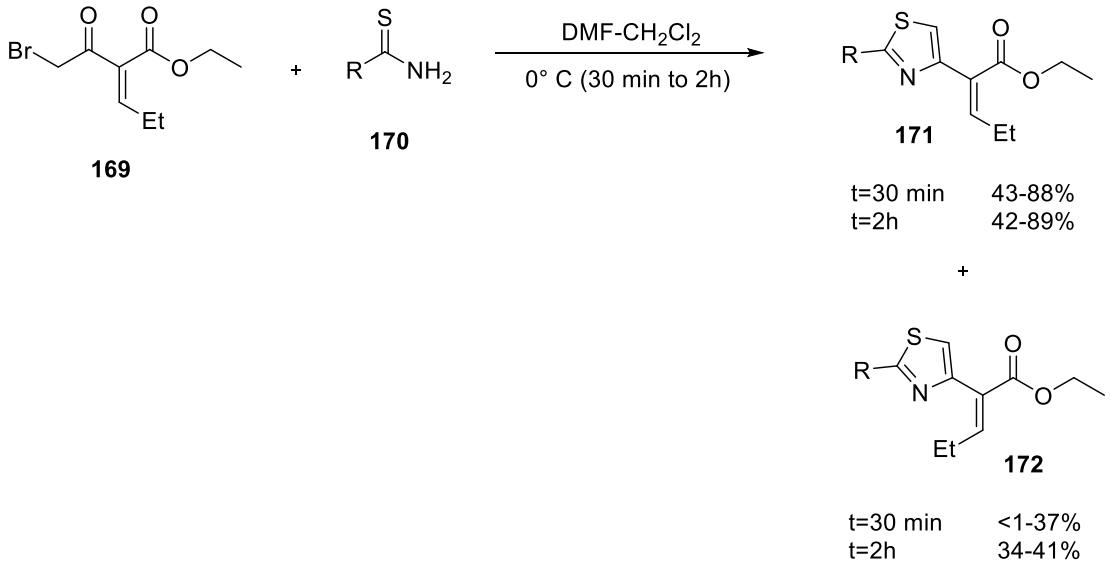
Scheme 1.41

A. Abdelhamid *et al*[50] designed the synthesis of indole-thiazole derivatives. The reaction starting from indole **164** reacted with thiosemicarbazide **165** and formed hydrazide like molecule **166**. The reaction of molecule **166** with two different derivatives of hydrazonoyl halide and phenacyl bromide by method B, both formed a novel thiazole molecule **168**. The two paths to synthesize thiazole-indole molecules are reported **(Scheme 1.42)**.



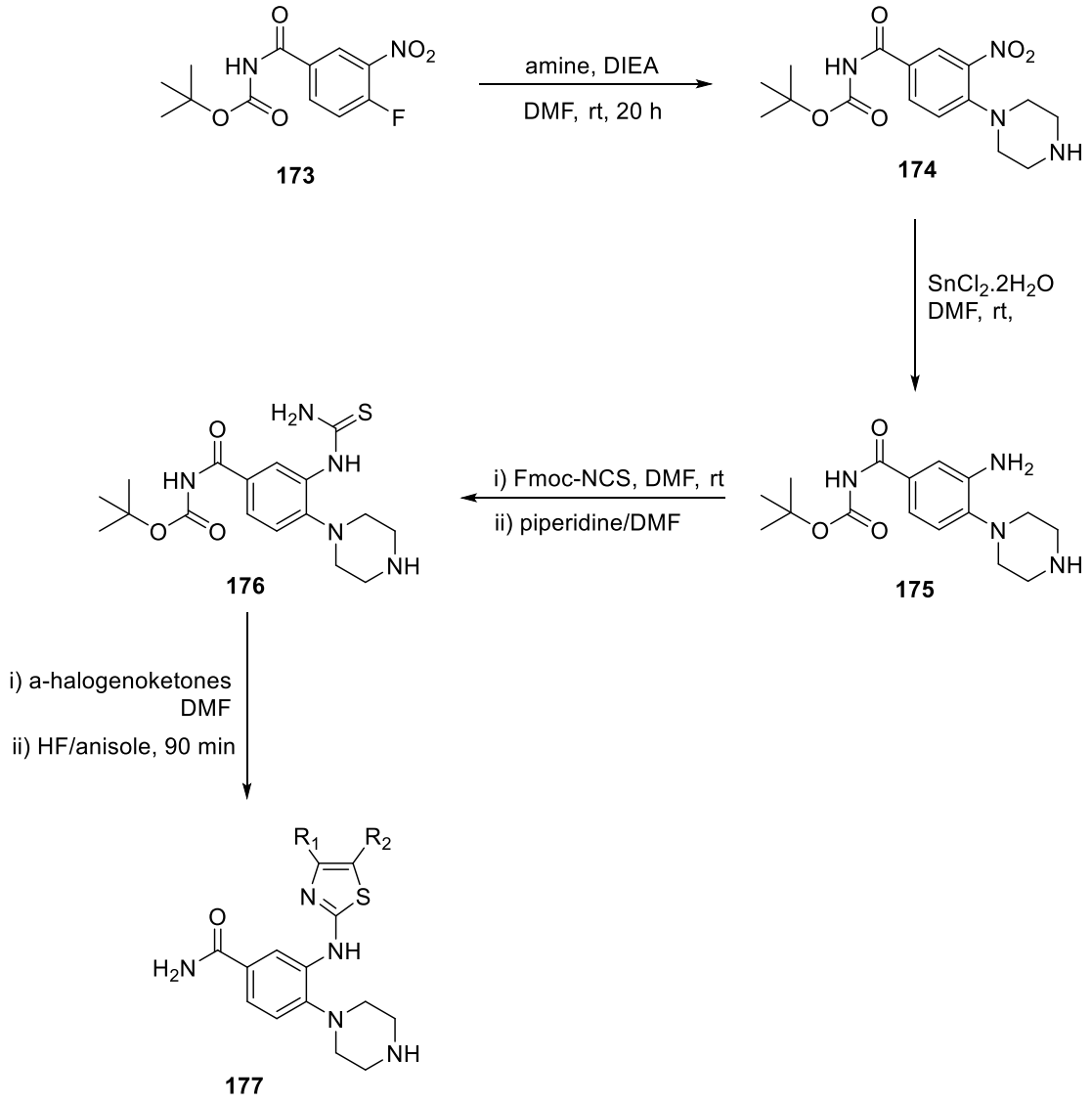
Scheme 1.42

J. Zhai and co-workers[51] reported isomers of new thiazole molecule. The reaction between the bromo pentanoate **169** molecule and the derivative thiourea **170** was reacted in DMF and DCM mixture at 0ºC to form two isomers of the thiazole molecules **171** and **172** in good yield **(Scheme 1.43)**.



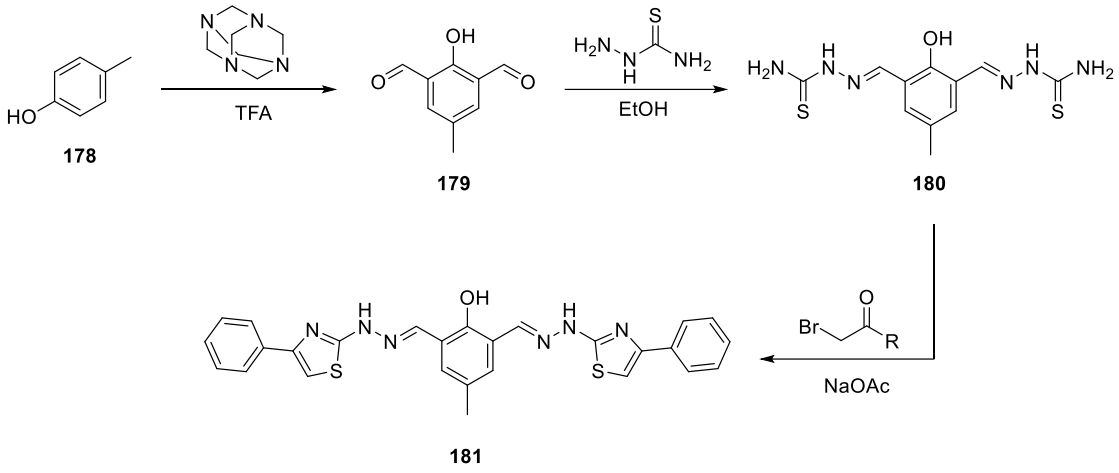
Scheme 1.43

A. Nefzi and S. Arutyunyan[52] reported the synthesis of piperazine-thiazole molecules. The reaction of molecule **173** with piperazine in excess amount in DMF formed molecule **174**, which was reacted with tin chloride to convert nitro group to amine **175**. Furthermore, the reaction of amine molecule **175** was done with isothiocyanate molecule to form compound **176**, which was cyclized via reaction with α-halo ketone molecules to form piperazine-thiazole molecule **177 (Scheme 1.44)**.

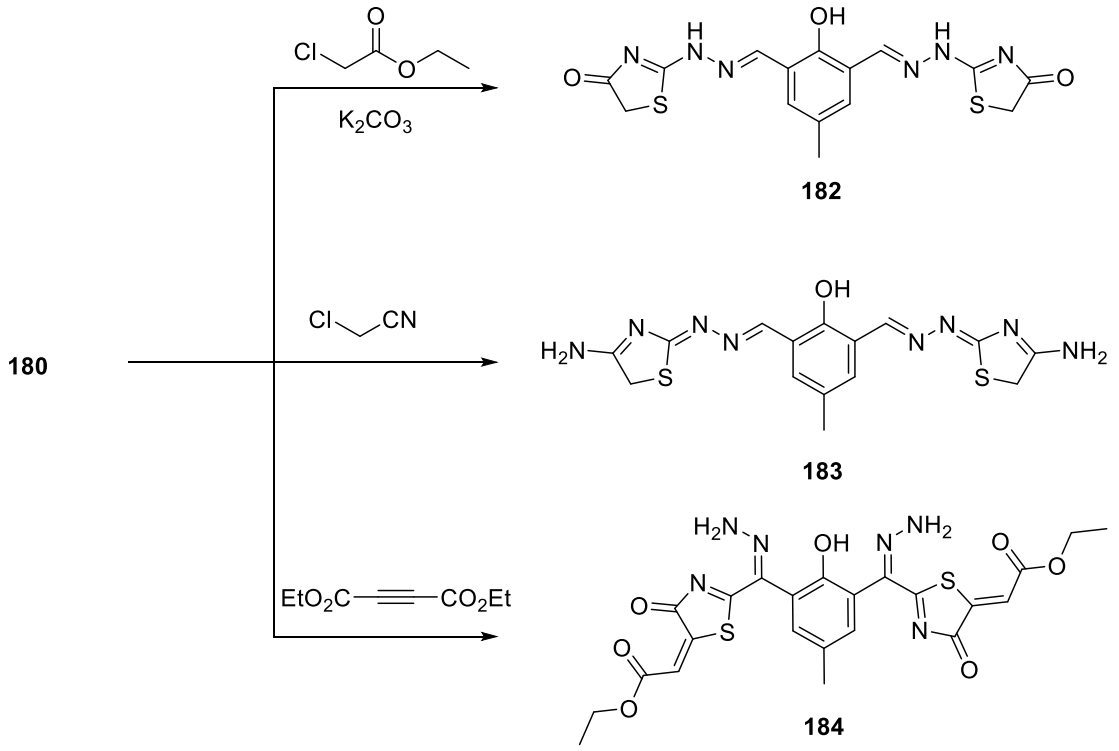


Scheme 1.44

W. Arafa *et al*[53] reported bis-thiazole derivatives. The reaction starts from 4-methyl phenol **178** reacted with hexamethylenetetramine in anhy. TFA to form di-aldehyde molecule **179**. Furthermore, the reaction of molecule **179** with thiosemicarbazide in ethanol resulted in intermediate product **180**, which was cyclized by rection with two mole equivalents of the phenacyl bromide derivative to from bis-thiazole molecule **181 (Scheme 1.45)**. Moreover, the reaction of molecule **180** with ethyl chloro acetate formed molecule **182**, with chloro acetonitrile formed molecule **183** and with diethyl acetylene dicarboxylate to form molecule **184 (Scheme 1.46)**.



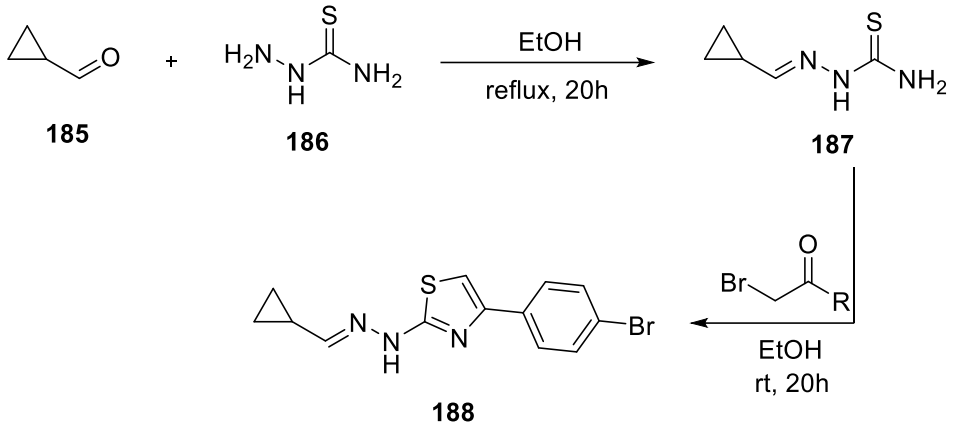
Scheme 1.45



Scheme 1.46

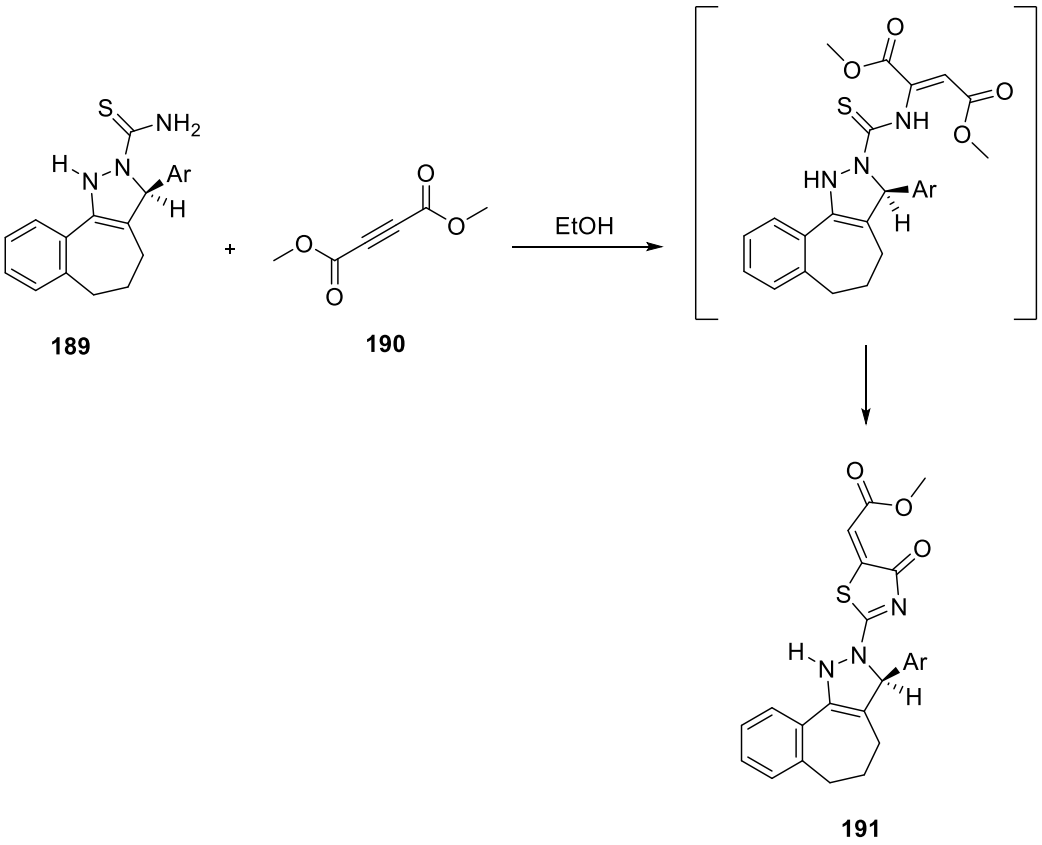
K. Laczkowski and co-workers[54] reported synthesis of cyclopropyl-thiazole molecules. The reaction between cyclopropane carboxaldehyde **185** and thiosemicarbazide **186** in ethanol at reflux for 20 hr formed intermediate product **187**, which was further cyclized via reaction with phenacyl bromide derivatives in ethanol at room temperature for 20 hr to form novel thiazole **188** molecules in good yield. The synthesized molecules here were screened for their antifungal, anticonvulsant and anti-*toxoplasma gondii* activities. In which, it was found that most of the molecules showed

very good inhibitory activity against *Candida* spp. Two of the synthesized molecules showed good anticonvulsant activity and three of them showed excellent *toxoplasma gondii* inhibition. The molecular docking studies also showed the synthesized molecules as a probable antifungal agent **(Scheme 1.47)**.



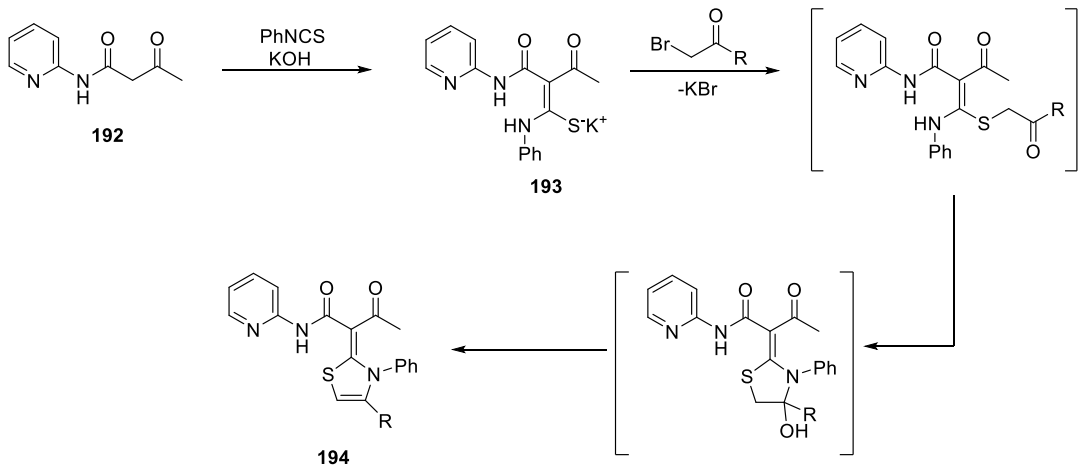
Scheme 1.47

V. Barseneva *et al*[55] reported thiazole synthesis by reacting the thioacetamide derivative **189** with dimethyl acetylene dicarboxylate **190** in ethanol to form molecule **191**. The synthesized molecules were screened for their anticancer activity against the HeLa cell line in which some of the molecules screened showed an excellent inhibition zone **(Scheme 1.48)**.



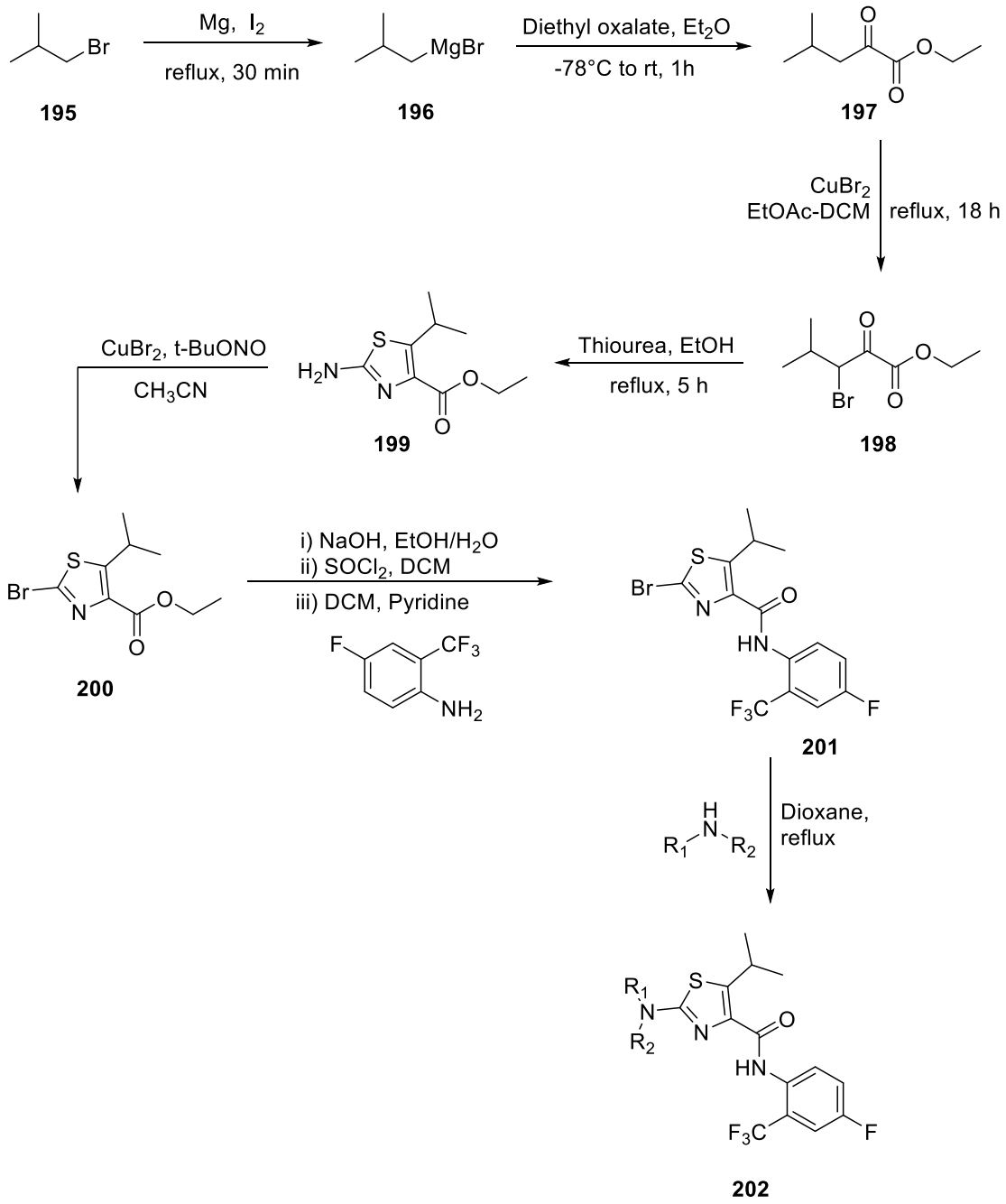
Scheme 1.48

A. Farag *et al*[56] reported synthesis of thiazole molecules by utilizing of potassium salt in the reaction **193**. The reaction between the active methylene molecule **192** and the phenyl isothiocyanate molecule formed compound **193**, which was reacted with the phenacyl bromide derivatives to form the thiazole-pyridine molecule **194 (Scheme 1.49)**.



Scheme 1.49

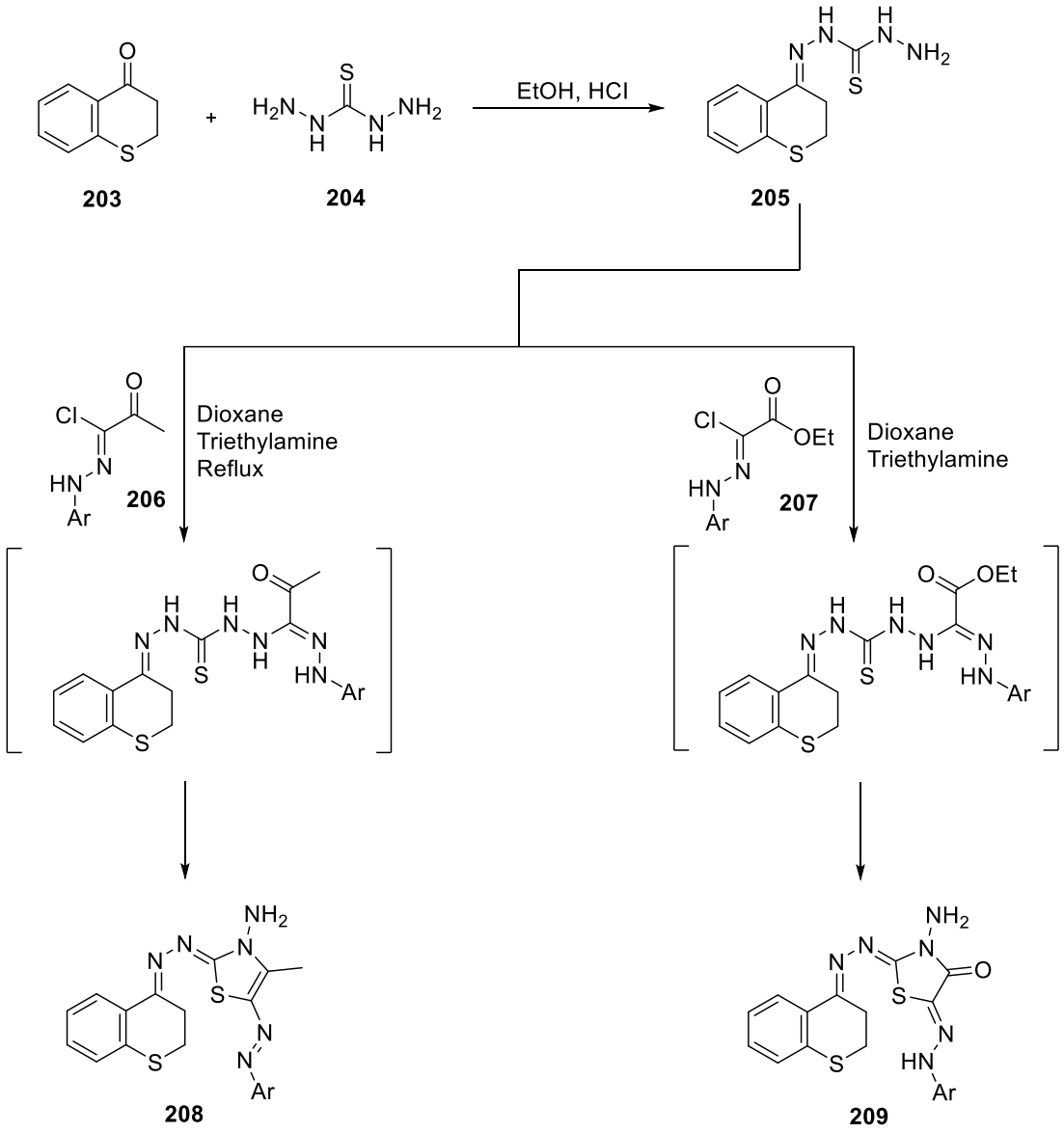
J. bueno *et al*[57] reported synthesis of thiazole molecules and screened them for antimalarial activity. The synthesis starts from the reaction of bromo methylpropane **195** with magnesium and iodine at reflux to form the isobutyl magnesium bromide **196** molecule. The molecule **196** was further reacted with diethyl oxalate in diethyl ether at -76°C to form ester molecule **197**. Moreover, the reaction of **197** with copper bromide in ethyl acetate and dichloromethane at reflux temperature for 18 hr resulted in brominated adduct **198**, which was cyclized via reaction with thiourea in ethanol at reflux for 5 hr to form thiazole molecule **199**. When **199** was reacted with copper bromide and *tert*-butyl nitrite to form bromo thiazole **200**. Then, a multistep reaction is performed where the ester of the thiazole molecule is reduced via a reaction with sodium hydroxide and converted to acid chloride via a reaction with thionyl chloride in DCM. Additionally, with this acid chloride, the fluorinated amine was attached to form molecule **201** and secondary amines were attached to it in dioxane at reflux temperature to form molecule **202**. The synthesized molecules were screened for their antimalarial activity, in which it was found that fluorinated molecules containing the piperazine moiety showed a greater inhibitory zone. The cytotoxicity of the synthesized molecules was also patterned in the HepG2 cell line **(Scheme 1.50)**.



Scheme 1.50

T. Farghaly *et al*[58] reported synthesis of thiazole incorporated thiochroman. The reaction starts by reacting thiochroman **203** molecule with hydrazine carbothio hydrazide **204** in ethanol containing HCl resulted in intermediate molecule **205**. The compound was further reacted with molecules **206** and **207** to synthesize the respective molecules **208** and **209** in good yields. The synthesized molecules were screened for their antifungal activity, where some of the molecules showed higher inhibitory activity

compared to standard drug utilized. Molecular docking study was also performed to check the antimicrobial properties of the molecules **(Scheme 1.51)**.

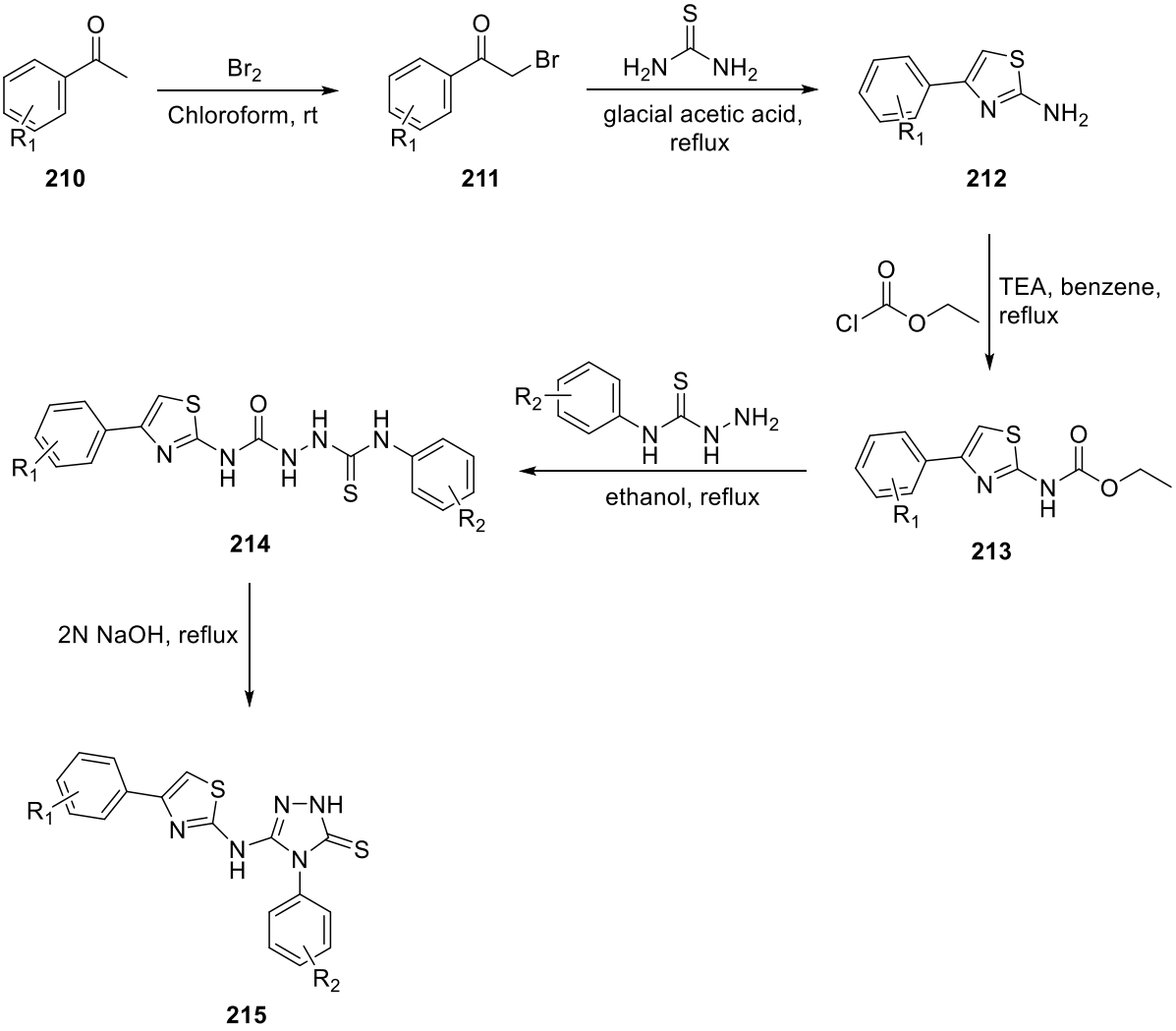


203 204 205 206 207 208 209

Scheme 1.51

N. Siddiqui and W. Ahsan[59] reported triazole-thiazole synthesis and screened the molecules for anticonvulsant activity. The reaction of substituted acetophenone with bromine in chloroform at room temperature formed phenacyl bromide derivatives which were cyclized via reaction with thiourea in glacial acetic acid to form amino phenyl thiazole **212** molecule. Furthermore, ethyl chloroacetate was reacted with molecule **212** to form ester thiazole molecule **213**. The reaction of carbothioamide with molecule **213** in ethanol at reflux temperature formed the intermediate product **214**, which was reacted with a solution of 2N sodium hydroxide solution to achieve cyclized

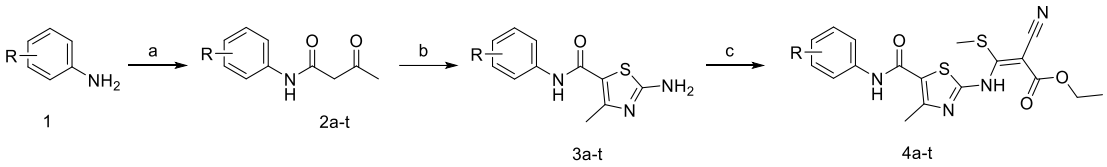
triazole-thiazole molecules **215**. The anticonvulsant activity showed that halogen, methyl and methoxy group increased the anticonvulsant activity **(Scheme 1.52)**.



Scheme 1.52

1.2 Results and Discussion

To find novel antidiabetic molecule and synthesis of different heterocyclic molecules, here, we report ten newly synthesized molecules with thiazole in their main structure. The compounds **4a-t** were elucidated through inspecting their spectroscopic data like ^{1}H-NMR, FTIR and Mass spectroscopy. In the first step, 3-oxo-*N*-arylbutanamide **2a-t** and *N*-bromosuccinimide reacted at ambient temperature to get 2-bromo-3-oxo-*N*-arylbutanamide. Then thiourea was added for ring closure to obtain 2-amino-*N*-aryl-4-methylthiazole-5-carboxamide **3a-t.**



Scheme 1 Reagents and conditions: (a) Ethyl acetoacetate, KOH, Reflux, 24 hr (b) NBS, Thiourea, MeOH, Reflux, 4 hr (c) ethyl 2-cyano-3,3-bis(methylthio)acrylate, K_2CO_3, DMF, rt, 1 hr.

Then compound **4a-t** was reacted with ethyl 2-cyano-3,3-bis(methylthio)acrylate with potassium carbonate in DMF to obtain novel and highly functionalized derivatives of thiazole **4a-t** as showed in **Scheme 1**.

The ^{1}H-NMR graph of molecules revealed that the methyl proton of ester seen at t 1.15-1.23 ppm (CH_3) which were triplet peaks, at s 2.28-2.32 ppm (SCH_3) for thiomethyl protons as a singlet peak. Thiazole methyl protons were detected at s 2.44-2.46 ppm (CH_3) as a singlet, ester methylene protons were seen at t 4.02 to 4.16 ppm (CH_3) which were triplet peaks. Aromatic region was seen between 6.89-7.80 ppm. A sharp singlet peak seen at s 9.02-9.86 ppm (NH) indicated the thiazole amine proton. Broad acetamide protons were observed at s 12.67-12.74 ppm (NH) as a singlet. To improve the experimental conditions for the preparation of molecules **4a-t**, several bases such as anhydrous potassium carbonate and triethylamine were used in different solvents such as methanol, ethanol, tetrahydrofuran and acetonitrile. As a result, we found that the reaction of **3a-t** with ethyl 2-cyano-3,3-bis(methylthio)acrylate was faster and gave thiazole derivatives **4a-t** in a good yield when potassium carbonate was used with DMF. The one-pot reaction of acetoacetanilide, *N*-bromosuccinimide and thiourea followed

by the addition of ethyl 2-cyano-3,3-bis(methylthio)acrylate was not successful and the reaction did not yield the desired product.

1.2.1 Optimizing reaction conditions

Table 1: Optimization of the reaction conditions

Entry	Solvent	Base	Temp. (°C)	Yield (%)	Purification Necessary / By-product formation
1	No Solvent	-	80	-	-
2	H_2O	-	rt	-	-
3	H_2O	K_2CO_3	rt	-	-
4	Acetone	Et_3N	rt	16	Yes
5	Acetone	K_2CO_3	rt	19	Yes
6	MeOH	Et_3N	rt	19	Yes
7	MeOH	K_2CO_3	rt	25	Yes
8	EtOH	Et_3N	rt	32	Yes
9	EtOH	K_2CO_3	rt	48	Yes
10	THF	Et_3N	rt	29	Yes
11	THF	K_2CO_3	rt	35	Yes
12	MeCN	Et_3N	rt	40	Yes
13	MeCN	K_2CO_3	rt	57	Yes
14	DMF	Et_3N	rt	72	Yes
15	DMF	K_2CO_3	rt	94	No

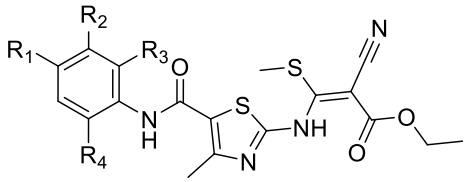
Firstly, the reaction conditions were kept neat with no use of solvent or catalyst at 80°C, but no product formation was seen (**Table 1**, entry 1). Therefore, the following trial was carried out with water as a solvent and at room temperature without a base for 1 hr. Once more, product formation was none (entry 2) so, with the addition of potassium carbonate, it was stirred for 1 hr but the result was again no product formation was seen (entry 3). Then the reaction was carried out using triethylamine as the base and acetone as a solvent and stirred at rt for 1 hr, resulting in the formation of 16% of the product (entry 4) and the formation of 19% of the product when potassium carbonate as a base

(entry 5). The reaction condition was then modified with respect to the solvent using methanol and triethylamine as a base. This resulted in the product being obtained with a yield of 19% (Entry 6) and as a base when potassium carbonate was used, the yield was 25% (entry 7). Furthermore, optimization of the reaction was done by using ethyl alcohol as a solvent and triethylamine as a base, the reaction mass was stirred at rt for 1 hr, the desired product was obtained in 32% yield (entry 8) and 48% yield was obtained when potassium carbonate was used as a base (entry 9). Subsequently, the use of tetrahydrofuran as solvent and triethylamine as a base yielded a product of 29% (entry 10) while using potassium carbonate yielded a 35% yield was obtained (entry 11). Surprisingly when using acetonitrile as solvent and triethylamine as a base, the yield was 40% (entry 12) and when potassium carbonate was used as a base, 57% yield (entry 13) was archived. When N, N-Dimethylformamide was used with triethylamine, this yielded a yield of 72% (entry 14), but using potassium carbonate as the base reaction mixture was stirred at rt for 30 min the resultant yield was 94% (entry 15). This variation led to no by-product formation and gave an excellent yield with high purity. It was clearly observed that when the triethylamine yield was low compared to that of potassium carbonate. Solvents such as methanol and acetone reduced the yield of the product, respectively (Entry 2 and 3), keeping water as a solvent, the reaction did not proceed further, probably because of the minor solubility of the reactants in water. So, with the optimized reaction environments, the technique was used to produce novel ten thiazole **4a-t** molecules (**Table 2**).

Newly prepared thiazole molecules were also subjected to molecular docking using autodock to discover various binding poses with affinity.

1.2.2 Physicochemical Properties

Table 2: Physicochemical characteristics of the novel thiazole derivatives **4a-t**



Entry	R^1	R^2	R^3	R^4	Molecular weight	Molecular formula	Yield (%)	Melting point (°C)
4a	OCH_3	H	H	H	432.51	$C_{19}H_{20}N_4O_4S_2$	82	129-131
4b	CH_3	H	H	H	416.51	$C_{19}H_{20}N_4O_3S_2$	89	150-152
4c	H	Cl	H	H	436.93	$C_{18}H_{17}ClN_4O_3S_2$	85	152-154
4d	H	H	H	H	402.49	$C_{18}H_{18}N_4O_3S_2$	82	127-129
4e	H	H	CH_3	CH_3	430.54	$C_{20}H_{22}N_4O_3S_2$	93	144-146
4f	F	H	H	H	420.48	$C_{18}H_{17}FN_4O_3S_2$	79	135-137
4g	Cl	H	H	H	436.93	$C_{18}H_{17}ClN_4O_3S_2$	86	152-154
4h	Br	H	H	H	481.38	$C_{18}H_{17}BrN_4O_3S_2$	91	172-174
4i	H	H	OCH_3	H	416.51	$C_{19}H_{20}N_4O_4S_2$	87	131-133
4j	CH_3	H	CH_3	H	430.54	$C_{20}H_{22}N_4O_3S_2$	79	148-150
4k	H	CF_3	H	H	470.49	$C_{19}H_{17}F_3N_4O_3S_2$	61	132-134
4l	H	OCH_3	H	H	432.51	$C_{19}H_{20}N_4O_4S_2$	83	125-127
4m	H	H	H	F	420.48	$C_{18}H_{17}FN_4O_3S_2$	59	129-131
4n	F	Cl	H	H	454.92	$C_{18}H_{16}ClFN_4O_3S_2$	65	141-143
4o	CH_3	CH_3	H	H	430.54	$C_{20}H_{22}N_4O_3S_2$	87	155-157
4p	H	H	Cl	Cl	471.37	$C_{18}H_{16}Cl_2N_4O_3S_2$	83	148-150
4q	Cl	Cl	H	H	471.37	$C_{18}H_{16}Cl_2N_4O_3S_2$	80	153-155
4r	H	F	H	H	420.48	$C_{18}H_{17}FN_4O_3S_2$	64	133-135
4s	H	Br	H	H	481.38	$C_{18}H_{17}BrN_4O_3S_2$	82	170-172
4t	H	CH_3	H	H	416.51	$C_{19}H_{20}N_4O_3S_2$	85	150-152

1.2.3 Molecular docking with α-amylase

In vitro outcomes revealed that the prepared molecules **4a-t** showed moderate to good inhibition towards α-amylase compared to acarbose. Molecule **4g** and **4h** exposed higher inhibition more than other prepared molecules. So, molecule **4g** and **4h** were utilized to determine the binding position and interactions accountable for α-amylase (2QV4) activity. Molecule **4g** with binding energy of -6.9 showed hydrogen bond interaction with Arg-398, Asp-402 and Ser-289 and pi-alkyl interactions with Tyr-333 and Arg-398 as displayed in **Fig. 3**. Molecule **4h** with a binding energy of -6.6 showed four hydrogen bond with Arg-252, Thr-6, Gly-9 and Asn-5, one pi-pi interaction with Phe-335 and pi-alkyl contacts with Pro-4 as shown in **Fig. 4**.

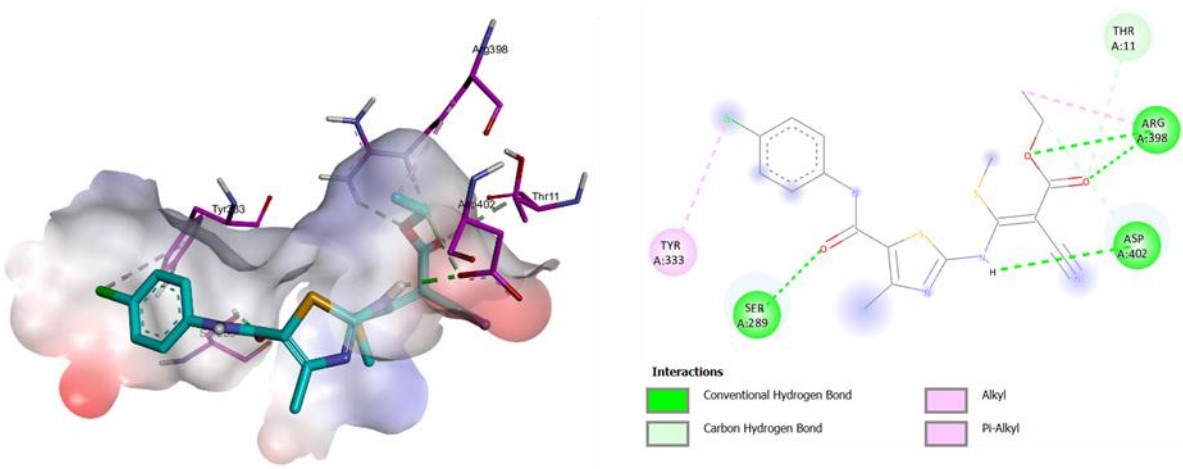


Fig 1: Docking pose of **4g** with human pancreatic α-amylase

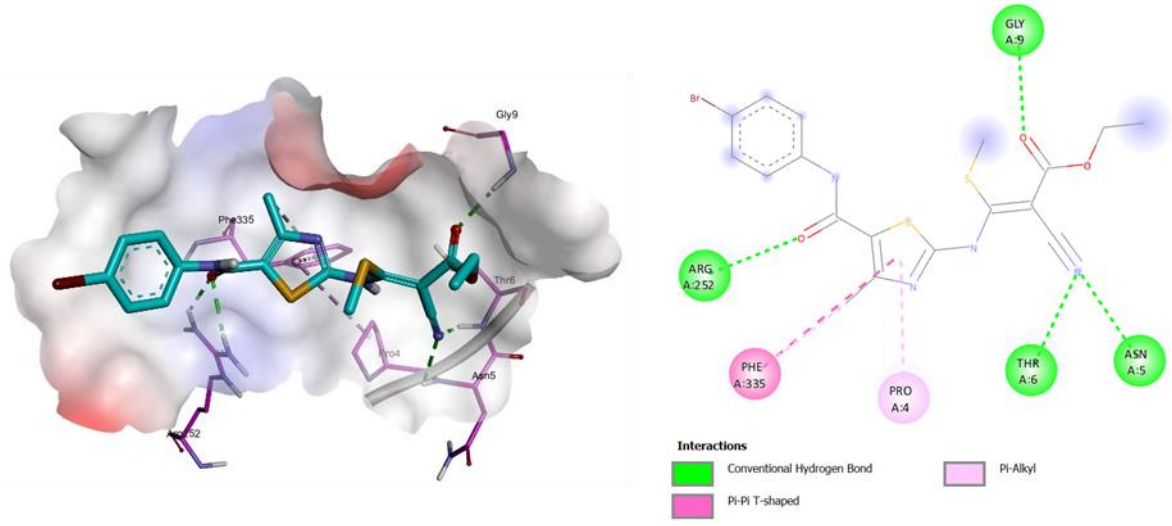


Fig 2: Docking pose of **4h** with human pancreatic α-amylase

1.2.4 In vitro α-amylase assay

The synthesized Thiazole molecules **4a-t** were assessed for in vitro activity study against α-amylase enzyme by utilizing acarbose as a control following Mor's technique. The outcomes of the α-amylase inhibitory study shown in **Table 2** exposed that tested molecules **4a-t** showed moderate to high % inhibition. Molecule **4b**, **4f**, **4g** and **4h** at 12.5 µg/mL, **4c**, **4d**, **4g** and **4h** at 25 µg/mL, **4b** at a concentration 50 µg/mL and **4g** at a concentration 100 µg/mL displayed more inhibition than the control drug acarbose at a concentration 100 µg/mL. Furthermore, the other molecules showed lesser inhibition compared to the control at various concentrations. Between the prepared molecules, **4b** (IC_{50}=22.20 µg/mL), **4g** (IC_{50}=11.73 µg/mL) and **4h** (IC_{50}=12.55 µg/mL) showed excellent inhibition compared to the acarbose (IC_{50}=23.62 µg/mL) as a control (**Table 4**).

Table 3: In vitro α-amylase assay of thiazole **4a-t**

Compounds	R^1	R^2	R^3	R^4	% Inhibition				IC_{50} (µg/mL)
					12.5 (µg/mL)	25 (µg/mL)	50 (µg/mL)	100 (µg/mL)	
4a	OCH_3	H	H	H	27.43	36.62	60.56	86.13	32.97
4b	CH_3	H	H	H	35.12	44.87	85.21	88.61	22.20
4c	H	Cl	H	H	23.14	51.61	70.14	86.38	26.30
4d	H	H	H	H	19.40	53.36	59.19	75.44	31.94
4e	H	H	CH_3	CH_3	14.74	30.13	39.42	59.57	69.47
4f	F	H	H	H	28.47	40.28	51.31	80.42	36.11
4g	Cl	H	H	H	58.85	66.11	74.82	92.57	11.73
4h	Br	H	H	H	53.38	68.71	78.63	82.61	12.55
4i	H	H	OCH_3	H	25.21	41.10	56.80	82.55	33.99
Acarbose	-	-	-	-	28.43	49.22	79.33	90.42	23.62

1.3 Conclusion

In conclusion, a series of novel ethyl (*E*)-2-cyano-3-((4-methyl-5-(arylcarbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate have been designed, synthesized and characterized by various analytical techniques such as FTIR, MS and ^{1}H NMR. Some of the synthesized compounds exhibited notable α-amylase inhibitory activity. This study furthermore marks that molecule **4g** (IC_{50}=11.73 μg/mL) and **4h** (IC_{50}=12.55 μg/mL) having a halogen group on the aromatic rings feature a more powerful inhibitory activity against α-amylase compared to reference compound acarbose (IC_{50}=23.62 μg/mL). Molecular docking studies have shown insight into the binding manners of prepared molecule with the target enzyme. Hence, these thiazole molecules are an esteem nominee deserving additional investigation for upcoming development of potential antidiabetic molecules.

1.4 Experimental Section

Melting points were determined on an electrothermal device using open capillaries and are uncorrected. Thin-layer chromatography was performed on precoated silica-gel 60 F254 (Merck), the compounds were visualized with UV light at 254 nm and 365 nm or with iodine vapor. The IR spectra were recorded on a Shimadzu FT-IR spectrometer using the ATR technique. ^{1}H NMR spectra were recorded on a Bruker AVANCE III (400 MHz) spectrometer in DMSO-d_6. Chemical shifts are expressed in δ ppm downfield from Tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded using a direct inlet probe on Shimadzu GCMS QP2010 Ultra mass spectrometer. All reactions were carried out under an ambient atmosphere. All reagents were purchased from Loba, Molychem, SRL and CDH and used without further purification.

- **General process for the synthesis of acetoacetanilides (2a-t)**

Substituted amine (10 mmol) and ethyl acetoacetate containing catalytic amount of Potassium or sodium hydroxide lie (10%) in toluene was refluxed for approximately 24 hr. On completion of the reaction, the mass was evaporated under vacuum and the residue was crystallized from methanol or ethanol to get pure acetoacetanilides.

- **General process for the synthesis of thiazoles (3a-t)**

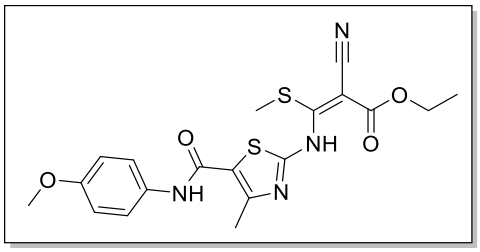
To a stirred solution of compound acetoacetanilide (10 mmol) **(2a-t)** in MeOH, *N*-bromosuccinimide (15 mmol) was added and stirred at room temperature for 30 min. To this reaction mass thiourea (20 mmol) was slowly added and refluxed for 4-5 hr. The reaction mixture was cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid product was filtered, washed with water and dried at room temperature to get an analytically pure compound (**3a-t**), as a light brown solid. Yield: 89%.

- **General procedure for the synthesis of ethyl (*E*)-2-cyano-3-((5-((arylphenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio) acrylate (4a-t)**

A mixture of **3a-t** (10 mmol) and ethyl 2-cyano-3,3-bis(methylthio)acrylate (10 mmol) in 10 mL of DMF and anhydrous potassium carbonate (10 mmol) was stirred at room temperature for 1 hr. After the completion of the reaction, the reaction mixture was

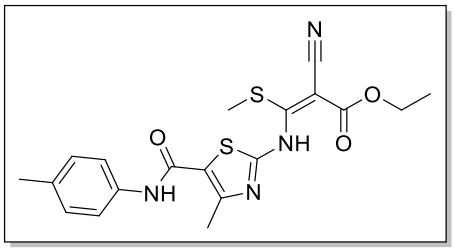
cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid was filtered, washed with water and purified by recrystallization from DMF to afford crystals **(4a-t)**.

Ethyl (*E*)-2-cyano-3-((5-((4-methoxyphenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-1)



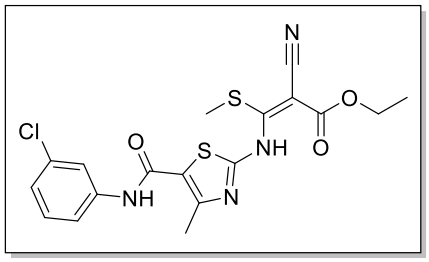
Yellow powder, Yield: 82%, mp 129-131°C; FTIR (ATR, ν_{max}, cm^{-1}): 1453, 1659, 2215, 2971; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (s, 1H), 9.63 (s, 1H), 7.51 (d, J = 9.1 Hz, 2H), 6.90 (d, J = 9.1 Hz, 2H), 4.14 (d, J = 7.3 Hz, 2H), 3.74 (s, 3H), 2.45 (s, 3H), 2.32 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H); MS (*m*/*z*): 432 (M^+); Anal. Calcd. For $C_{19}H_{20}N_4O_4S_2$: C, 52.76; H, 4.66; N, 12.95; Found: C, 52.81; H, 4.65; N, 12.98.

Ethyl (*E*)-2-cyano-3-((4-methyl-5-(p-tolylcarbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-2)



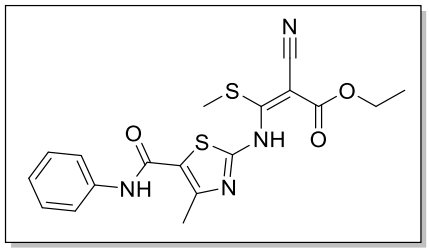
Yellow Powder, Yield: 89%, mp 150-152°C; FTIR (ATR, ν_{max}, cm^{-1}): 1451, 1663, 2217, 2970; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H), 9.68 (s, 1H), 7.50 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 8.1 Hz, 2H), 4.15 (d, J = 7.5 Hz, 2H), 2.45 (s, 3H), 2.32 (s, 3H), 2.28 (s, 3H), 1.22 (t, J = 7.3 Hz, 3H); MS (*m*/*z*): 416 (M^+); Anal. Calcd. For $C_{19}H_{20}N_4O_3S_2$: C, 54.79; H, 4.84; N, 13.45; Found: C, 54.68; H, 4.80; N, 13.34.

Ethyl (*E*)-3-((5-((3-chlorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-2-cyano-3-(methylthio)acrylate (OETTH-3)



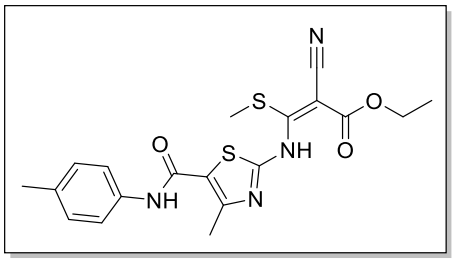
Yellow Powder, Yield: 85%, mp 152-154°C; FTIR (ATR, ν_{max}, cm^{-1}): 1481, 1670, 2210, 2973; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 9.86 (s, 1H), 7.80 (s, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.36 (t, J = 8.1 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H), 2.46 (s, 3H), 2.32 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H); MS (*m*/*z*): 436 (M^+); Anal. Calcd. For $C_{18}H_{17}ClN_4O_3S_2$: C, 49.48; H, 3.92; N, 12.82; Found: C, 49.62; H, 4.02; N, 12.91.

Ethyl (*E*)-2-cyano-3-((4-methyl-5-(phenylcarbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-4)



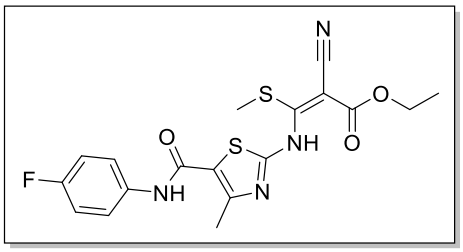
Yellow Powder, Yield: 82%, mp 127-129°C; FTIR (ATR, ν_{max}, cm^{-1}): 1487, 1658, 2217, 2971; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H), 9.63 (s, 1H), 7.62 (d, J = 8.3 Hz, 2H), 7.50 (d, J = 8.6 Hz, 2H), 7.32 (t, J = 7.7 Hz, 2H), 7.09 (t, J = 7.4 Hz, 2H), 4.05 (q, J = 7.1 Hz, 2H), 2.45 (s, 3H), 2.29 (s, 3H), 1.17 (t, J = 7.0 Hz, 3H); MS (*m*/*z*): 402 (M^+); Anal. Calcd. For $C_{18}H_{18}N_4O_3S_2$: C, 53.72; H, 4.51; N, 13.92; Found: C, 53.82; H, 4.55; N, 13.89.

Ethyl (*E*)-2-cyano-3-((5-((2,6-dimethylphenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-5)



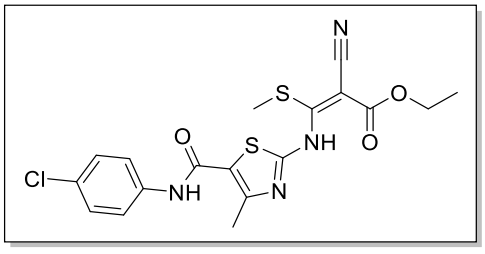
Yellow Powder, Yield: 93%, mp 144-146°C; FTIR (ATR, ν_{max}, cm^{-1}): 1463, 1665, 2214, 2972; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H), 9.20 (s, 1H), 7.11 (s, 3H), 4.14 (q, J = 7.1 Hz, 2H), 2.46 (s, 3H), 2.33 (s, 3H), 2.15 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H); MS (*m*/*z*): 430 (M^+); Anal. Calcd. For $C_{20}H_{22}N_4O_3S_2$: C, 54.79; H, 4.84; N, 13.45; Found: C, 54.65; H, 4.84; N, 13.41.

Ethyl (*E*)-2-cyano-3-((5-((4-fluorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-6)



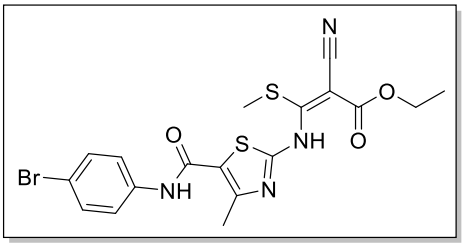
Yellow Powder, Yield: 79%, mp 135-137°C; FTIR (ATR, ν_{max}, cm^{-1}): 1250, 1408, 1676, 2214, 2975; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H), 9.79 (s, 1H), 7.63 (dd, J = 8.9, 5.0 Hz, 2H), 7.17 (t, J = 8.9 Hz, 2H), 4.14 (d, *J* = 7.0 Hz, 1H), 2.46 (s, 3H), 2.32 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H); MS (*m*/*z*): 420 (M^+); Anal. Calcd. For $C_{18}H_{17}FN_4O_3S_2$: C, 51.42; H, 4.08; N, 13.32; Found: C, 51.32; H, 3.96; N, 13.35.

Ethyl (*E*)-3-((5-((4-chlorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-2-cyano-3-(methylthio)acrylate (OETTH-7)



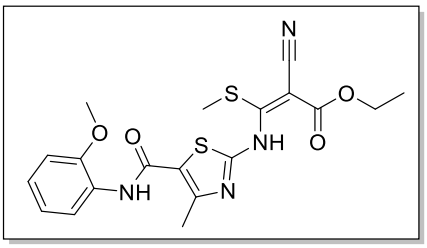
Yellow Powder, Yield: 86%, mp 152-154°C; FTIR (ATR, ν_{max}, cm^{-1}): 1590, 1658, 2218, 2972; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (1H, s, H-18), 9.86 (1H, s, H-10), 7.66 (2H, d, J = 8.9 Hz, Ar-H), 7.39 (2H, d, J = 8.9 Hz, Ar-H), 4.14 (2H, q, J = 7.1 Hz, H-27), 2.45 (3H, s, H-16), 2.32 (3H, s, H-7), 1.21 (3H, t, J = 7.1 Hz, H-28); MS (m/z): 436 (M^+); Anal. Calcd. For $C_{18}H_{17}ClN_4O_3S_2$: C, 49.48; H, 3.92; N, 12.82; Found: C, 49.18; H, 3.76; N, 12.96.

Ethyl (*E*)-3-((5-((4-bromophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-2-cyano-3-(methylthio)acrylate (OETTH-8)



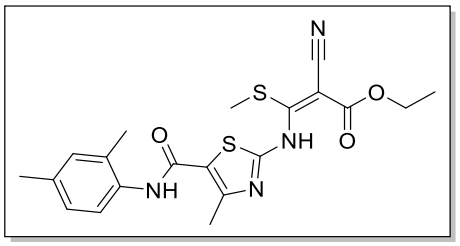
Yellow Powder, Yield: 91%, mp 172-174°C; FTIR (ATR, ν_{max}, cm^{-1}): 1488, 1667, 2214, 2979; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H), 9.86 (s, 1H), 7.60 (d, J = 9.0 Hz, 2H), 7.51 (d, J = 8.9 Hz, 2H), 4.14 (d, J = 7.4 Hz, 2H), 2.45 (s, 3H), 2.31 (s, 3H), 1.21 (t, J = 7.0 Hz, 3H); MS (m/z): 481 (M^+); Anal. Calcd. For $C_{18}H_{17}BrN_4O_3S_2$: C, 44.91; H, 3.56; N, 11.64; Found: C, 44.87; H, 3.19; N, 11.43.

Ethyl (*E*)-2-cyano-3-((4-methyl-5-(o-tolylcarbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-9)



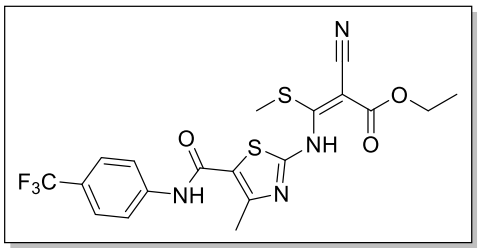
Yellow Powder, Yield: 87%, mp 131-133°C; FTIR (ATR, ν_{max}, cm^{-1}): 1451, 1665, 2208, 2974; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H), 9.38 (s, 1H), 7.30-7.12 (m, 4H), 4.14 (q, J = 7.1 Hz, 3H), 2.47 (s, 3H), 2.32 (s, 3H), 2.19 (s, 3H), 1.21 (t, J = 7.0 Hz, 3H); MS (m/z): 416 (M^+); Anal. Calcd. For $C_{19}H_{20}N_4O_4S_2$: C, 52.76; H, 4.66; N, 12.95; Found: C, 52.79; H, 4.82; N, 13.01.

Ethyl (*E*)-2-cyano-3-((5-((2,4-dimethylphenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-10)



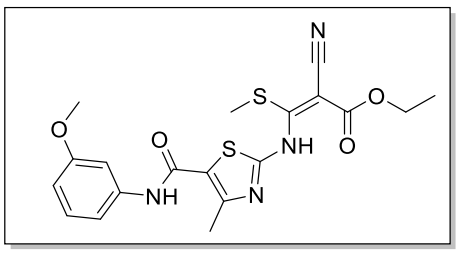
Yellow Powder, Yield: 79%, mp 148-150°C; FTIR (ATR, ν_{max}, cm^{-1}): 1464, 1665, 2212, 2973; MS (*m*/*z*): 430 (M^+); Anal. Calcd. For $C_{20}H_{22}N_4O_3S_2$: C, 55.79; H, 5.15; N, 13.01; Found: C, 55.62; H, 5.32; N, 13.09.

Ethyl (*E*)-2-cyano-3-((4-methyl-5-((3-(trifluoromethyl)phenyl)carbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-11)



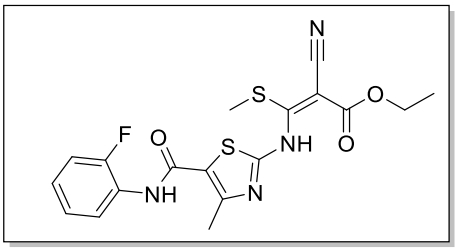
Yellow Powder, Yield: 61%, mp 132-134°C; FTIR (ATR, ν_{max}, cm^{-1}): 1448, 1665, 2213, 2972; MS (*m*/*z*): 470 (M^+); Anal. Calcd. For $C_{19}H_{17}F_3N_4O_3S_2$: C, 48.51; H, 3.64; N, 11.91; Found: C, 48.17; H, 3.55; N, 11.87.

Ethyl (*E*)-2-cyano-3-((5-((3-methoxyphenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-12)



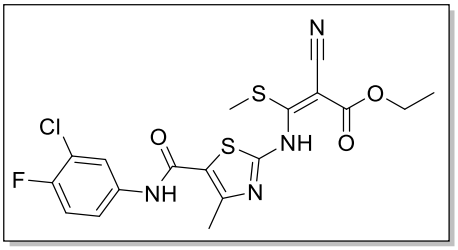
Yellow Powder, Yield: 83%, mp 125-127°C; FTIR (ATR, ν_{max}, cm^{-1}): 1455, 1658, 2212, 2972; MS (*m*/*z*): 432 (M^+); Anal. Calcd. For $C_{19}H_{20}N_4O_4S_2$: C, 52.76; H, 4.66; N, 12.95; Found: C, 52.34; H, 4.41; N, 12.85.

Ethyl (*E*)-2-cyano-3-((5-((2-fluorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-13)



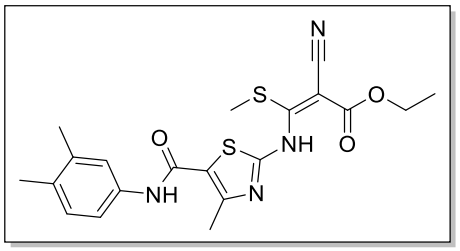
Yellow Powder, Yield: 59%, mp 129-131°C; FTIR (ATR, ν_{max}, cm^{-1}): 1249, 1607, 1675, 2215; MS (*m*/*z*): 420 (M^+); Anal. Calcd. For $C_{18}H_{17}FN_4O_3S_2$: C, 51.42; H, 4.08; N, 13.32; Found: C, 51.32; H, 4.09; N, 13.18.

Ethyl (*E*)-3-((5-((3-chloro-4-fluorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-2-cyano-3-(methylthio)acrylate (OETTH-14)



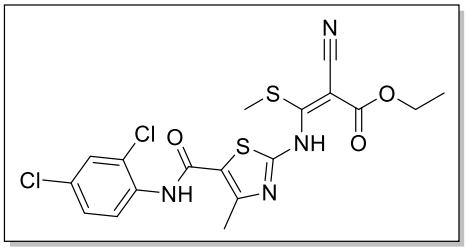
Yellow Powder, Yield: 65%, mp 141-143°C; FTIR (ATR, ν_{max}, cm^{-1}): 1252, 1403, 1673, 2218; MS (*m*/*z*): 454 (M^+); Anal. Calcd. For $C_{18}H_{16}ClFN_4O_3S_2$: C, 47.52; H, 3.55; N, 12.32; Found: C, 47.76; H, 3.21; N, 12.56.

Ethyl (E)-2-cyano-3-((5-((3,4-dimethylphenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-15)



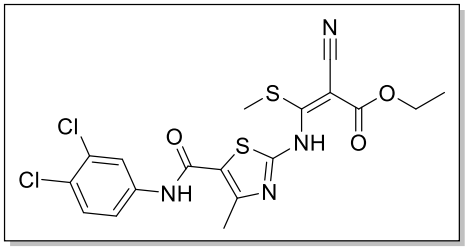
Yellow Powder, Yield: 87%, mp 155-157°C; FTIR (ATR, ν_{max}, cm^{-1}): 1468, 1668, 2214, 2975; MS (*m*/*z*): 430 (M^+); Anal. Calcd. For $C_{20}H_{22}N_4O_3S_2$: C, 55.79; H, 5.15; N, 13.01; Found: C, 55.91; H, 5.29; N, 13.08.

Ethyl (*E*)-2-cyano-3-((5-((2,6-dichlorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-16)



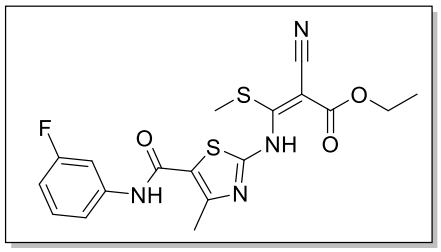
Yellow Powder, Yield: 83%, mp 148-150°C; FTIR (ATR, ν_{max}, cm^{-1}): 1592, 1651, 2213, 2974; MS (*m*/*z*): 471 (M^+); Anal. Calcd. For $C_{18}H_{16}Cl_2N_4O_3S_2$: C, 45.87; H, 3.42; N, 11.89; Found: C, 45.96; H, 3.61; N, 11.93.

Ethyl (*E*)-2-cyano-3-((5-((3,4-dichlorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-17)



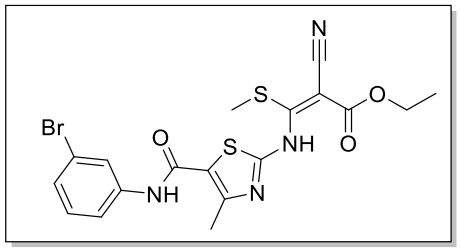
Yellow Powder, Yield: 80%, mp 153-155°C; FTIR (ATR, ν_{max}, cm^{-1}): 1594, 1654, 2217, 2973; MS (*m*/*z*): 471 (M^+); Anal. Calcd. For $C_{18}H_{16}Cl_2N_4O_3S_2$: C, 45.87; H, 3.42; N, 11.89; Found: C, 45.79; H, 3.43; N, 11.82.

Ethyl (*E*)-2-cyano-3-((5-((3-fluorophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-18)



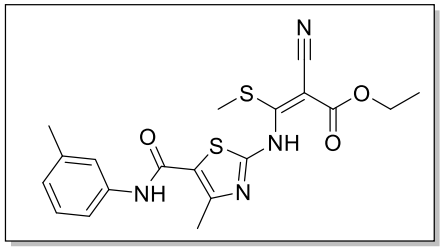
Yellow Powder, Yield: 64%, mp 133-135°C; FTIR (ATR, ν_{max}, cm^{-1}): 1254, 1602, 1677, 2215; MS (*m*/*z*): 420 (M^+); Anal. Calcd. For $C_{18}H_{17}FN_4O_3S_2$: C, 51.42; H, 4.08; N, 13.32; Found: C, 51.11; H, 3.96; N, 13.22.

Ethyl (*E*)-3-((5-((3-bromophenyl)carbamoyl)-4-methylthiazol-2-yl)amino)-2-cyano-3-(methylthio)acrylate (OETTH-19)



Yellow Powder, Yield: 82%, mp 170-172°C; FTIR (ATR, ν_{max}, cm^{-1}): 1489, 1665, 2213, 2976; MS (*m*/*z*): 481 (M^+); Anal. Calcd. For $C_{18}H_{17}BrN_4O_3S_2$: C, 44.91; H, 3.56; N, 11.64; Found: C, 44.91; H, 3.57; N, 11.62.

Ethyl (*E*)-2-cyano-3-((4-methyl-5-(m-tolylcarbamoyl)thiazol-2-yl)amino)-3-(methylthio)acrylate (OETTH-20)



Yellow Powder, Yield: 85%, mp 150-152°C; FTIR (ATR, ν_{max}, cm^{-1}): 1464, 1666, 2213, 2974; MS (*m*/*z*): 416 (M^+); Anal. Calcd. For $C_{19}H_{20}N_4O_3S_2$: C, 54.79; H, 4.84; N, 13.45; Found: C, 54.72; H, 4.77; N, 13.60.

1.4.1 Experimental protocol of molecular docking study

The ChemSketch 2021.2.0 software was used for the generation of ligand structures. Furthermore, the energy minimization of every molecule was performed using the Dundee PRODRG2 server and Autodock Vina 1.1.2 was used to for the docking studies. Human pancreatic α-amylase was obtained from PDB with ID 2QV4. The grid box size was set to 40, 40 and 40 Å for x, y and z correspondingly. The grid center was set to 17.390, 61.804 and 15.925 for x, y and z individually. Value of exhaustiveness was set to 40. Powerful molecular graphics viewer Discovery Studio Visualizer v21.0 was used to figure out the most probable binding mode.

1.4.2 In vitro α-amylase inhibition

Mor's technique was used to assess the inhibition activity of α-amylase in vitro using acarbose as reference compound. The molecule **4a-t** were added to 5 mL DMSO and dissolved at ambient temperature to give concentrations ranging from 12.5, 25, 50 and 100 µg/mL. In 25 mL 0.4 M NaOH solution, 500 mg starch was dissolved at 100°C for 5 min and used as a substrate solution, following cooling to room temperature, pH 7 was attained by addition of 2 M HCl solution and 100 mL water was added to make the volume. In microplates, samples (20L) and substrate (40L) were mixed and incubated at 37°C for three minutes. Afterward, 20 µL α-amylase solution (50 µg/mL) was added to each well followed by incubation for 15 min. To stop the reaction, 0.1 M HCl (80 µL) was added. 1 mM iodine solution (200 µL) was added to the reaction mass and the absorbance was measured at 650 nm using Elisa microplate reader. The α-amylase inhibitory activity was demonstrated as percentage inhibition.

% inhibition = $\{1 - (Abs2 - Abs1)/(Abs4 - Abs3) \times 100\}$

1.5 Spectral Data

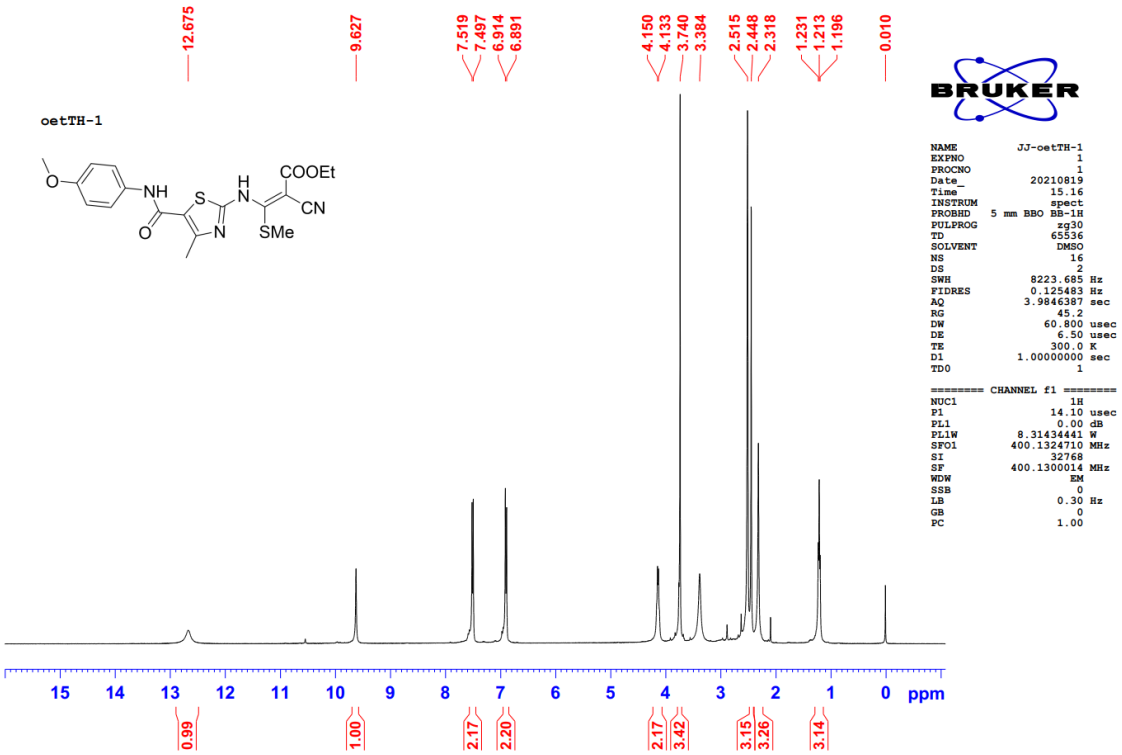


Fig. 1: Representative ^{1}H NMR spectrum of compound OETTH-1

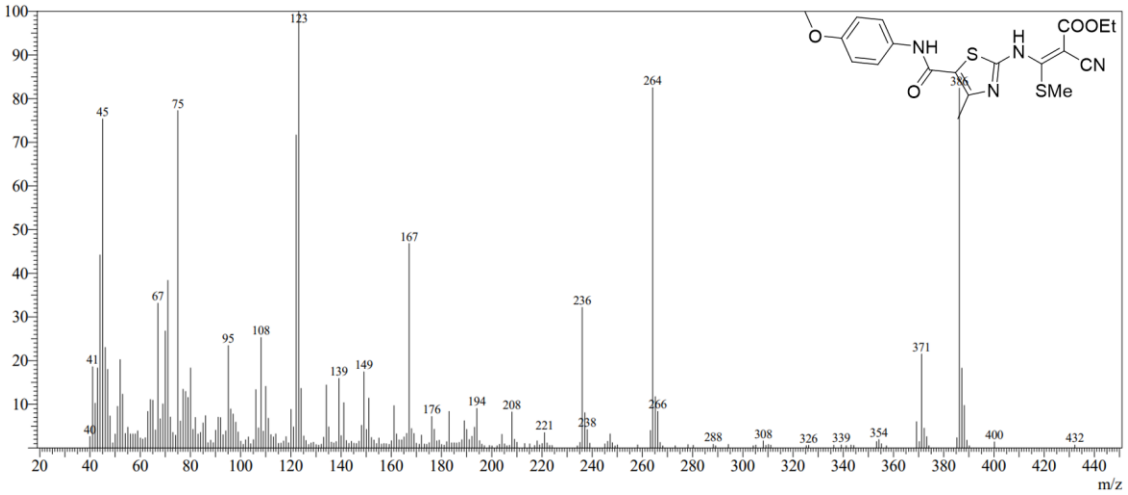


Fig. 2: Representative mass spectrum of compound OETTH-1

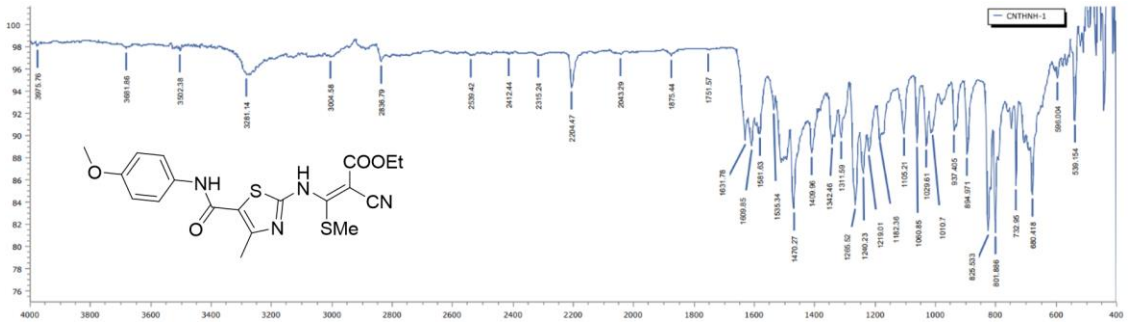


Fig. 3: Representative IR spectrum of compound OETTH-1

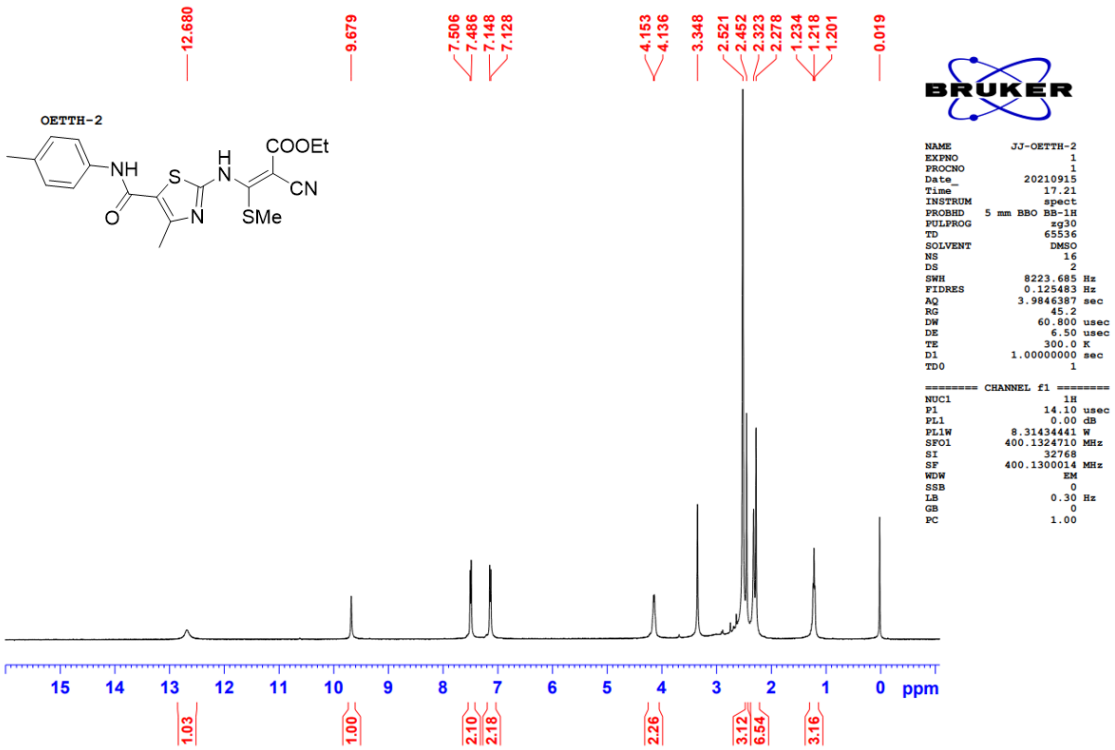


Fig. 4: Representative ^{1}H NMR spectrum of compound OETTH-2

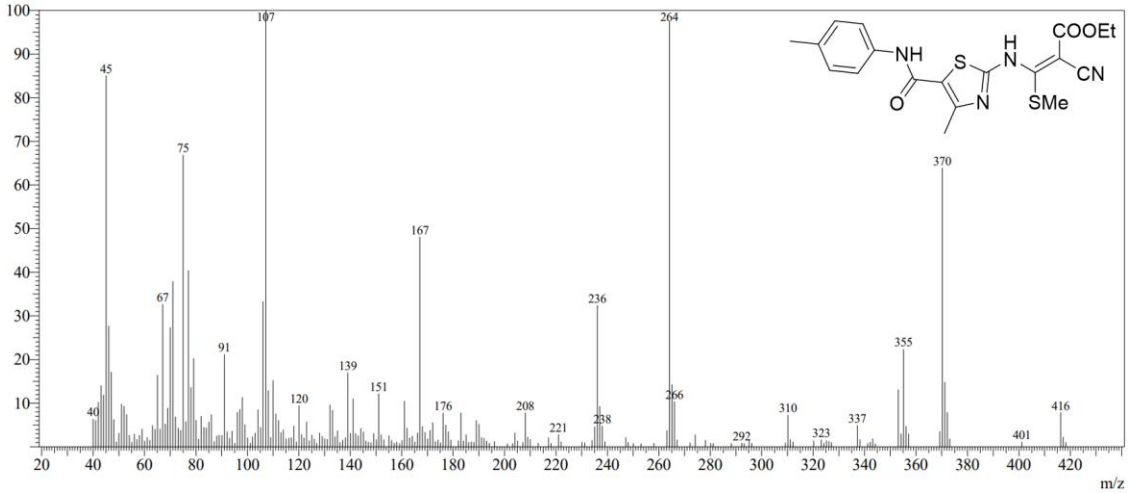


Fig. 5: Representative mass spectrum of compound OETTH-2

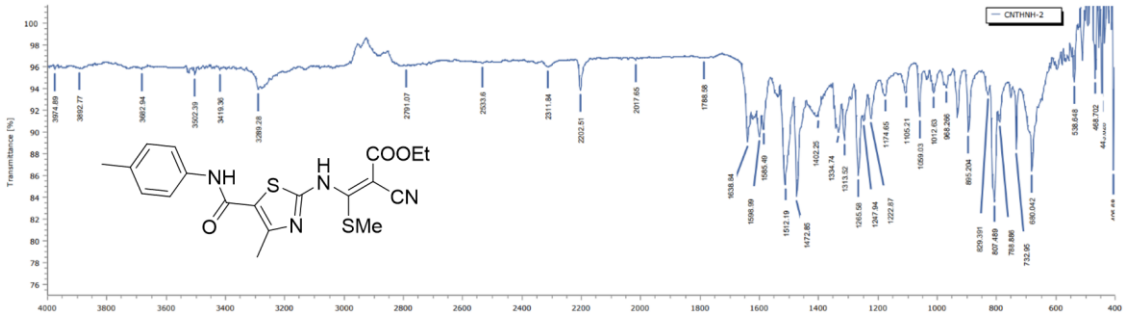


Fig. 6: Representative IR spectrum of compound OETTH-2

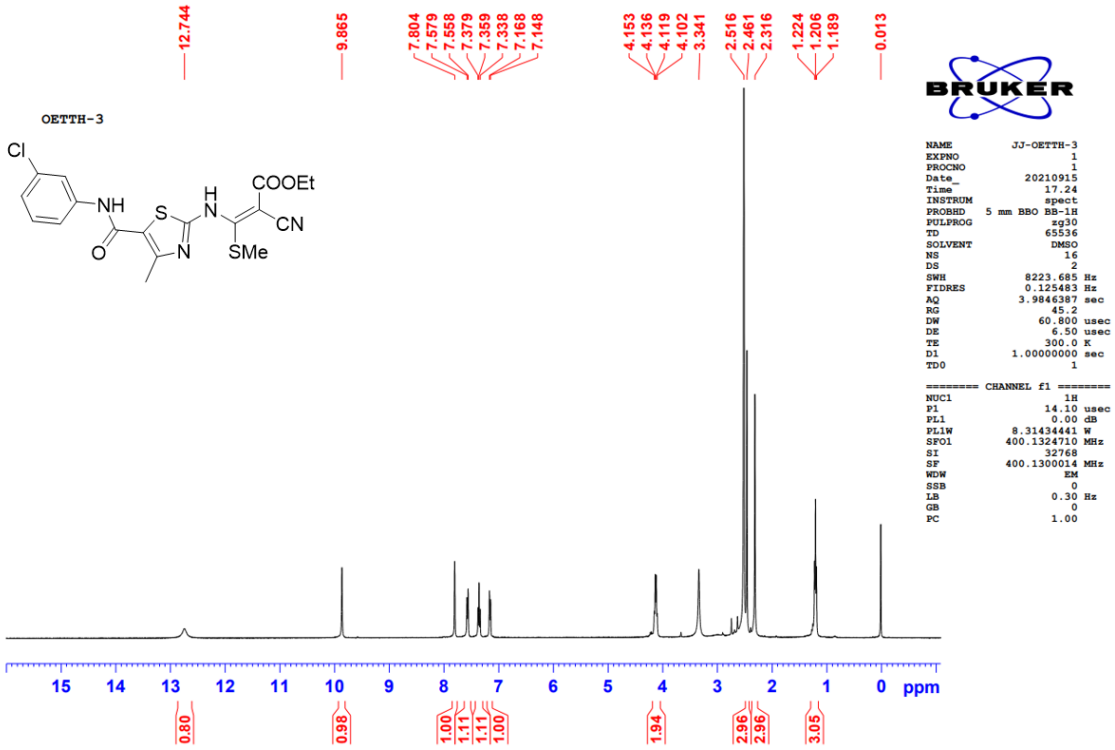


Fig. 7: Representative ^{1}H NMR spectrum of compound OETTH-3

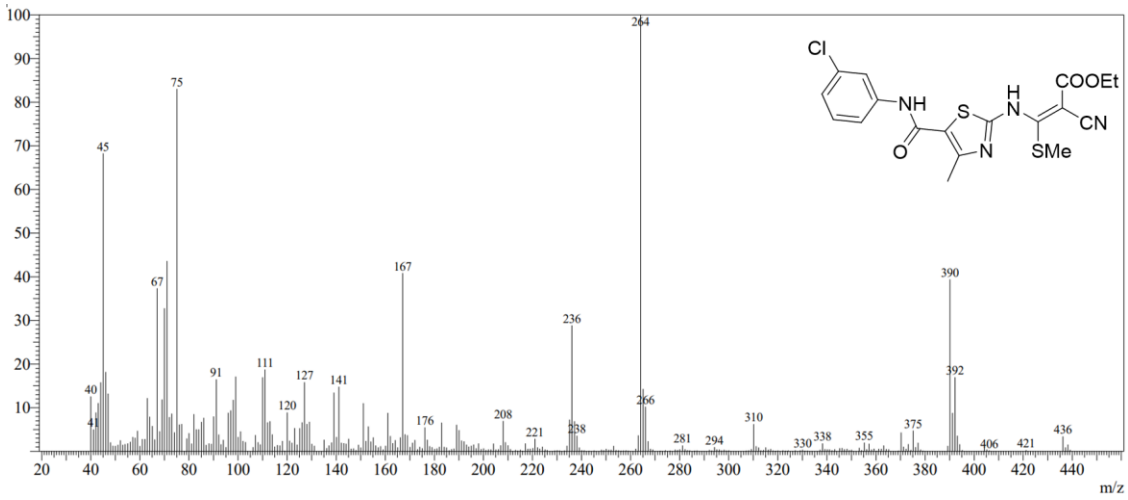


Fig. 8: Representative mass spectrum of compound OETTH-3

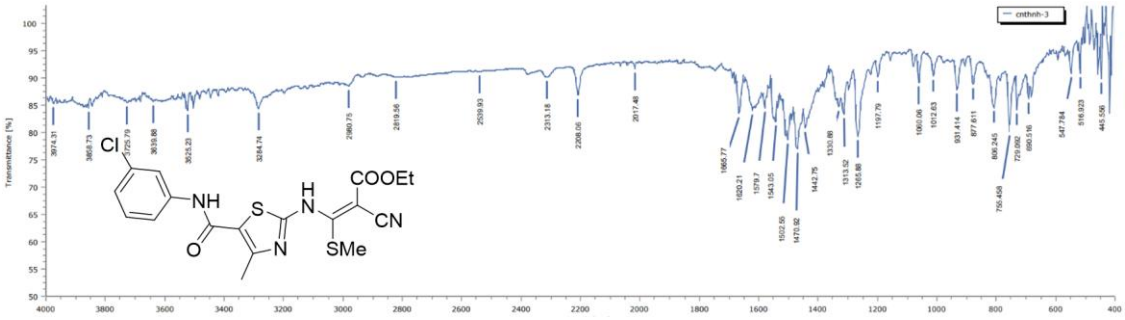


Fig. 9: Representative IR spectrum of compound OETTH-3

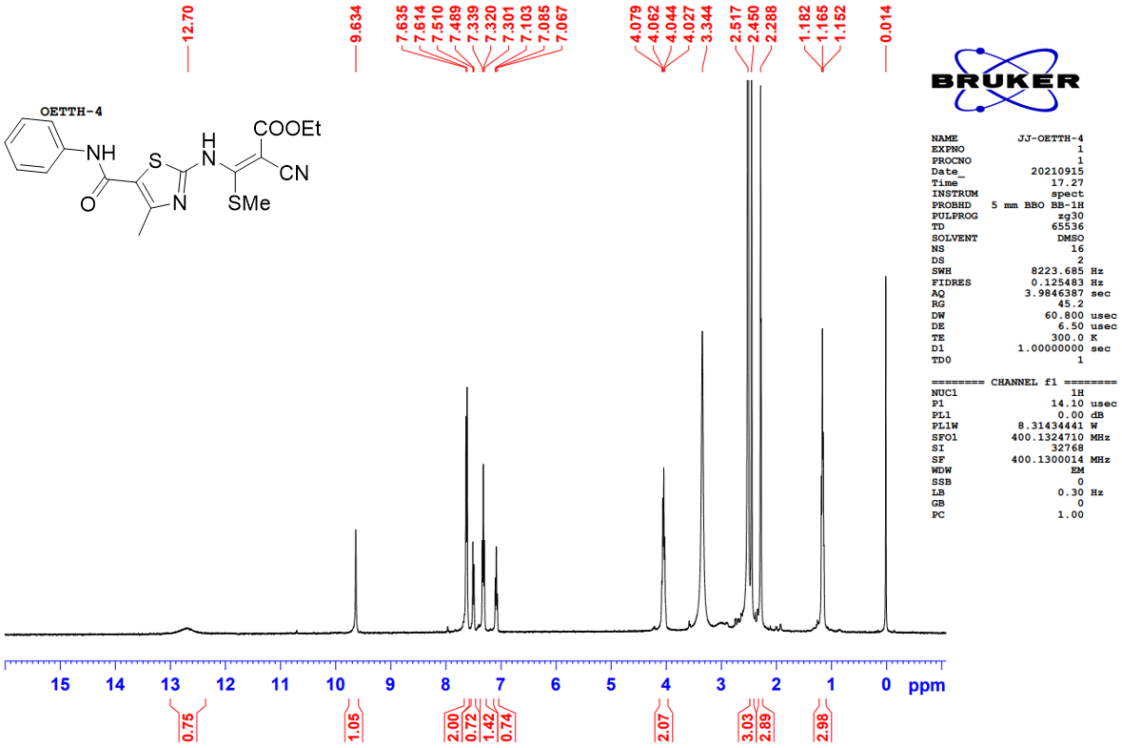


Fig. 10: Representative ^{1}H NMR spectrum of compound OETTH-4

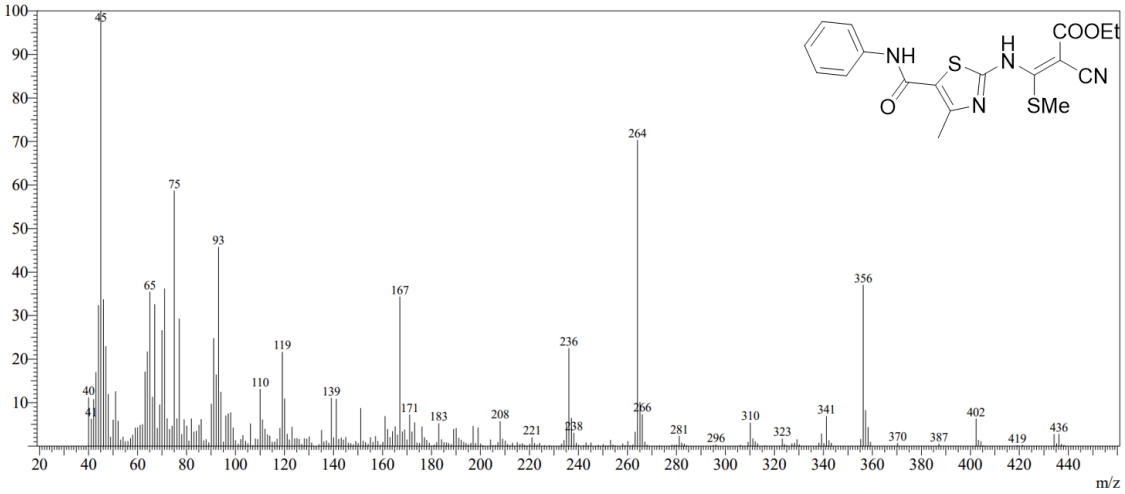


Fig. 11: Representative mass spectrum of compound OETTH-4

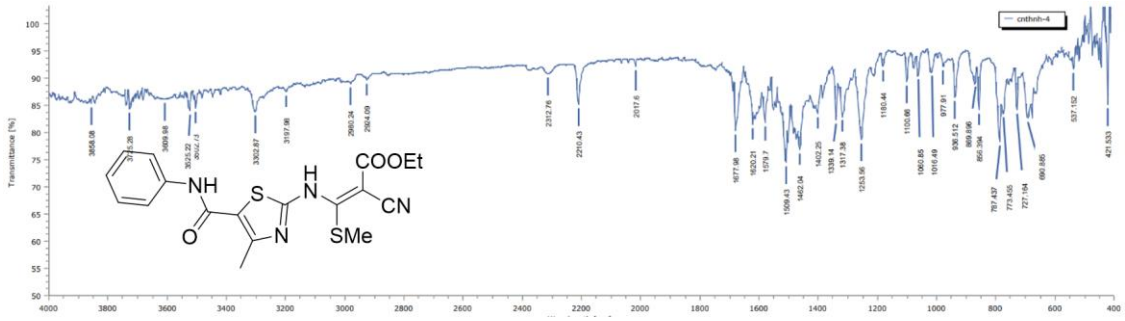


Fig. 12: Representative IR spectrum of compound OETTH-4

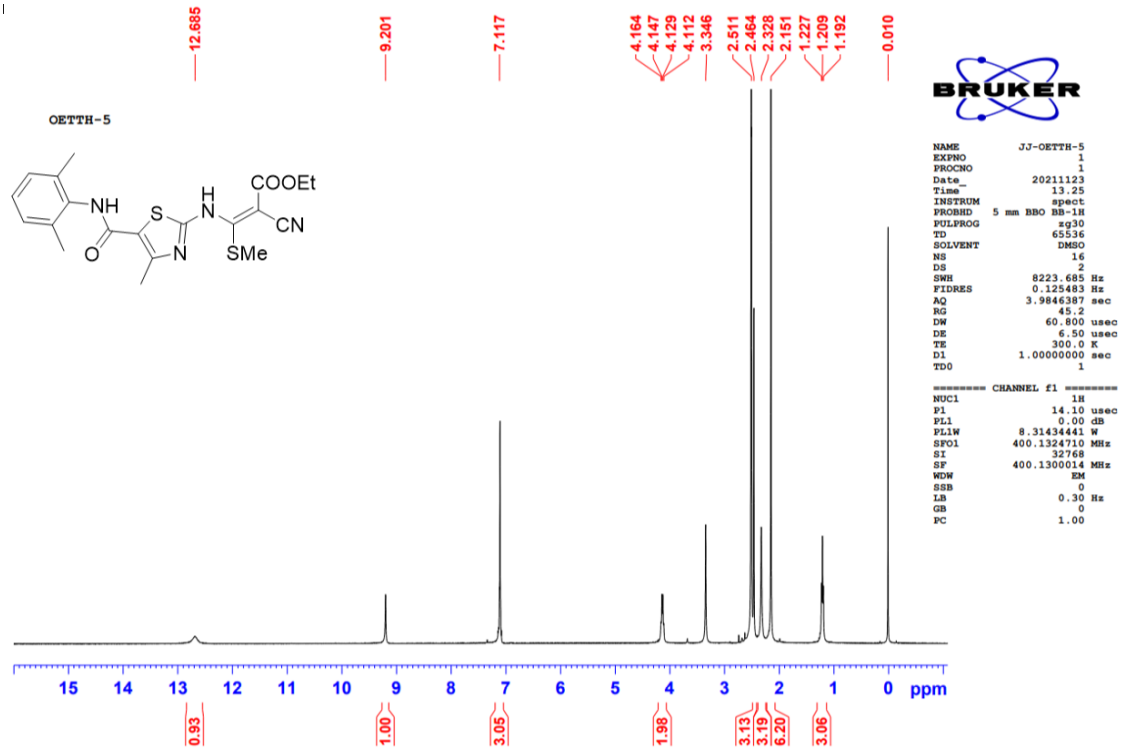


Fig. 13: Representative ^{1}H NMR spectrum of compound OETTH-5

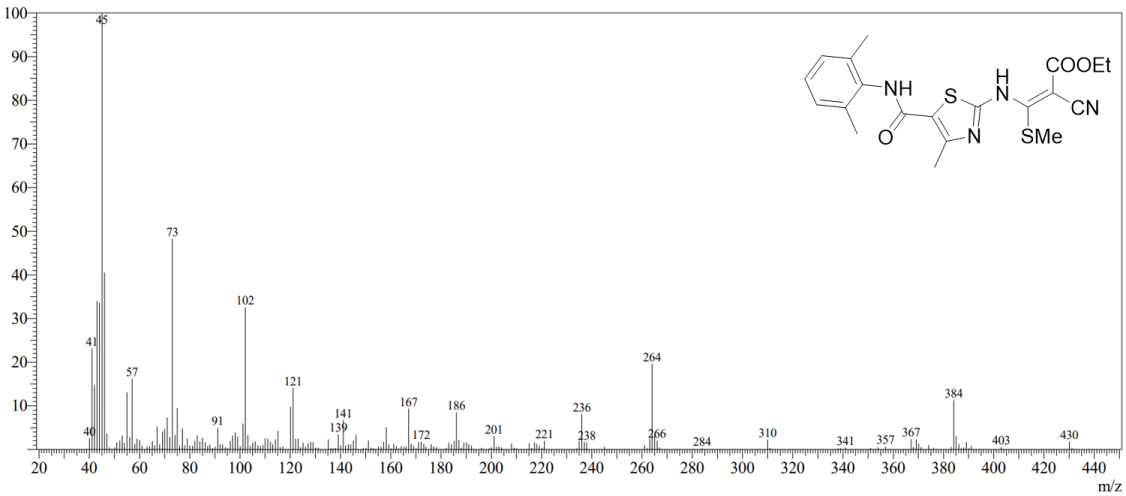


Fig. 14: Representative mass spectrum of compound OETTH-5

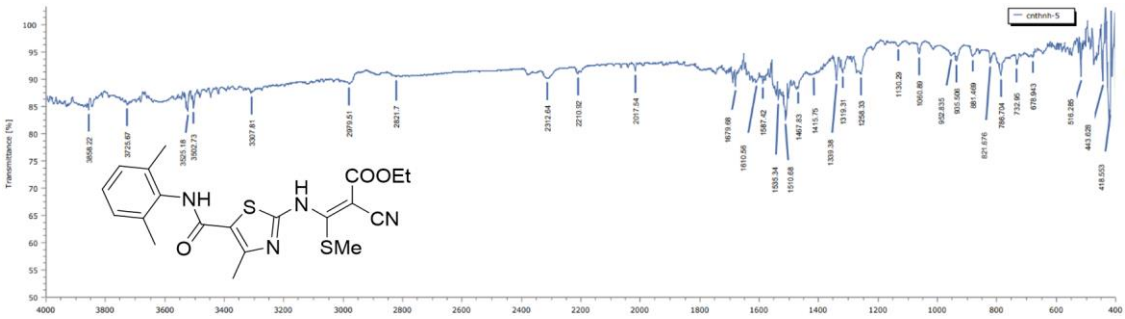


Fig. 15: Representative IR spectrum of compound OETTH-5

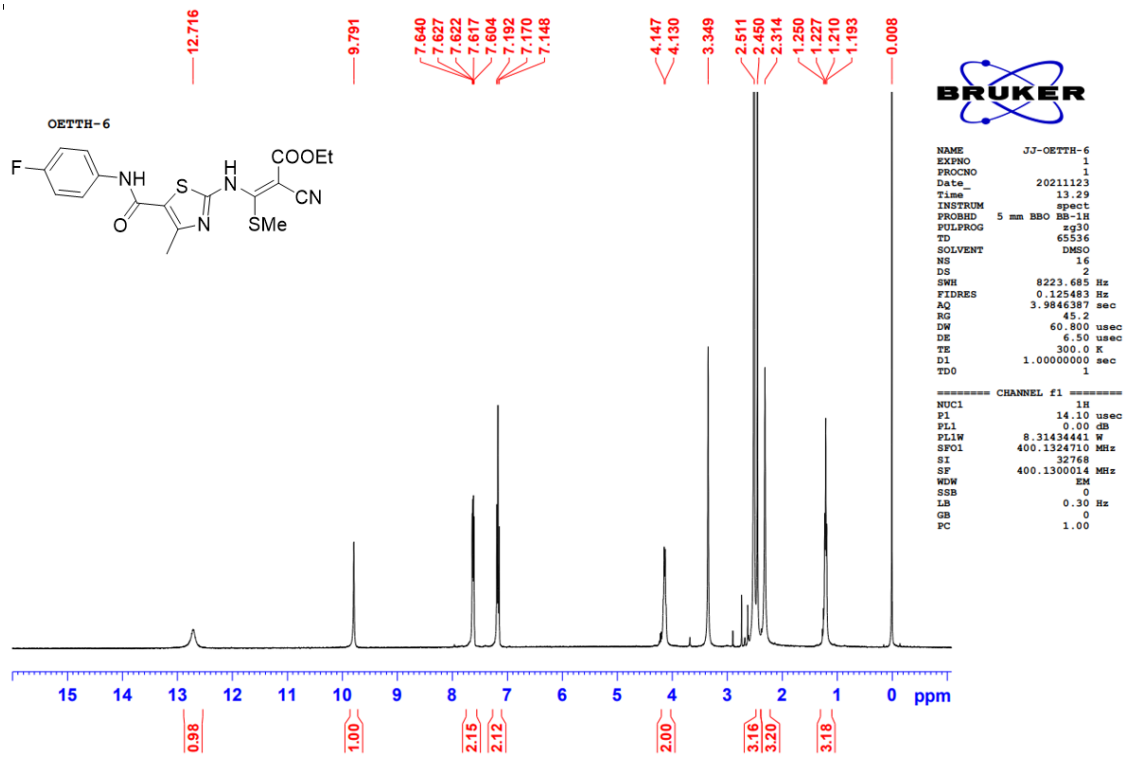


Fig. 16: Representative ^{1}H NMR spectrum of compound OETTH-6

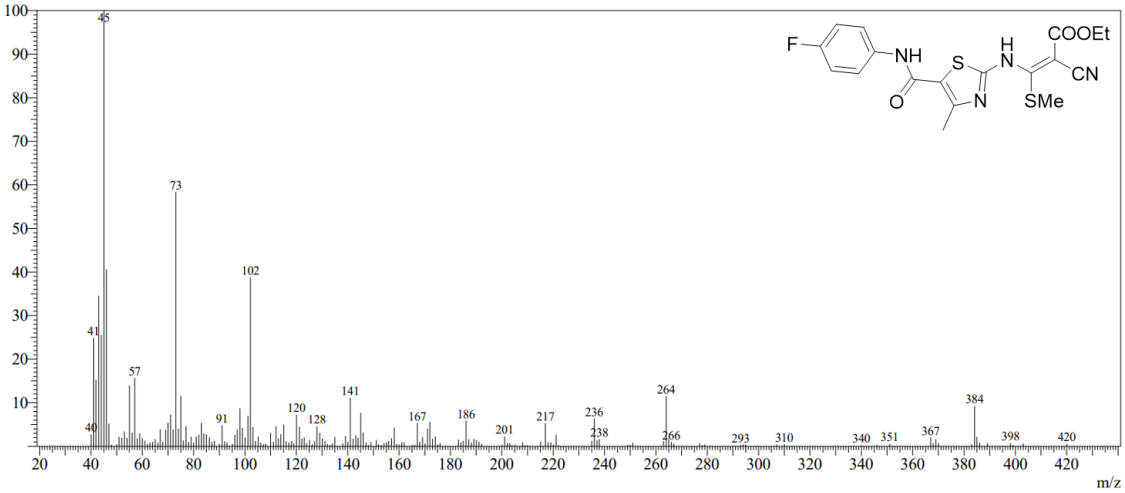


Fig. 17: Representative mass spectrum of compound OETTH-6

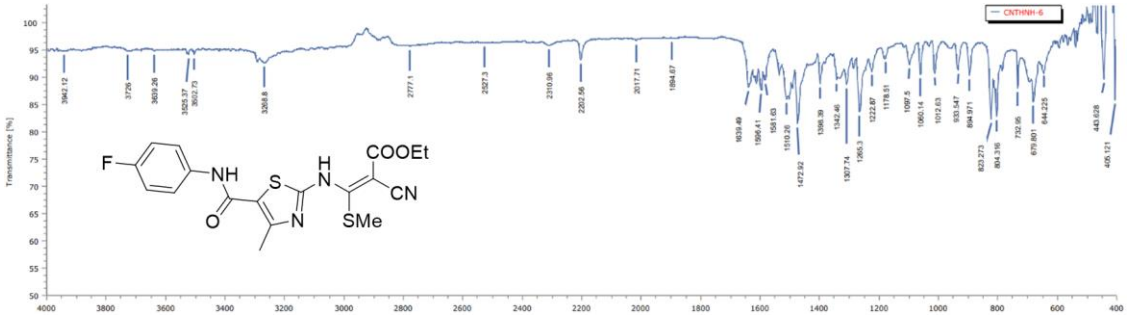


Fig. 18: Representative IR spectrum of compound OETTH-6

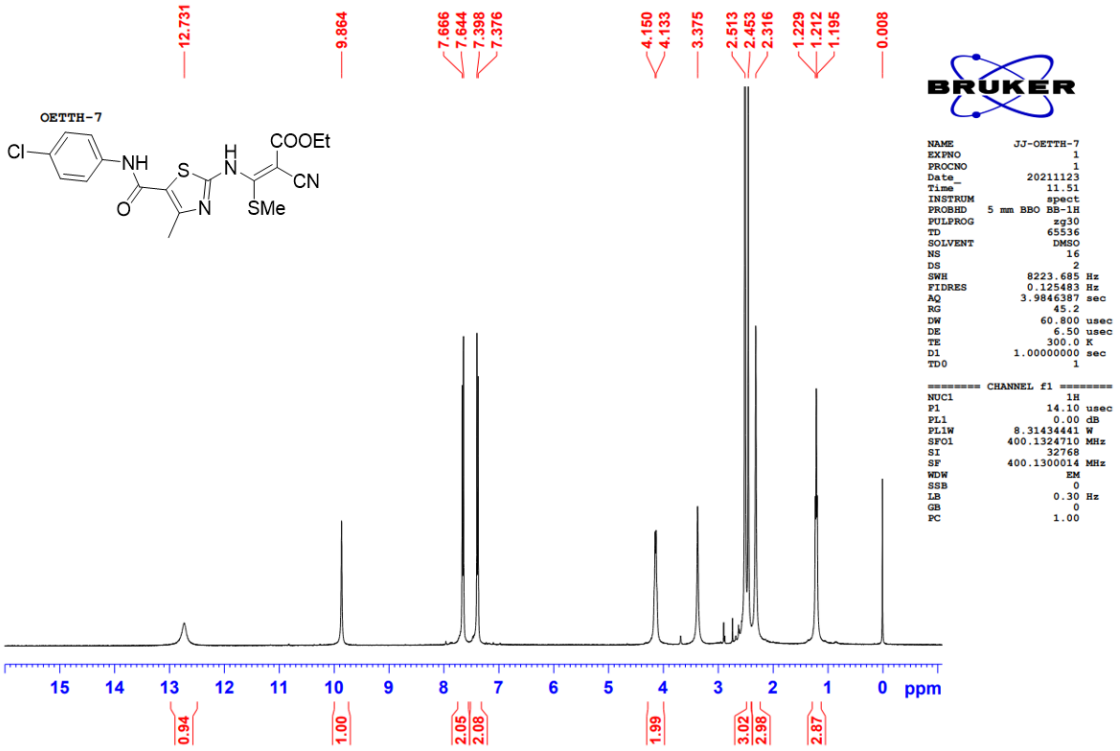


Fig. 19: Representative ^{1}H NMR spectrum of compound OETTH-7

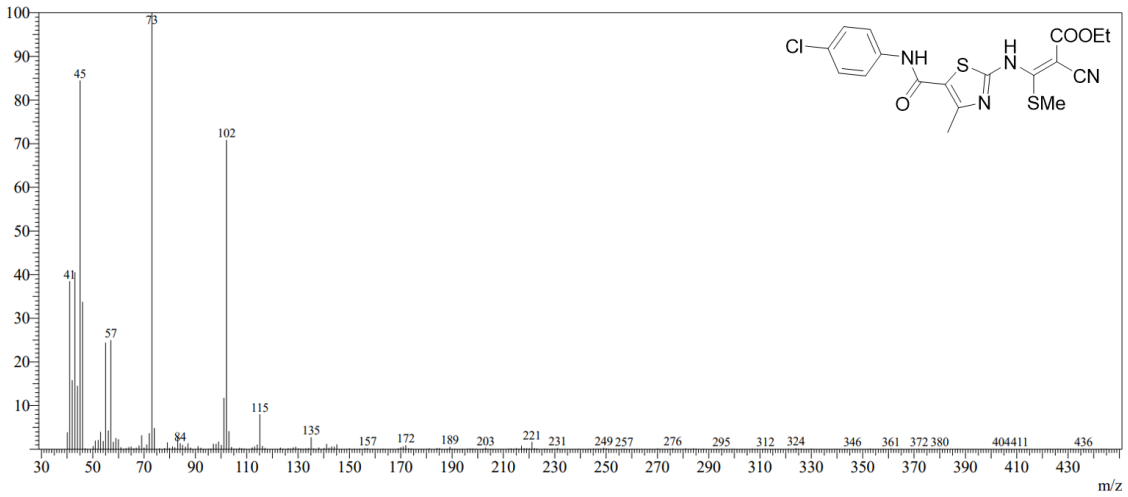


Fig. 20: Representative mass spectrum of compound OETTH-7

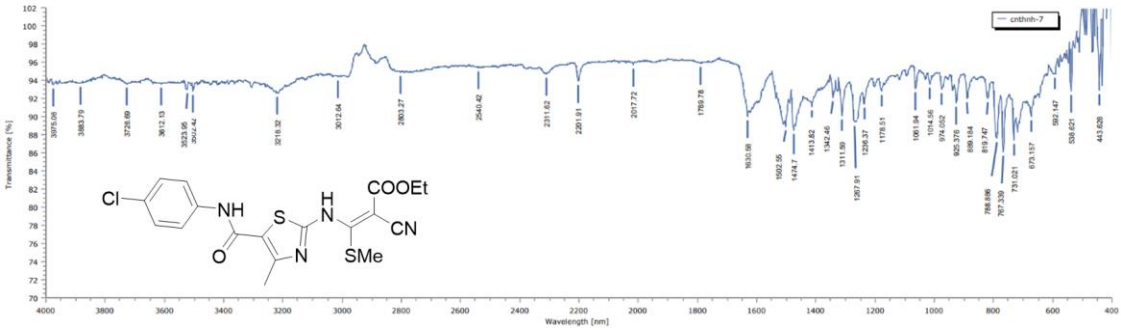


Fig. 21: Representative IR spectrum of compound OETTH-7

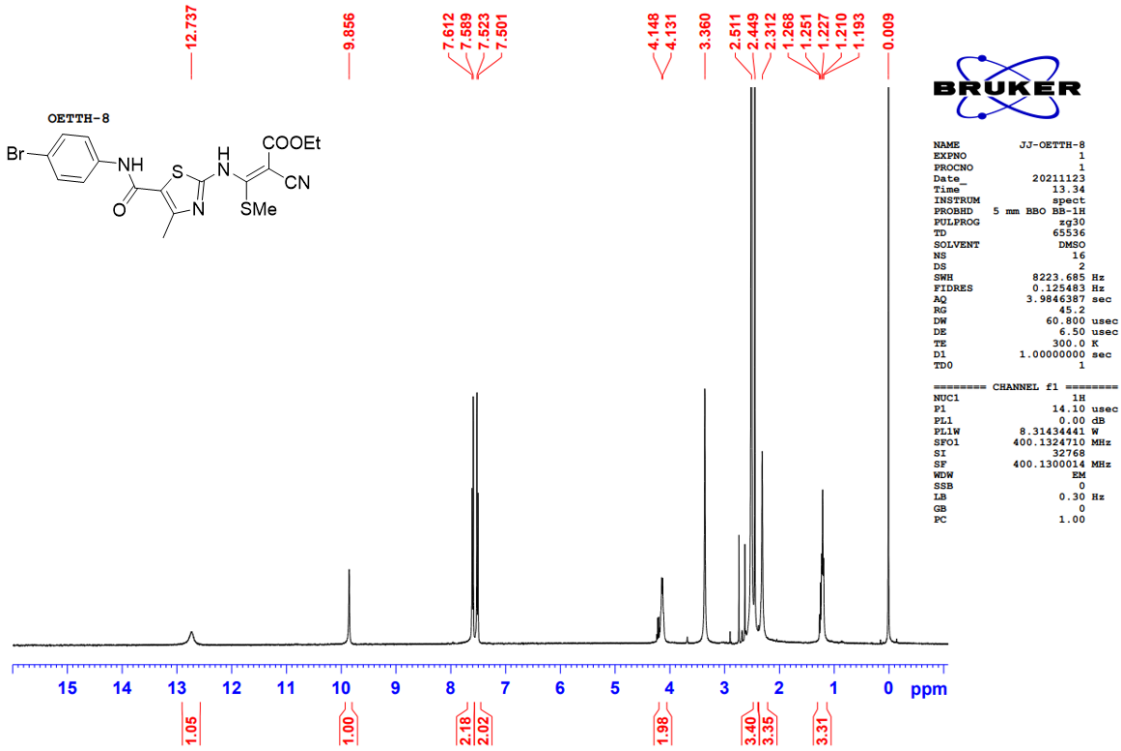


Fig. 22: Representative ^{1}H NMR spectrum of compound OETTH-8

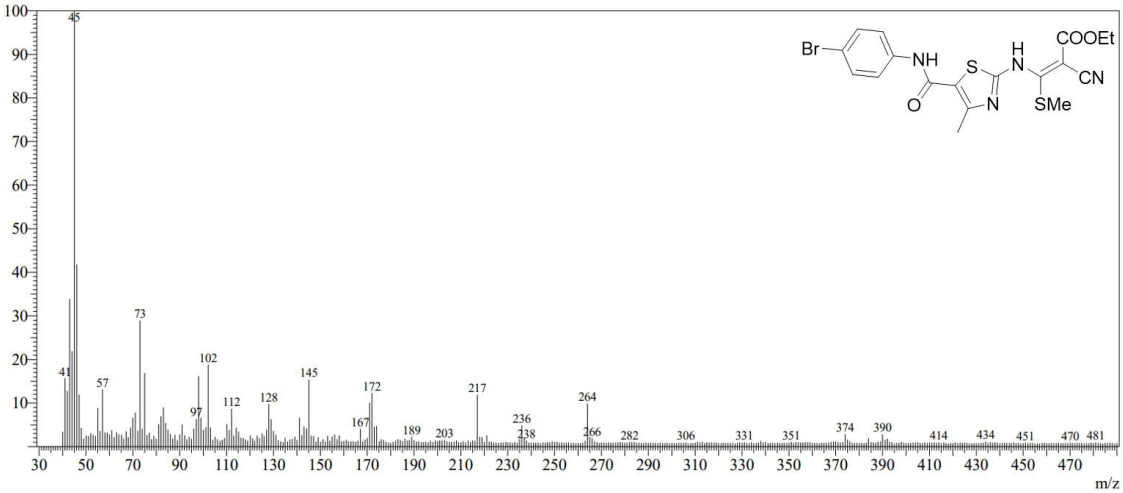


Fig. 23: Representative mass spectrum of compound OETTH-8

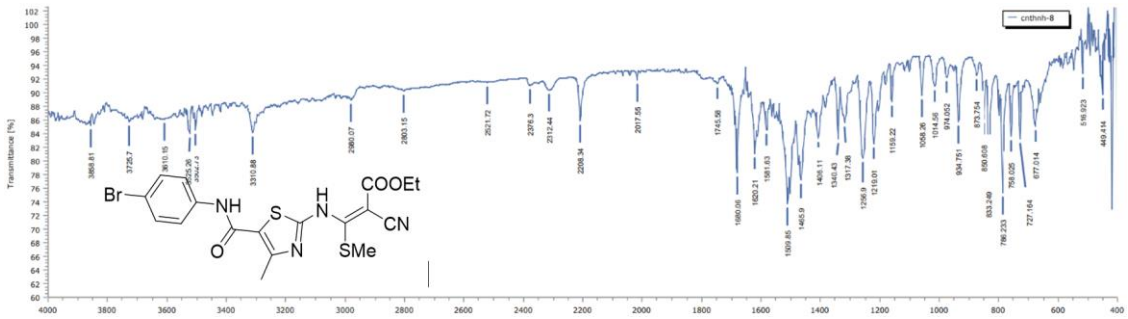


Fig. 24: Representative IR spectrum of compound OETTH-8

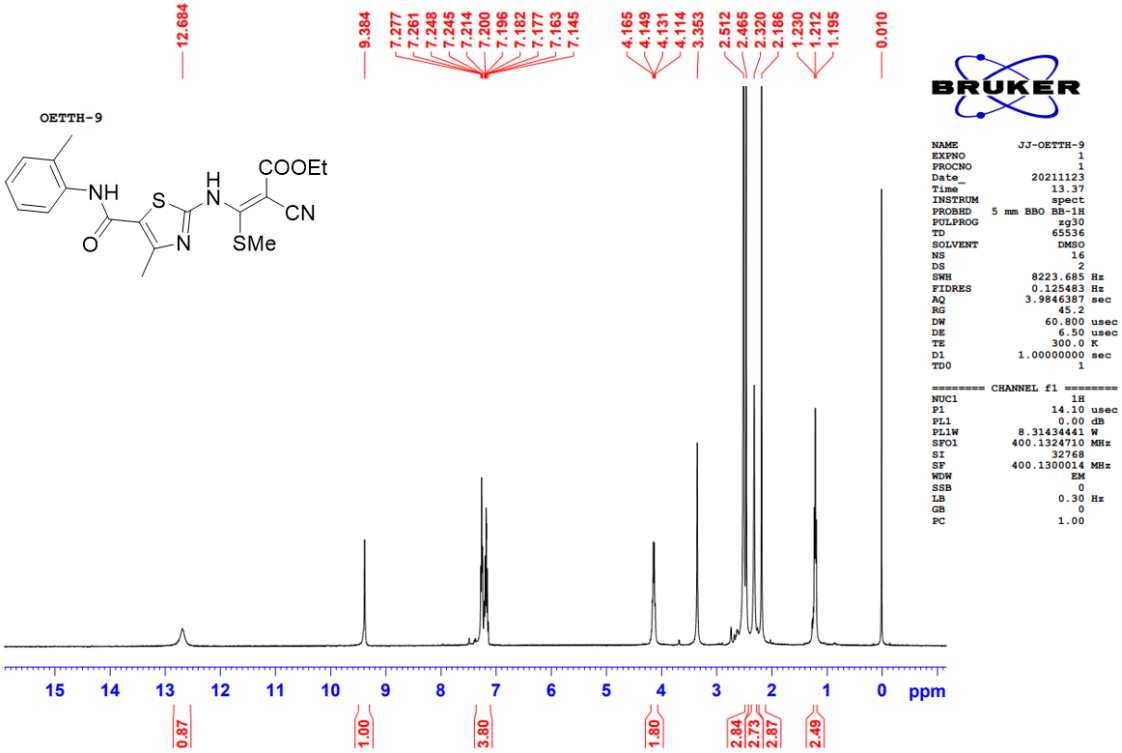


Fig. 25: Representative ^{1}H NMR spectrum of compound OETTH-9

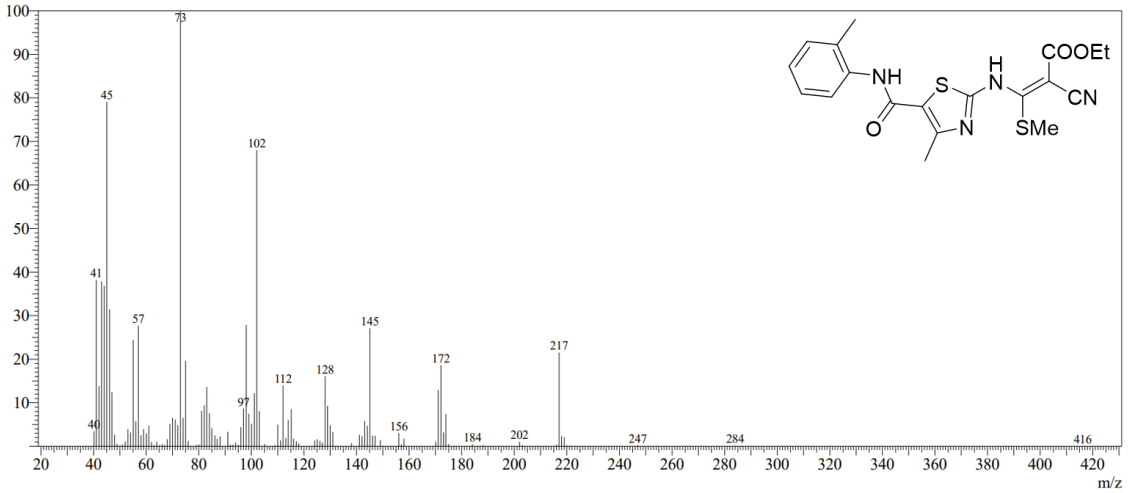


Fig. 26: Representative mass spectrum of compound OETTH-9

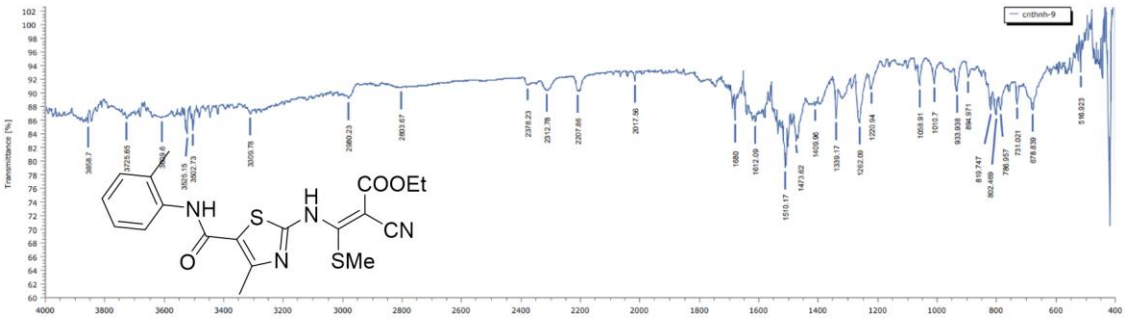


Fig. 27: Representative IR spectrum of compound OETTH-9

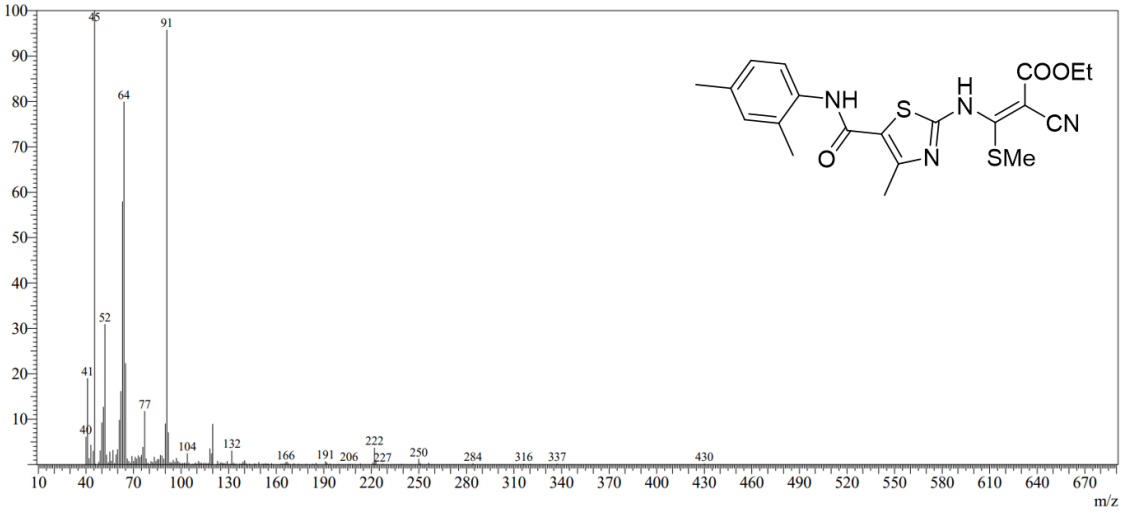


Fig. 28: Representative mass spectrum of compound OETTH-10

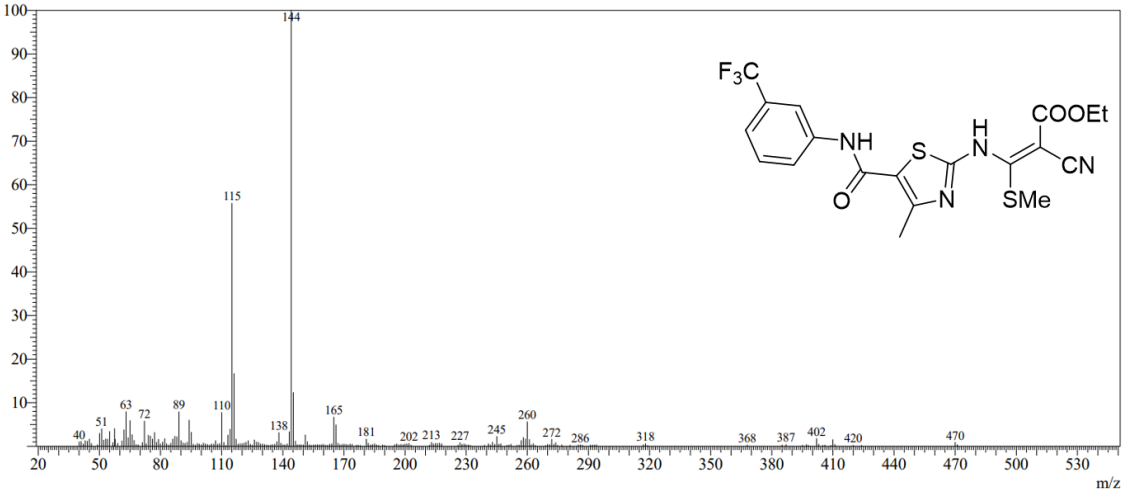


Fig. 29: Representative mass spectrum of compound OETTH-11

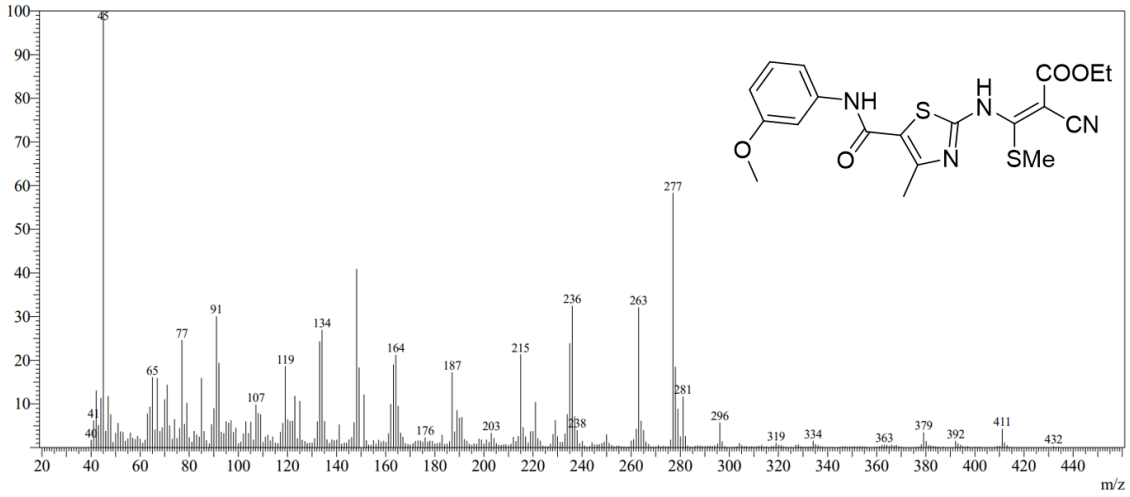


Fig. 30: Representative mass spectrum of compound OETTH-12

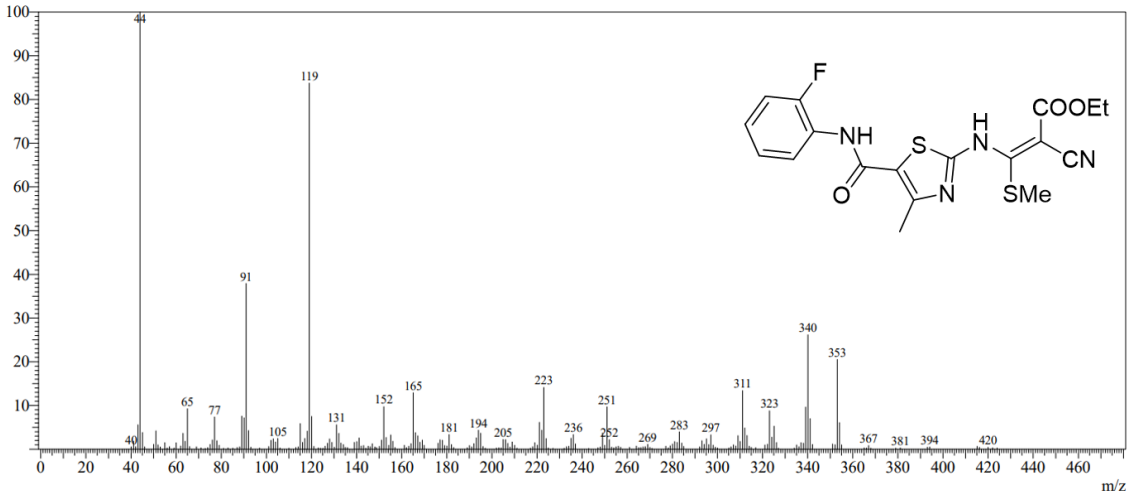


Fig. 31: Representative mass spectrum of compound OETTH-13

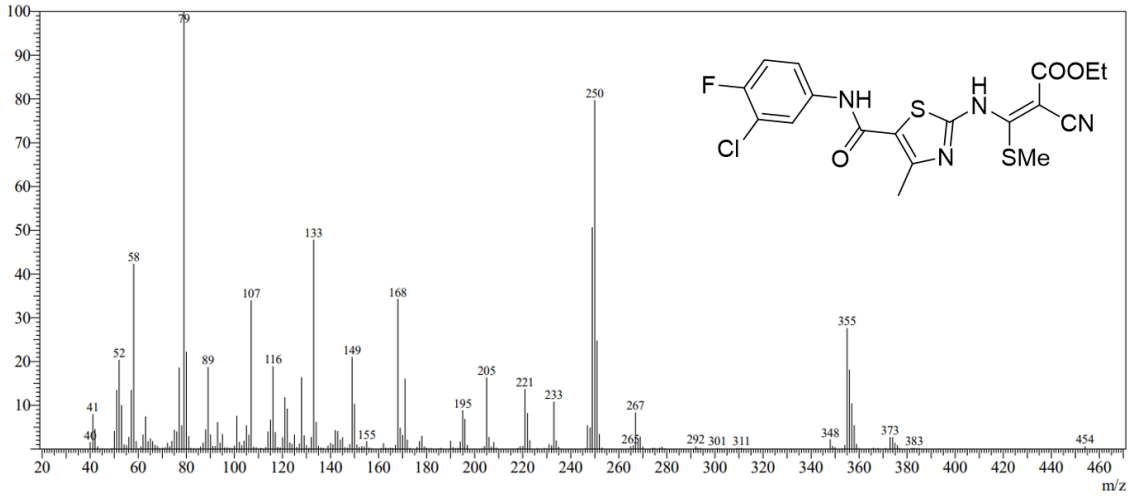


Fig. 32: Representative mass spectrum of compound OETTH-14

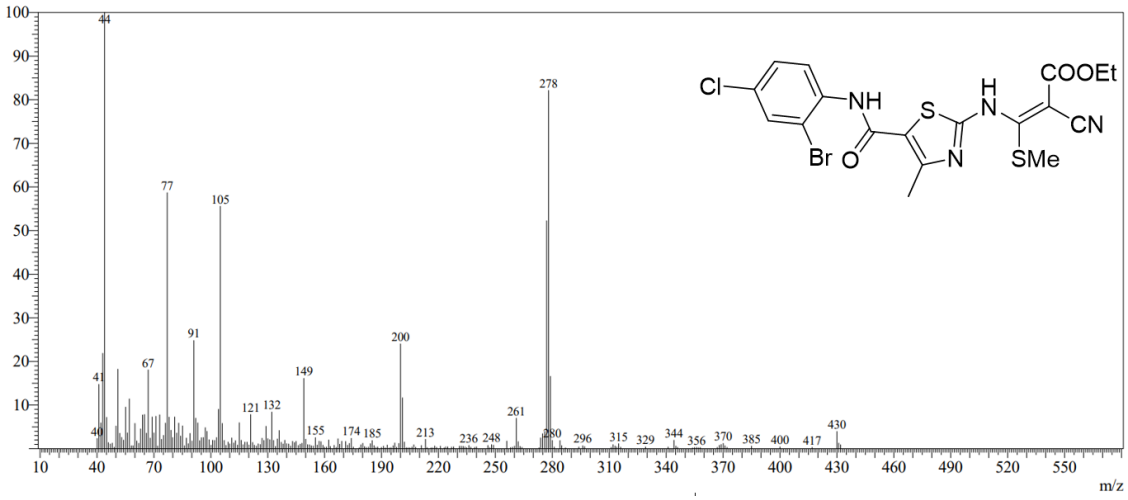


Fig. 33: Representative mass spectrum of compound OETTH-15