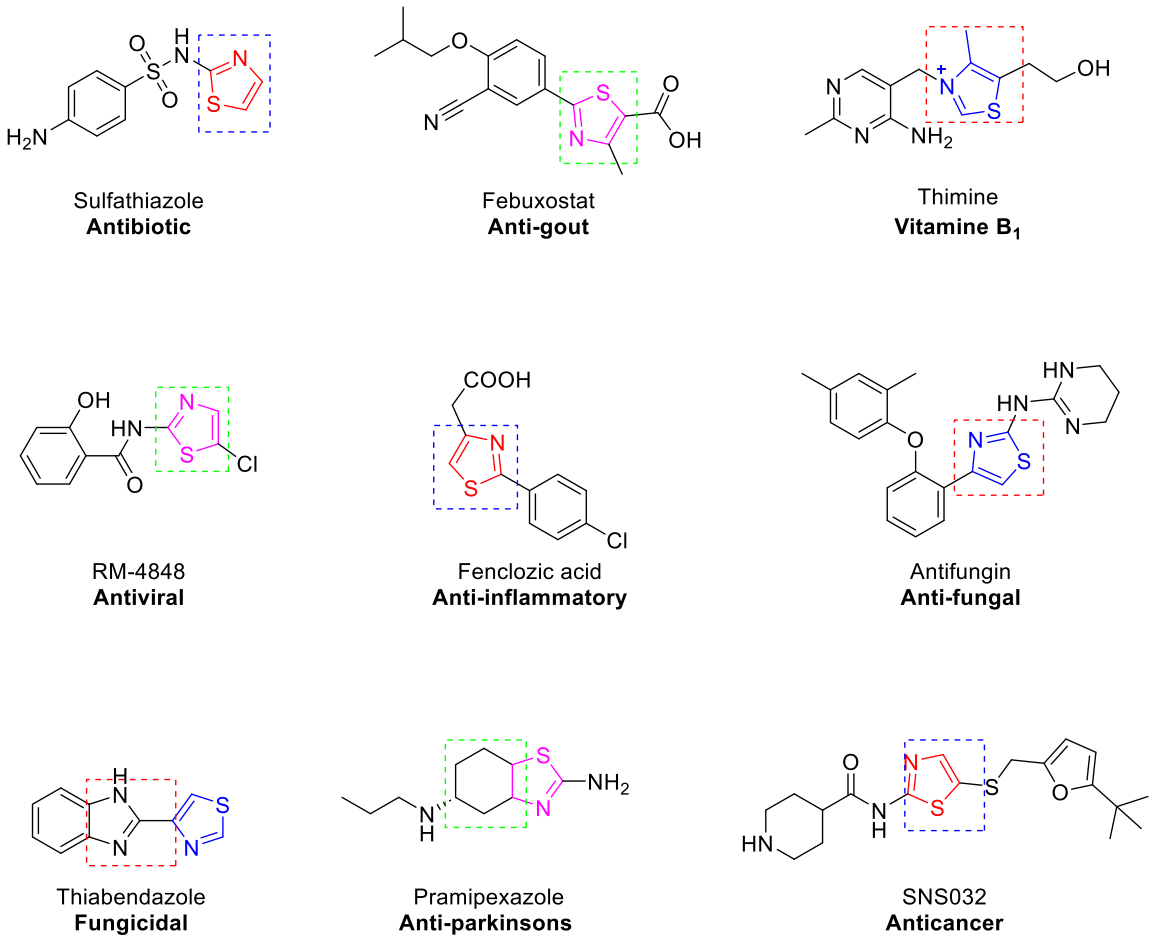
Chapter 3

Synthesis, *In-Vitro* Antimicrobial Evaluation, ADMET Properties and Molecular Docking Studies of Novel Thiazole Derivatives

3.1. Introduction

The treatment of severe diseases brought on by bacteria and fungus poses a substantial challenge to the global community. Antimicrobial resistance (AMR) is a hazard to human life, but it can also be used to treat bacterial and fungal illnesses. To accomplish this, organic and medicinal chemist must deliberately design and create novel medications and tactics. In addition to this, we have chosen thiazole for the creation of fresh AMR Fighting candidates[56].

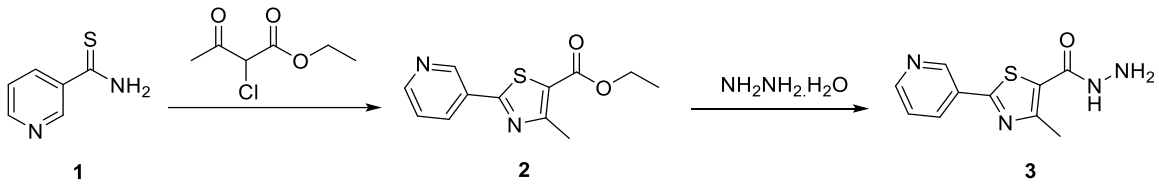


Scheme 1. Several bioactive thiazole

Thiazole is a five-membered heterocyclic ring made up of sulfur and nitrogen atoms, occupies a significant position in chemistry[57]. Due to its numerous uses in variety of liquid crystals[58,59], dyes[60], rubber vulcanization[61], chromophores[62,63], pigments[64], catalysts[65] sunscreens[66] and sensors[67] derivatives of thiazole and its isomers have attracted a great deal of attention recently. The clinical field relies heavily on heterocyclic thiazole molecule to treat a variety of bacterial illnesses in the human body because of its biological activity and availability in nature[68]. Hantzsch and weber were the first authors to provide a detailed explanation of thiazole ring formation[69]. The use of chemoenzymatic one pot multicomponent synthesis has been describe in numerous studies as a classic approach for the synthesizing thiazole[70]. Many natural products, including penicillin and thiamine which have molecules with thiazole moieties as a key structural component[71]. In-depth studies on the thiazole ring during the last three or four decades have demonstrated that it has a number of biologically active qualities, including antiviral[72, 73], antioxidant[74], anticonvulsant[75], antibacterial[76], antitumor[77], anticancer[78–80], antitubercular[81, 82], anti-inflammatory[83, 84], antifungal[85, 86], antimicrobial[87, 88], antiproliferative[89]. The biological activity of 2-amino thiazole is increased due to presence of amino group attached to the ring[90]. **Scheme 1** illustrates several bioactive thiazole molecules, highlighting their potential significance in chemistry. Out continue efforts in search of novel heterocyclic compounds[91, 92] for medicinal interest, based on this, our effort attempted to synthesis novel heterocyclic systems annulated with thiazole ring as core moiety to enhance their biological activity.

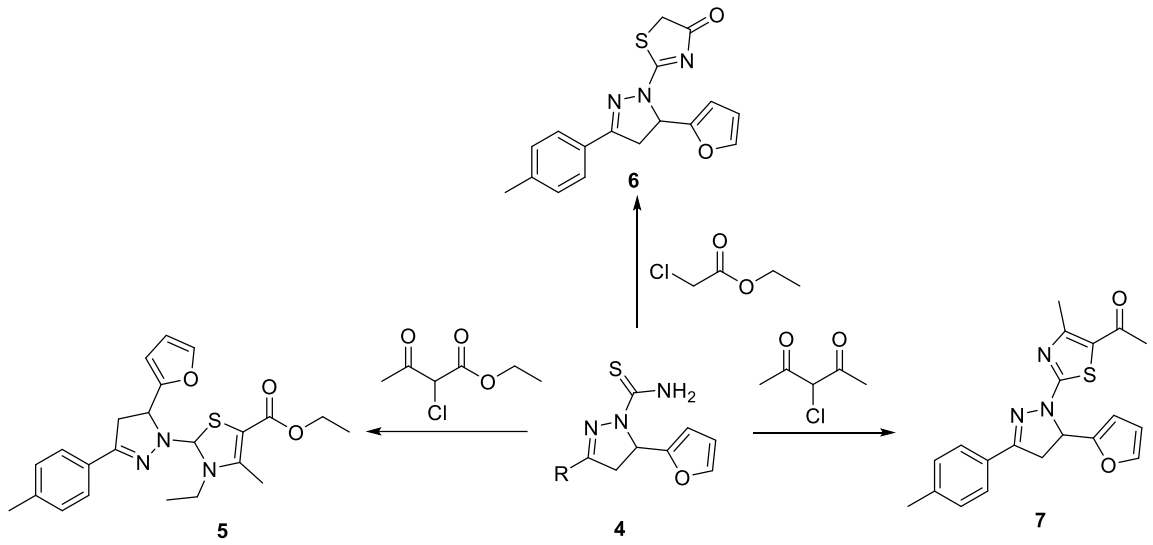
3.1.1. Synthetic methods for substituted thiophene scaffold and its biological significance

Thiazole derivative **3** was produced by a process described by T. Salman *et al*[93], wherein pyridine-3-carbothioamide **1** was reacted with ethyl 2-chloroacetoacetate. Hydrazine hydrate then reacted with compound **2** to synthesized the hydrazide compound **3**. Mild steel inhibitory activity against HCl environment was demonstrated by the synthesized compound **(Scheme 3.1)**.

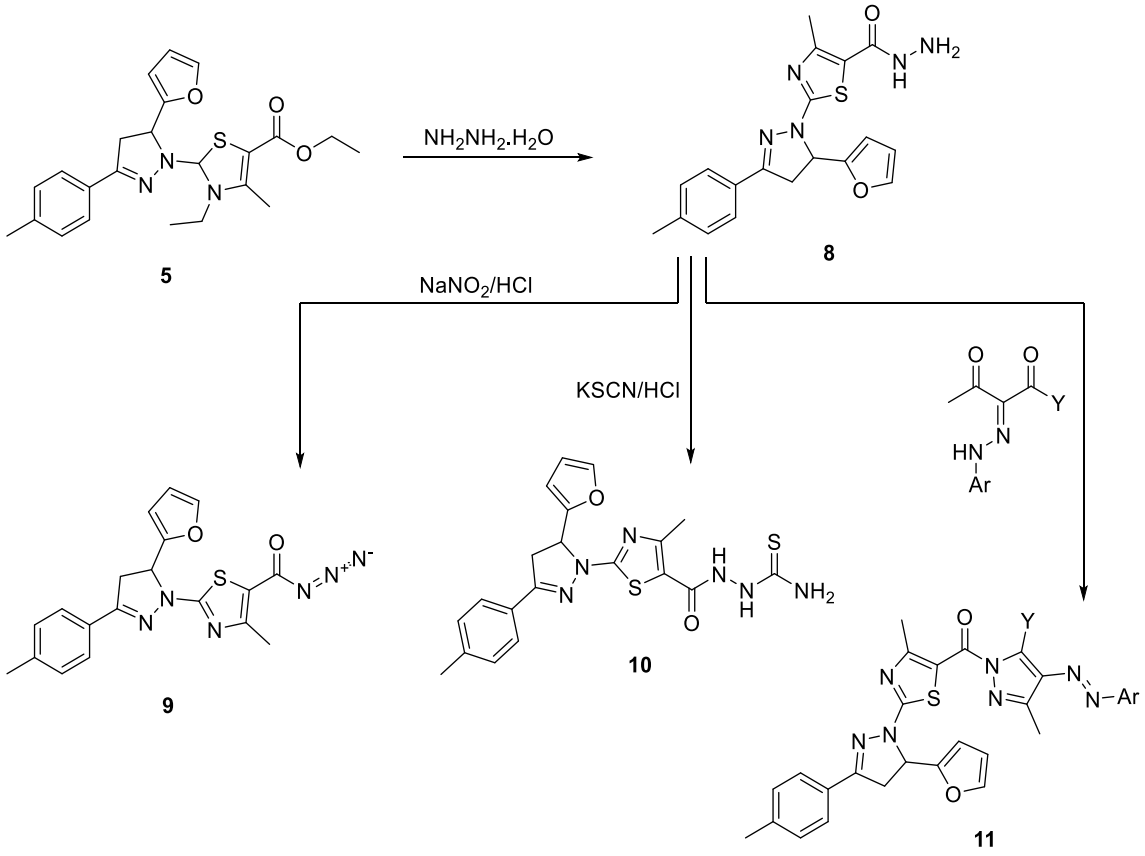


Scheme 3.1

A work by A. Abdelhamid *et al*[94] described the synthesis of 5-(furan-2-yl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide **4** to produce thiazole derivatives. The interaction of compound **4** with ethyl 2-chloro-3-oxobutanoate led to the synthesis of compound **5** in subsequent reactions. In the same way, compound **6** was produced by using ethyl 2-chloroacetate, while compound **7** was produced by using 3-chloropentane-2,4-dione. These processes used a catalytic quantity of triethylamine in ethanol. Additionally, the ester group of compound **5** underwent transformation, leading to the formation of hydrazide **6**. Azide molecule **7** was then created by the reaction of hydrazide **6** with hydrochloric acid and sodium nitrite (**Scheme 3.2**). It interacted with potassium thiocyanate in subsequent processes, which led to the synthesis of the thiourea bond in molecule **7**. The interaction between ethyl 2-(2-arylhydrazono)-3-oxobutanoate and compound **8** yielded compound **11**. As shown in **Scheme 3.3**, the results showed that several of the compounds had modest inhibitory action when compared to gentamycin and ampicillin.



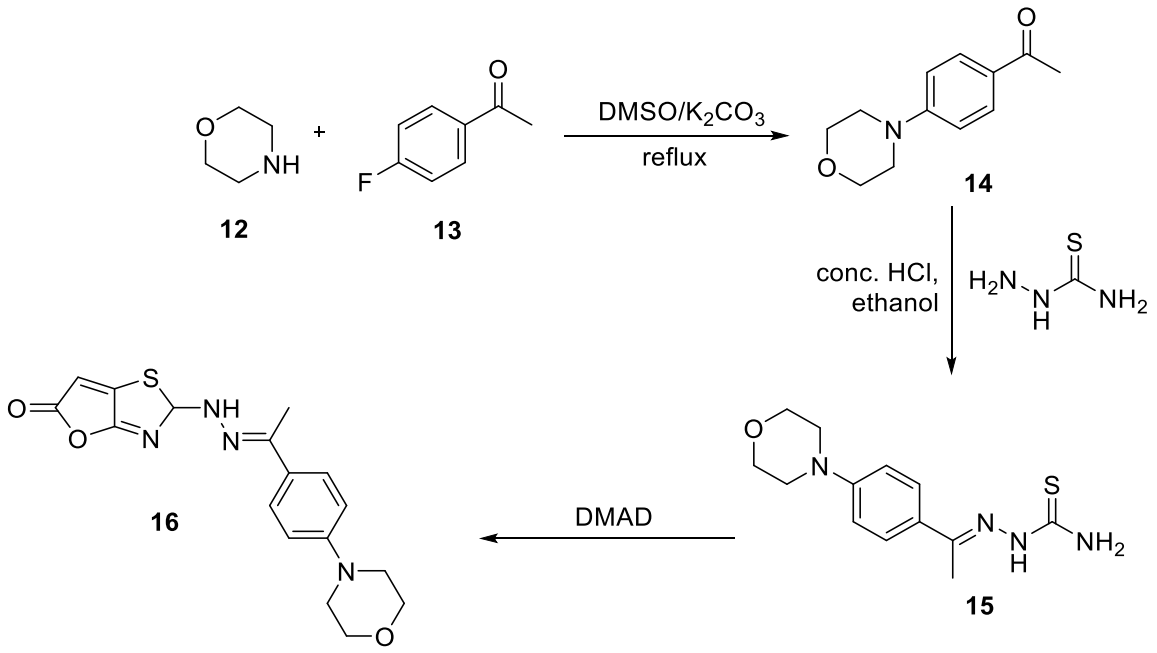
Scheme 3.2



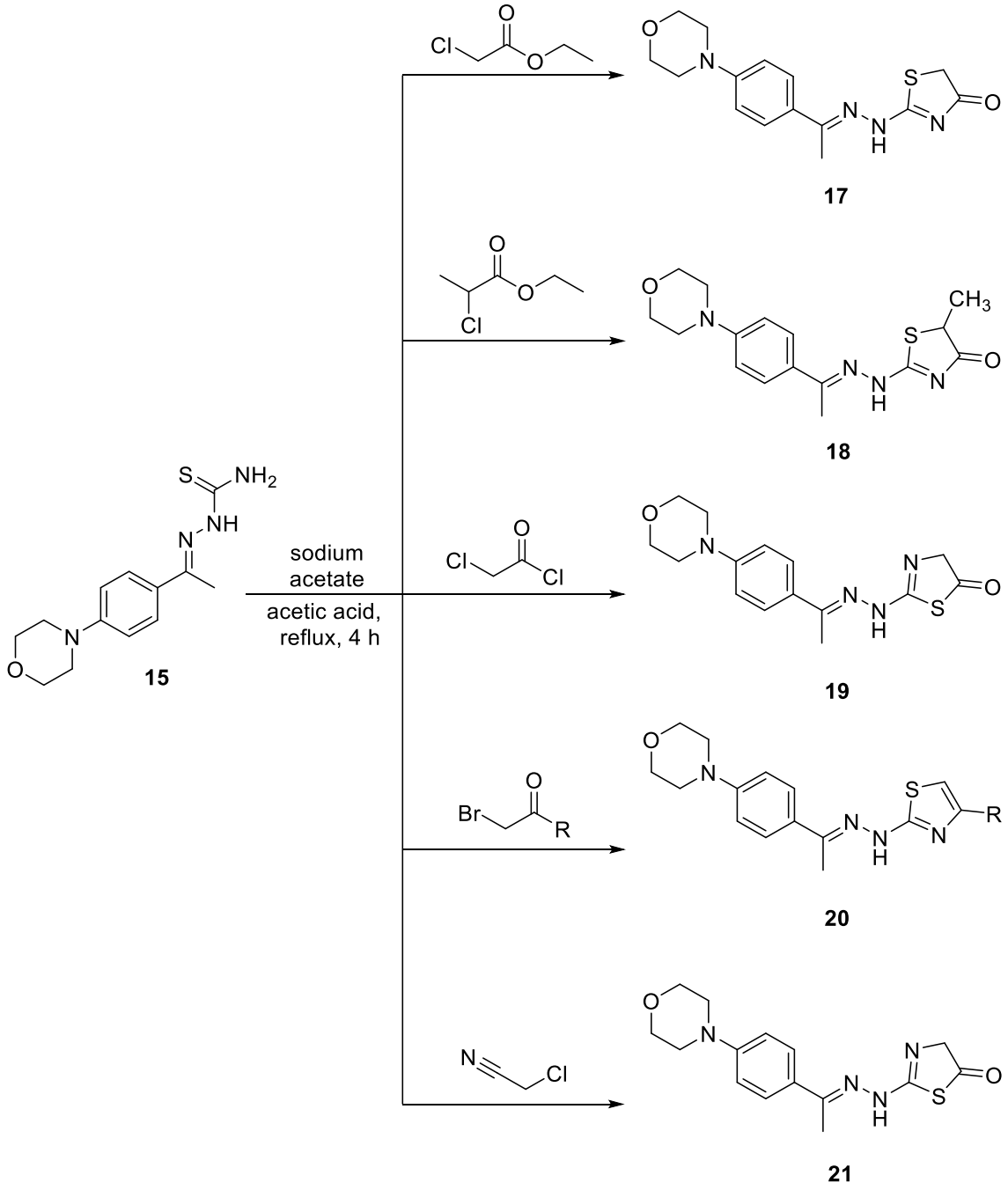
Scheme 3.3

The synthesis of a thiazole derivative **16** was described by M. Helal *et al*[95] and co-workers. The reaction started with morpholine and 4-fluoroacetophenone in DMSO, using

potassium bicarbonate as a catalytic amount. The outcome of this reaction was the synthesis of compound **14**. Compound **14** then reacted further with thiosemicarbazide to synthesized compound **15**. After reacting with dimethyl acetylene dicarboxylate, the resulting compound underwent another reaction that formed fused thiazole ring **16 (Scheme 3.4)**. Following reactions with chloroacetonitrile, phenacyl bromide, ethyl 2-chloro propionate, ethyl chloroacetate, chloroacetone and chloroacetyl chloride, compound **15** form a series of thiazole derivatives known as **17** to **21 (Scheme 3.5)**.



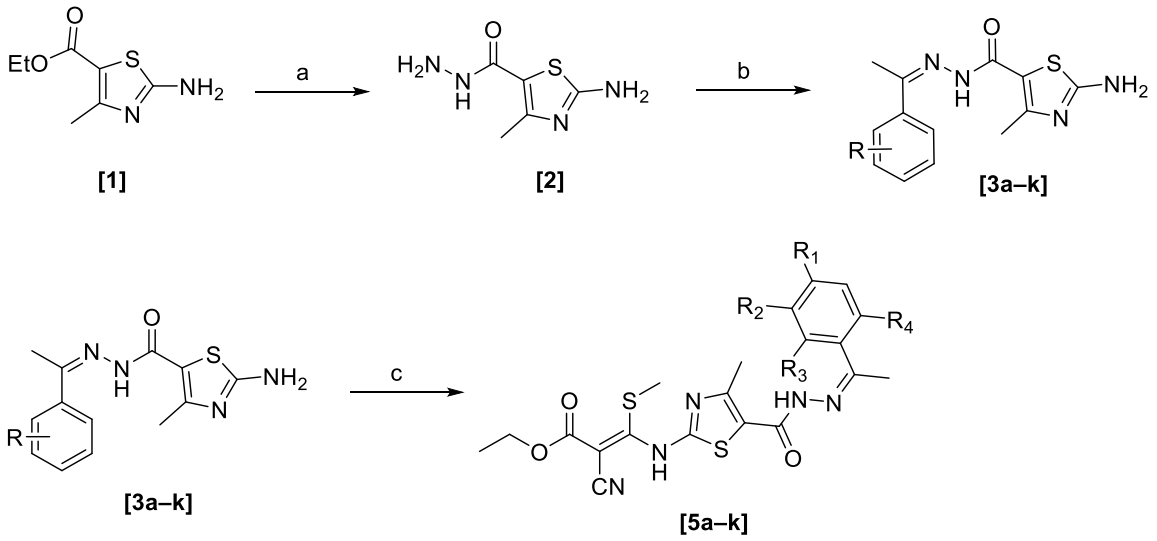
Scheme 3.4



Scheme 3.5

3.2. Results and Discussion

The synthesis of novel and highly functionalized thiazoles containing ketene N, S-acetal, which includes thiomethyl, ethyl ester, nitrile and amide linkage have been shown **Scheme 2**. Initially ethyl 2-amino-4-methylthiazole-5-carboxylate **1** were synthesized by using NBS, ethyl acetoacetate and thiourea or its *N*-substituted derivatives, which is easily available. Next compound 2-amino-4-methylthiazole-5-carbohydrazide **2** were synthesized by reaction of compound **1** with hydrazine hydrate in MeOH at reflux temperature. Next compound (*Z*)-2-amino-4-methyl-*N*'-(1-arylethylidene)thiazole-5-carbohydrazide **3a–k** obtain by reaction of compound **2** with various acetophenone. After that compound (*Z*)-2-amino-4-methyl-*N*'-(1-arylethylidene)thiazole-5-carbohydrazide **3a–k** reacted with ethyl 2-cyano-3,3-bis(methylthio)acrylate **4** and potassium carbonate in DMF to generate desired thiazole derivatives.



(a) $NH_2NH_2.H_2O$, MeOH, reflux, 1h (b) Substituted acetophenone, CH_3COOH, MeOH, reflux, 1h (c) ethyl 2-cyano-3,3-bis(methylthio)acrylate, K_2CO_3, DMF, rt, 1h.

Scheme 2. Reagents and conditions

According to their elemental analyses and spectrum data, new compounds structural assignment was made. We Presents the elemental analysis information as well as some physical characteristics of these novel compounds in which we displayed NMR, Mass, IR spectra of these novel substance. It was determined that compounds **5a–k** are characterized

by a proton singlet of two NH group seen between 10.81–12.77 ppm and triple peaks of methyl proton of ester seen between 1.15–1.23 ppm and between 2.26–2.56 ppm methyl protons detected as singlet. The aromatic region was seen between 6.96–7.85 ppm. Each compound's mass spectra displayed a molecular ion, confirming its molecular weight. The mass spectra showed a molecular ion peak at 444 that corresponded to the molecular formula $C_{20}H_{21}N_5O_3S_2$.

3.2.1. Optimizing reaction conditions

Table 1: Optimization of the reaction conditions

Entry	Solvent	Base	Temp. (C)	Yield (%)	Purification
1	No solvent	-	rt	-	-
2	H_2O	K_2CO_3	90	-	-
3	H_2O	-	rt	-	-
4	MeCN	K_2CO_3	rt	49	Yes
5	MeCN	Et_3N	rt	41	Yes
6	THF	K_2CO_3	rt	38	Yes
7	THF	Et_3N	rt	31	Yes
8	EtOH	K_2CO_3	rt	55	Yes
9	EtOH	Et_3N	rt	41	Yes
10	MeOH	K_2CO_3	rt	50	Yes
11	MeOH	Et_3N	rt	45	Yes
12	Acetone	K_2CO_3	rt	80	Yes
13	Acetone	Et_3N	rt	85	Yes
14	DMF	Et_3N	rt	89	Yes
15	DMF	K_2CO_3	rt	90	No

To improve the experiment condition for the preparation of molecules **5a–k**, different solvents, including acetone, methanol, tetrahydrofuran, ethanol and IPA were utilized with a variety of bases, including piperidine and triethylamine were used. Therefore, we discovered that when potassium carbonate was used with DMF, the reaction between (*Z*)-2-amino-4-methyl-*N*'-(1-arylethylidene)thiazole-5-carbohydrazide **3a–k** and

ethyl 2-cyano-3,3-bis(methylthio)acrylate **4** proceeded more quickly and yielded a satisfactory yield of thiazole derivatives ethyl (*Z*)-2-cyano-3-{[4-methyl-5-(2-((*Z*)-1-arylethylidene)hydrazine-1-carbonyl)thiazol-2-yl]amino}-3-(methylthio)acrylate **5a–k**.

Initially, the reaction was attempted without any solvent or catalyst at room temperature, but no product was formed (**Table 1**, entry 1). As a result, a new experiment was conducted using water as a solvent with potassium carbonate at room temperature, but no product was obtained (entry 2). To further investigate, the reaction was carried out with potassium carbonate as a base and acetonitrile as a solvent, resulting in 49% product yield (entry 4) and 41% yield when triethylamine was used as the base (entry 5). Next, the solvent was changed to tetrahydrofuran with potassium carbonate as a base, yielding a 38% product yield (entry 6) and a 31% yield when triethylamine was used as the base (entry 7). Further optimization was conducted with ethyl alcohol as a solvent and potassium carbonate as a base, leading to a 55% yield (entry 8) and 41% yield with triethylamine as the base (entry 9). When methanol was used as the solvent with potassium carbonate as the base, a product yield of 50% was obtained (entry 10), and a 45% yield was obtained when triethylamine was used as the base (entry 11). Surprisingly, the use of acetone as the solvent and potassium carbonate as the base resulted in an 80% yield (entry 12), and 85% yield was obtained when triethylamine was used as the base (entry 13). When DMF was used with triethylamine, a yield of 89% was obtained (entry 14), and a 90% yield was achieved when potassium carbonate was used as the base and the reaction mixture was stirred at room temperature for 1 hour (entry 15). The results indicate that using potassium carbonate with DMF yielded a satisfactory yield of thiazole derivatives, and the reaction proceeded more rapidly. Utilizing the optimized reaction conditions, our methodology was employed to generate novel thiazole derivatives, as demonstrated in **Table 2**.

3.2.2. Physicochemical characteristeristics

Table 2: Novel thiazole derivative's physicochemical characteristeristics

Entry	R_1	R_2	R_3	R_4	Molecular Formula	Molecular Weight	Yield (%)	Melting Point (°C)
5a	OCH_3	H	H	H	$C_{21}H_{23}N_5O_4S_2$	473.57	89	289–291
5b	Cl	H	H	H	$C_{20}H_{20}ClN_5O_3S_2$	477.98	86	274–276
5c	F	H	H	H	$C_{20}H_{20}FN_5O_3S_2$	461.53	84	255–257
5d	Br	H	H	H	$C_{20}H_{20}BrN_5O_3S_2$	522.44	81	288–291
5e	H	H	H	H	$C_{20}H_{21}N_5O_3S_2$	443.54	85	212–214
5f	CH_3	H	H	H	$C_{21}H_{23}N_5O_3S_2$	457.57	88	236–239
5g	Cl	H	H	Cl	$C_{20}H_{19}C_{l2}N_5O_3S_2$	512.42	83	266–268
5h	H	H	H	OH	$C_{20}H_{21}N_5O_4S_2$	459.54	85	225–227
5i	H	OH	H	H	$C_{20}H_{21}N_5O_4S_2$	459.54	87	230–232
5j	OH	H	H	H	$C_{20}H_{21}N_5O_4S_2$	459.54	88	244–246
5k	NO_2	H	H	H	$C_{20}H_{20}N_6O_5S_2$	488.54	85	255–257

Compound **1** was created using the method describe by meng[96]. We followed the reported procedure[97] to react compound ethyl 2-amino-4-methylthiazole-5-carboxylate **1** with hydrazine hydrate.

3.2.3. Molecular Docking

To determine the potential binding sites of potent molecules and evaluate their affinity, molecular docking using Auto Dock Vina was conducted on *E. coli* dihydropteroate synthase. The crystal structure of *E. coli* dihydropteroate synthase (PDB id 5U0Y) was obtained from the Protein Data Bank. In the context of folic acid synthesis in bacteria such as *E. coli*, dihydropteroate synthase (DHPS) is the protein of interest for molecular docking studies. DHPS is an enzyme that plays a crucial role in the biosynthesis of folate, specifically in the synthesis of dihydropteroate. The docking results revealed hydrogen bonding interactions between the synthesized compounds and several amino acids, including ARG A: 220, ARG A: 235, ARG A:63, ARG A:255, ARG A: 280, THR A: 62, HIS A:257, HIS A:208, SER A:222, ASN A:22, GLY A:250, ALA A:251. The 3D structures of the compounds were energy minimized and used for the docking studies. All newly prepared compounds were subjected to docking, and **Table 3** presents the binding energies, number of H-bond and active site residues. The prepared molecules exhibited favorable binding energies with the target, ranging from –6.5 to –7.3 kJ mol^{-1}. Amongst the various compound, compound **5a** and **5k** exhibited the highest docking score of –7.3. Among the compounds tested, molecule **5a** demonstrated hydrogen bonding interactions with ARG A:63, SER A:222 with docking score of –7.3 (**Fig. 1**), while molecule **5b** exhibited hydrogen bonding with ARG A:235, ARG A:63, THR A:62, ASN A:22. Molecule **5c** formed two hydrogen bonds with ARG A:280, HIS A:208. Furthermore, molecule **5e** formed conventional hydrogen bonds with ARG A:220, ARG A:235, ARG A:63, ARG A:255, THRA:62, while molecule **5f** created three hydrogen bonds with the amino acids ARG A:235, ARG A:63, THRA:62. On other hand, molecule **5g** engaged in a conventional hydrogen bond with ARG B:235.

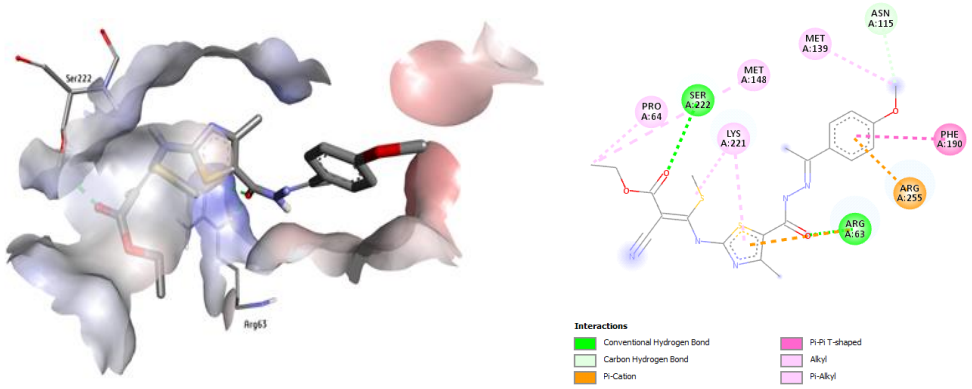


Fig 1. Docking pose of **5a** with *E. coli* dihydropteroate synthase

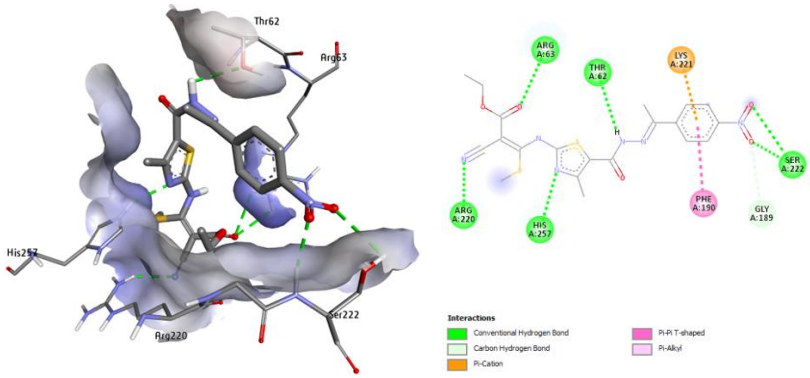


Fig 2. Docking pose of **5k** with *E. coli* dihydropteroate synthase.

Molecule **5h** established two hydrogen bonds with ARG A:280, HIS A:208, while molecule **5i** formed a hydrogen bond with ARG A:63, ARG A:255, THR A:62, HIS A:257. Molecule **5j** demonstrated hydrogen bonding interactions with ARG A:280, HIS A:208, GLY A:250, ALA A:251. The most potent molecule **5k** formed 5 conventional hydrogen bonds with ARG A:63, ARG A:220, THR A:62, SER A:222, HIS A:257 with docking score of –7.3 (**Fig. 2**).

Table 3. Docking of Thiazole Molecule

Compound	Binding energy (KJ/mol)	Active site residues	No. of H-bonds
5a	–7.3	ARG A:63, SER A:222	2
5b	–6.5	ARG A:235, ARG A:63, THR A:62, ASN A:22	4
5c	–6.6	ARG A:280, HIS A:208	2
5e	–6.5	ARG A:220, ARG A:235, ARG A:63, ARG A:255, THRA:62	5
5f	–6.7	ARG A:235, ARG A:63, THRA:62	3
5g	–6.7	ARG B:235	1
5h	–6.6	ARG A:280, HIS A:208	2
5i	–7.1	ARG A:63, ARG A:255, THR A:62, HIS A:257	4
5j	–6.9	ARG A:280, HIS A:208, GLY A:250, ALA A:251	4
5k	–7.3	ARG A:63, ARG A:220, THR A:62, SER A:222, HIS A:257	5

3.2.4. Antimicrobial activity of synthesized compound

The Synthesized molecules **5a**–**k** were screened for potential antimicrobial activity against three fungal strains (*Aspergillus niger, Aspergillus clavatus, Candida albicans*), gram-positive bacteria (*Streptococcus Pyogenes, Staphylococcus Aureus*) and gram-negative bacteria (*Escherichia coli, Pseudomonas Aeruginosa*). The inhibition zone (mm) was tested against ampicillin as a standard for antibacterial activity and nystatin as a standard for antifungal activity. The results of the experiments indicate that the substances tested, with an inhibition zone of 5–24 mm and exhibited substantial action against all species of bacterial and fungal. In comparison with the reference drugs ampicillin and nystatin, the synthesized molecule demonstrated higher and moderate action. The tested compounds antimicrobial activity was determined by using a 100 µg/ml concentration of a selected molecule in the solvent dimethyl sulfoxide (DMSO). **Table 4** presents the results of the antimicrobial activity evaluation for molecules **5a–k**, highlighting their potential as antimicrobial agent. **Figure 3** provides a graphical representation of the antimicrobial activity data.

Table 4. Antimicrobial activity

Antibacterial Activity					Antifungal Activity		
Name	*E. Coli*	*P. Aeruginosa*	*S. Pyognes*	*S. Aureus*	*A. Niger*	*A. Clavatus*	*C. Albicans*
5a	15	14	13	11	21	20	20
5b	8	7	9	8	17	16	15
5c	9	8	9	7	18	19	19
5d	9	10	5	8	19	18	17
5e	7	9	9	10	18	17	17
5f	11	10	10	14	19	22	21
5g	10	12	11	10	22	23	22
5h	9	8	10	9	20	20	21
5i	14	11	12	8	23	21	19
5j	13	14	12	10	22	21	19
5k	15	14	13	11	24	21	20
Ampicillin	19	18	18	16	-	-	-
Nystatin	-	-	-	-	27	25	24

It was noted that, among the compound **5a**–**5d**, Compound **5a** showed good activity against *E. coli*, *P. Aeruginosa*, *S. Pyogenes*. Compound **5b**–**5d** showed moderate activity against *S. Pyogenes*, *S. Aureus*, *E. coli* and *P. Aeruginos.* Compound **5a** showed good antibacterial and antifungal activity, while compound **5b**–**d** showed moderate activity.

It was noted that, among the compounds **5e**–**5h**, Compound **5e** showed moderate activity against *S. Pyogenes*, *S. Aureus*, *E. coli* and *P. Aeruginosa*. Compound **5f** showed moderate activity against *P. Aeruginosa* and good activity *S. Aureus.* Compound **5g** showed good activity against *P. Aeruginosa*. Compound **5h** showed moderate activity against *S. Pyogenes*, *S. Aureus*, *E. coli* and *P. Aeruginosa*. Compound **5f** and **5g** showed good activity against *A. niger*, *A. clavatus*, *C. albican*, while compound **5e** showed moderate activity and compound **5h** showed good antifungal activity.

It was noted that, among the compounds **5i**–**5k**, Compound **5i** showed good activity against *E. coli* and moderate activity against *S. Aureus*. Compound **5j** showed good activity against *S. Pyogenes*, *E. coli*, *P. Aeruginosa* and moderate activity against *S. Aureus.* Compound **5k** showed good activity against *S. Pyogenes*, *S. Aureus*, *E. coli* and *P. Aeruginosa*. Compound **5i**–**5k** showed good antibacterial and antifungal activity.

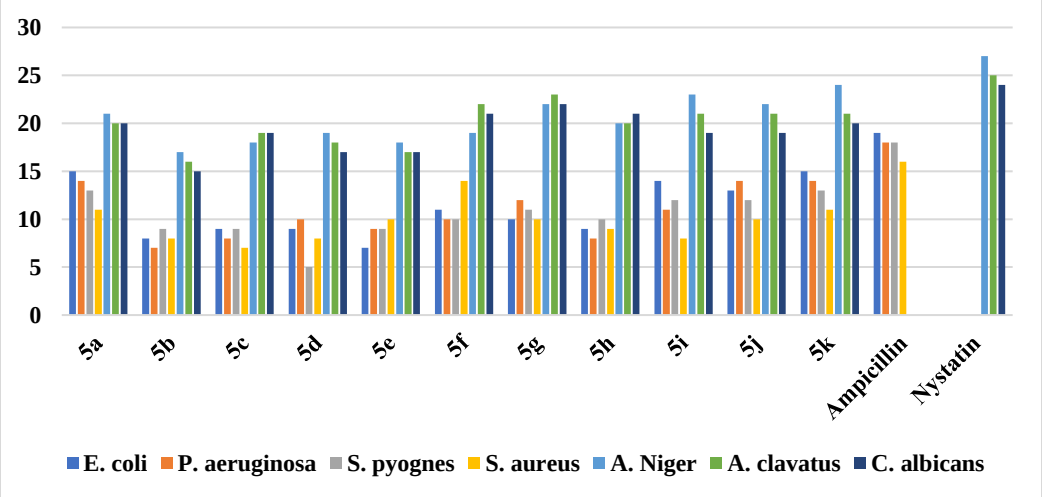


Fig 3. Antimicrobial activity activity of compound **5a–k**

3.2.5. Prediction of the ADMET properties

Many potential drug candidates fail in drug discovery process due to their poor physicochemical and pharmacokinetic properties. These drawbacks could be addressed at early investigation stage by using analysis of newly developed molecules through computational ADMET methods. The drug likeness ADMET parameters such as H-bond acceptor (HBA), H-bond donor (HBD) Topological polar surface area (TPSA), Lipophilicity (Log Po/w), Water Solubility (Log S), Human intestinal absorption (HIA), Rat Oral Acute Toxicity (ROA), Lipinski's Rule of five (RoF (V)), and Synthetic Accessibility (SA) of newly synthesized thiazole derivatives **5a**–**k** have been shown in **Table 5**. The bioavailability of almost all synthesized compounds could be assumed based on their Lipophilicity (Log Po/w) lower than 5 (in the range of 3.19 to 4.25) and water solubility (Log S) higher than –6 (–4.66 to –5.94) except 5g having Log S value 6.57. Almost all the synthesized compound follows Lipinski's Rule of five except 5g because of molecular weight higher than 500. Moreover, complexity of molecular structure of the newly synthesized were assessed through synthetic accessibility and result show that all the thiazole derivatives does not have complex synthetic route based on their score in the range of 2.86–3.00 which is much good value than the standard drug Doxycycline score 4.534. The computational data are carried out using Swiss ADME and ADMET lab 2.0[98].

Table 5. Physicochemical, Pharmacokinetic and Medicinal Chemistry Properties of the synthesized Molecule 5a–k

	Physicochemical Properties						Pharmacokinetics		Medicinal Chemistry	
Compound	**MW**	**HBA**	**HBD**	**TSPA**	**LOG $P_{o/w}$**	**Log S**	**HIA**	**ROA**	**RoF (V)**	**SA**
5a	473.57	7	2	179.24	3.50	−5.79	0.358	0.026	Yes (0)	2.861
5b	477.98	6	2	170.01	4.14	−5.86	0.073	0.029	Yes (0)	2.884
5c	461.53	7	2	170.01	4.05	−5.63	0.197	0.053	Yes (0)	2.889
5d	522.44	6	2	170.01	4.25	−5.94	0.563	0.039	Yes (0)	2.92
5e	443.54	6	2	170.01	3.49	−5.50	0.195	0.022	Yes (0)	2.844
5f	457.57	6	2	170.01	3.80	−5.86	0.244	0.024	Yes (0)	2.873
5g	512.42	6	2	170.01	4.79	−6.57	0.047	0.030	No (0)	3.000
5h	459.54	7	3	190.24	3.19	−5.16	0.326	0.022	Yes (0)	2.992
5i	459.54	7	3	190.24	3.19	−4.66	0.388	0.021	Yes (0)	2.989
5j	459.54	7	3	190.24	3.19	−4.67	0.334	0.022	Yes (0)	2.942
5k	488.54	8	2	215.83	3.40	−5.31	0.240	0.027	Yes (0)	2.973
Doxycycline	444.40	9	6	181.62	–0.35	−2.35	0.022	0.044	Yes (0)	4.534

3.3. Conclusion

This study has designed and synthesized a novel series of thiazole derivatives and characterized through NMR, FTIR and MS spectral analysis. All synthesized compound were tested for their antimicrobial and antifungal activity. Several of the synthetic thiazole compounds exhibited fair to excellent anti-bacterial activity against gram positive bacteria (*S. Pyogenes, S. Aureus*), gram negative bacteria (*E. coli, P. Aeruginosa*) and antifungal activity against *A. niger, A. clavatus, C. albicans.* It was determined that compounds **5a** and **5k** show high anti-bacterial properties and compounds **5g**, **5i** and **5k** show high anti-fungal activity. Our process has the advantages of being simplicity, using affordable reagent and having gentle reaction condition. Molecular docking study demonstrates that compound **5a** and **5k** exhibited the highest docking score of –7.3 and good binding affinities towards *E. coli* dihydropteroate synthase.

3.4. Experimental Section

The electro thermal device with open capillaries was used to determine the melting points, and the values have not been adjusted. To perform thin-layer chromatography, silica-gel 60 F254 precoated plates from Merck were used. The compounds were visualized either with UV light at 254 nm and 365 nm or with iodine vapor. The ATR technique was employed to record the IR spectra using a Shimadzu FT-IR spectrometer. For the ^{1}H spectra, a Bruker AVANCE III (400 MHz) spectrometer was utilized in DMSO-d_6. Chemical shifts are expressed in δ ppm relative to Tetramethylsilane (TMS), which served as the internal standard. To obtain mass spectra, a direct inlet probe was used with a Shimadzu GCMS QP2010 Ultra mass spectrometer. All reactions were conducted under ambient atmospheric conditions, and all reagents were purchased from Loba, SRL, Merk, Spectrochem, Combi-Blocks and CDH and used without further purification.

- **General procedure for synthesis of 2-amino-4-methylthiazole-5-carbohydrazide (2).**

Compound **1** (2.28 g, 20 mmol) was dissolved in 20 ml EtOH, to this hydrazine hydrate (1.5 g, 40 mmol) was added and refluxed for 14 h. After completion of the reaction, the reaction mixture was cooled and solid appeared in the flask was filtered and recrystallized from ethanol to yield pure product.

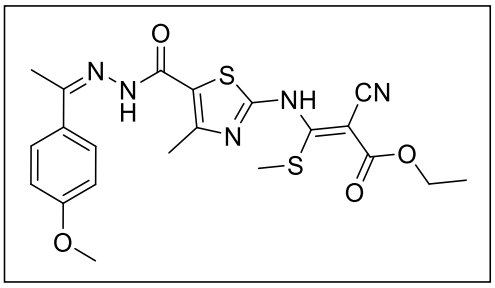
- **General procedure for synthesis of (*Z*)-2-amino-4-methyl-*N*'-(1-arylethylidene)thiazole-5-carbohydrazide (3a–k).**

A mixture of **2** (20 mmol) and substituted acetophenone (20 mmol) in 20 ml MeOH and catalytic quantity of glacial acetic acid was heated to reflux temperature for 1 h. once the reaction is completed. The reaction mixture was cooled to room temperature and poured in to crushed ice. After that neutralized the reaction mixture with dil. HCl. Filtration was used to capture the solid product and washed with water and purified by recrystallization from EtOH to afford pure product.

- **General procedure for synthesis of ethyl (*Z*)-2-cyano-3-{[4-methyl-5-(2-((*Z*)-1-arylethylidene)hydrazine-1-carbonyl)thiazol-2-yl]amino}-3-(methylthio)acrylate (5a–k).**

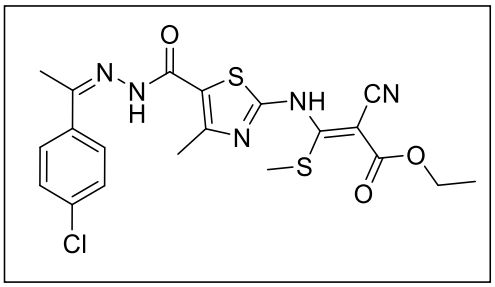
Anhydrous potassium carbonate (20 mmol) was agitated at room temperature for one hour with a combination of (*Z*)-2-amino-4-methyl-*N*'-(1-arylethylidene)thiazole-5-carbohydrazide **3a–k** (20 mmol) and ethyl 2-cyano-3,3-bis(methylthio)acrylate **4** (20 mmol) in 30 mL of DMF. Once the reaction is completed, the suspension had been added to the ice-cold water. The final product was filter and repeatedly washed in cold water and purified by recrystallization from DMF to give yellow color compounds **5a–k**.

Ethyl (*Z*)-2-cyano-3-{[5-(2-((*Z*)-1-(4-methoxyphenyl)ethylidene)hydrazine-1-carbonyl)-4-methylthiazol-2-yl]amino}-3-(methylthio)acrylate (5a).



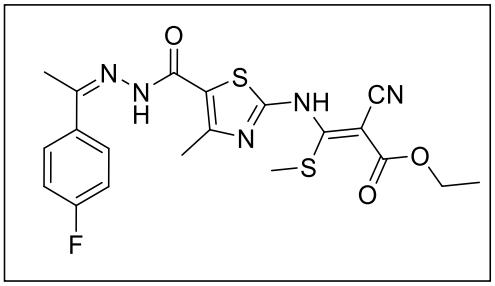
Yield 89%, mp 289–291 °C. IR spectrum, *v*, cm^{-1}: 3155.65 (-NH), 2206.64 (CN), 1697.41 (C=O), 2978.19 (CH_3). ^1H NMR spectrum, *δ*, ppm: 1.18 t (3H, CH_3, CH_2CH_3), 2.26–2.29 s (6H, $2CH_3$, SCH_3, acetophenone), 2.51–2.56 s (3H, CH_3, thiazole), 3.81 s (3H, CH_3, OCH_3), 4.11 q (2H, CH_2), 6.96 d (2H, 2CH, acetophenone), 7.33–7.75 d (2H, 2CH, acetophenone), 10.81 s (1H, NH). Found, %: C 53.31; H 4.81; N 14.91. $C_{21}H_{23}N_5O_4S_2$. Calculated, %: C 53.26; H 4.90; N 14.79. *M* 474.

Ethyl (*Z*)-3-{[5-(2-((*Z*)-1-(4-chlorophenyl)ethylidene)hydrazine-1-carbonyl)-4-methylthiazol-2-yl]amino}-2-cyano-3-(methylthio)acrylate (5b).



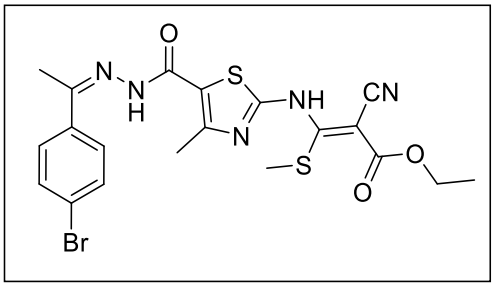
Yield 86%, mp 274–276 °C. IR spectrum, *v*, cm^{-1}: 3155.65 (-NH), 2214.35 (CN), 1735.99 (C=O), 2924.18 (CH_3). ^1H NMR spectrum, *δ*, ppm: 1.19–1.23 t (3H, CH_3, CH_2CH_3), 2.55 s (3H, CH_3, thiazole), 2.86 s (6H, $2CH_3$, SCH_3, acetophenone), 4.12–4.16 q (2H, CH_2), 7.42–7.44 d (2H, 2CH, acetophenone), 7.80–7.82 d (2H, 2CH, C_6H_4), 10.96 s (1H, NH), 12.76 s (1H, NH). Found, %: C 50.41; H 4.15; N 14.61. $C_{20}H_{20}ClN_5O_3S_2$. Calculated, %: C 50.26; H 4.22; N 14.65. *M* 478.

Ethyl (*Z*)-2-cyano-3-{[5-(2-((*Z*)-1-(4-fluorophenyl)ethylidene)hydrazine-1-carbonyl)-4-methylthiazol-2-yl]amino}-3-(methylthio)acrylate (5c).



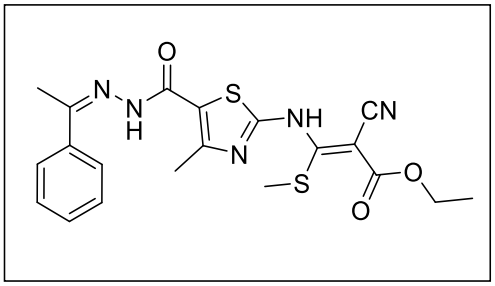
Yield 84%, mp 255–257 °C. IR spectrum, *v*, cm^{-1}: 3152.71 (-NH), 2211.71 (CN), 1732.99 (C=O), 2911.71 (CH_3). ^1H NMR spectrum, *δ*, ppm: 1.12–1.21 t (3H, CH_3, CH_2CH_3), 2.28 s (6H, $2CH_3$, SCH_3, acetophenone), 2.55 s (3H, CH_3, thiazole), 4.09–4.15 q (2H, CH_2), 7.20 s (2H, 2CH, acetophenone), 7.84–7.86 t (2H, 2CH, acetophenone), 10.93 s (1H, NH), 12.75 s (1H, NH). Found, %: C 51.92; H 4.41; N 15.08. $C_{20}H_{20}ClN_5O_3S_2$. Calculated, %: C 52.05; H 4.37; N 15.17. *M* 462.

Ethyl (*Z*)-3-{[5-(2-((*Z*)-1-(4-bromophenyl)ethylidene)hydrazine-1-carbonyl)-4-methylthiazol-2-yl]amino}-2-cyano-3-(methylthio)acrylate (5d).



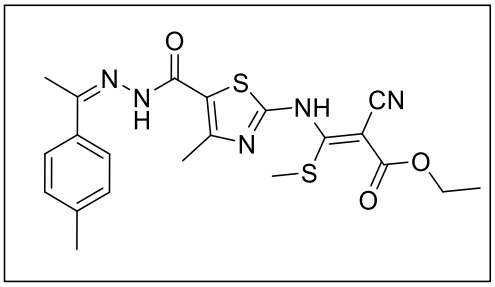
Yield 81%, mp 288–291°C. IR spectrum, *v*, cm^{-1}: 3157.76 (-NH), 2216.46 (CN), 1737.68 (C=O), 2216.46 (CH_3). ^1H NMR spectrum, *δ*, ppm: 1.19–1.24 t (3H, CH_3, CH_2CH_3), 2.28 s (6H, $2CH_3$, SCH_3, acetophenone), 2.50–2.55 s (3H, CH_3, thiazole), 4.13–4.18 q (2H, CH_2), 7.56–7.76 d (2H, 2CH, acetophenone), 7.73–7.75 d (2H, 2CH, acetophenone), 10.96 s (1H, NH), 12.78 s (1H, NH). Found, %: C 46.07; H 3.71; N 13.57. $C_{20}H_{20}BrN_5O_3S_2$. Calculated, %: C 45.98; H 3.86; N 13.41. *M* 522.

Ethyl (*Z*)-2-cyano-3-{[4-methyl-5-(2-((*Z*)-1-phenylethylidene)hydrazine-1-carbonyl)thiazol-2-yl]amino}-3-(methylthio)acrylate (5e).



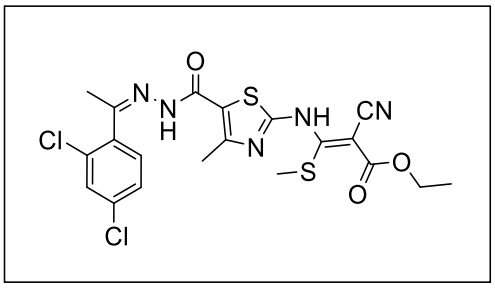
Yield 85%, mp 212–214 °C. IR spectrum, *v*, cm^{-1}: 3152.25 (-NH), 2204.09 (CN), 1695.12 (C=O), 2975.91 (CH_3). ^1H NMR spectrum, *δ*, ppm: 1.15–1.23 t (3H, CH_3, CH_2CH_3), 2.28–2.29 s (6H, $2CH_3$, SCH_3, acetophenone), 2.56 s (3H, CH_3, thiazole), 4.10–4.15 q (2H, 2CH), 7.40 d (2H, 2CH, acetophenone), 7.78 d (2H, 2CH, acetophenone), 10.89 s (1H, NH), 12.71 s (1H, NH). Found, %: C 54.31; H 4.71; N 15.88. $C_{20}H_{21}N_5O_3S_2$. Calculated, %: C 54.16; H 4.77; N 15.79. *M* 444.

Ethyl (*Z*)-2-cyano-3-{[4-methyl-5-(2-((*Z*)-1-(p-tolyl)ethylidene)hydrazine-1-carbonyl)thiazol-2-yl]amino}-3-(methylthio)acrylate (5f).



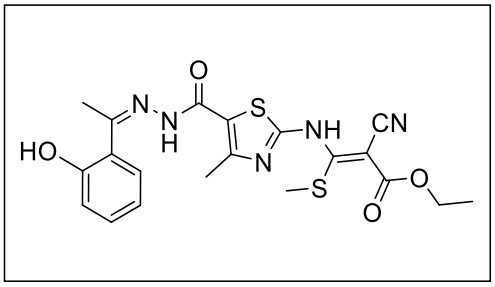
Yield 88%, mp 236–239 °C. IR spectrum, *v*, cm^{-1}: 3163.36 (-NH), 2214.35 (CN), 1735.99 (C=O), 2924.18 (CH_3). Found, %: C 55.21; H 5.01; N 15.23. $C_{21}H_{23}N_5O_3S_2$. Calculated, %: C 55.12; H 5.07; N 15.31. *M* 458.

Ethyl (*Z*)-2-cyano-3-{[5-(2-((*Z*)-1-(2,4-dichlorophenyl)ethylidene)hydrazine-1-carbonyl)-4-methylthiazol-2-yl]amino}-3-(methylthio)acrylate (5g).



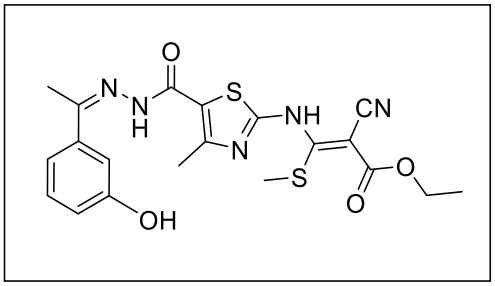
Yield 83%, mp 266–268 °C. IR spectrum, *v*, cm^{-1}: 3160.89 (-NH), 2217.54 (CN), 1738.21 (C=O), 2929.52 (CH_3). Found, %: C 46.69; H 3.79; N 13.61. $C_{20}H_{19}Cl_2N_5O_3S_2$. Calculated, %: C 46.88; H 3.74; N 13.67. *M* 512.

Ethyl (*Z*)-2-cyano-3-{[5-(2-((*Z*)-1-(2-hydroxyphenyl)ethylidene)hydrazine-1-carbonyl)-4-methylthiazol-2-yl]amino}-3-(methylthio)acrylate (5h).



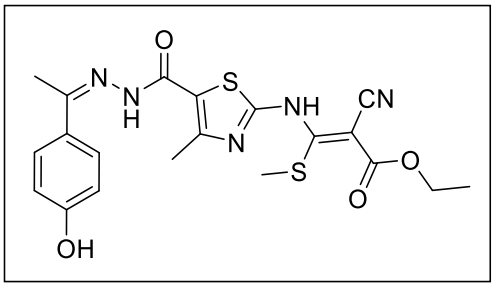
Yield 85%, mp 225–227 °C. IR spectrum, *v*, cm^{-1}: 3170.52 (-NH), 2211.78 (CN), 3036.85 (CH_3). Found, %: C 52.21; H 4.49; N 15.34. $C_{20}H_{21}N_5O_4S_2$. Calculated, %: C 52.27; H 4.61; N 15.24. *M* 459.

Ethyl (*Z*)-2-cyano-3-{[5-(2-((*Z*)-1-(3-hydroxyphenyl)ethylidene)hydrazine-1-carbonyl)-4-methylthiazol-2-yl]amino}-3-(methylthio)acrylate (5i).



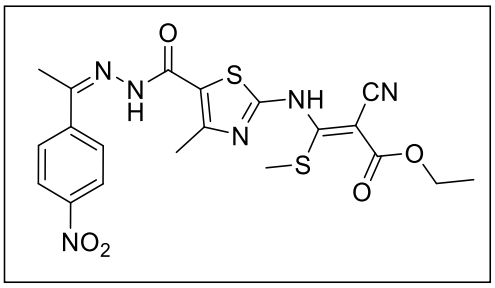
Yield 87%, mp 230–232 °C. IR spectrum, *v*, cm^{-1}: 3175.74 (-NH), 2217.23 (CN), 3041.25 (CH_3). Found, %: C 52.39; H 4.53; N 15.31. $C_{20}H_{21}N_5O_4S_2$. Calculated, %: C 52.27; H 4.61; N 15.24. *M* 459.

Ethyl (*Z*)-2-cyano-3-{[5-(2-((*Z*)-1-(4-hydroxyphenyl)ethylidene)hydrazine-1-carbonyl)-4-methylthiazol-2-yl]amino}-3-(methylthio)acrylate (5j).



Yield 88%, mp 244–246 °C. IR spectrum, *v*, cm^{-1}: 3171.08 (-NH), 2214.35 (CN), 3039.91 (CH_3). Found, %: C 52.41; H 4.49; N 15.33. $C_{20}H_{21}N_5O_4S_2$. Calculated, %: C, 52.27; H, 4.61; N, 15.24. *M* 459.

Ethyl (*Z*)-2-cyano-3-{[4-methyl-5-(2-((*Z*)-1-(4-nitrophenyl)ethylidene)hydrazine-1-carbonyl)thiazol-2-yl]amino}-3-(methylthio)acrylate (5k).



Yield 85%, mp 255–257 °C. IR spectrum, *v*, cm^{-1}: 3166.81 (-NH), 2217.21 (CN), 2927.37 (CH_3). Found, %: C, 49.21; H, 4.25; N, 17.11. $C_{20}H_{20}N_6O_5S_2$. Calculated, %: C, 49.17; H, 4.13; N, 17.20. *M* 488.

3.4.1. Experimental protocol of molecular docking study

The design of ligand structures was done using The ChemSketch 2022.2.3. Furthermore, the docking investigations were also conducted using Autodock Vina 1.5.7[99]. The PDB database was used to download *E. coli* dihydropteroate synthase (5U0Y). To ensure that the structural receptor was free of any ligand before docking, heteroatoms were excluded. In order to prepare the protein, kollaman charge and polar hydrogens were added, and water was removed. The grid box sizes for x, y, and z were set to 40, 40, and 40 Å, respectively. The grid center for x, y, and z were set to 17.838, –1.962, and 7.511. The exhaustiveness was equal to 40, and the spacing among grid points was 0.375 angstroms. The probable binding mode was determined using Discovery studio v21.1.0.20298[100].

3.5. Spectral Data

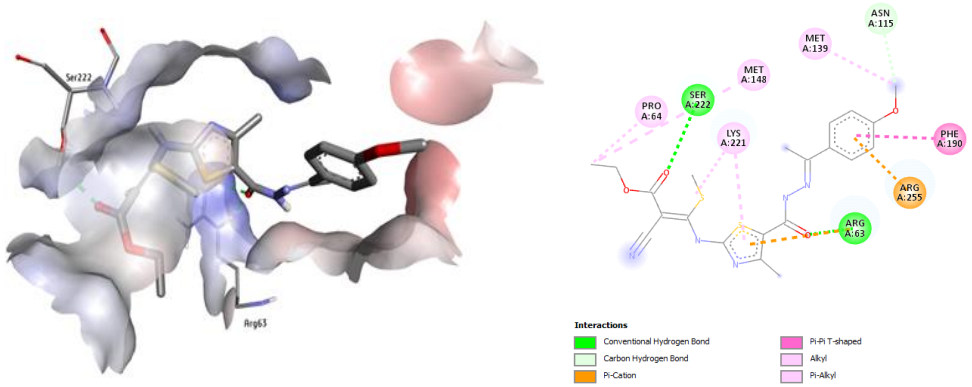


Fig. 1: Representative 2D & 3D molecular docking data of compound **JTAE-1**

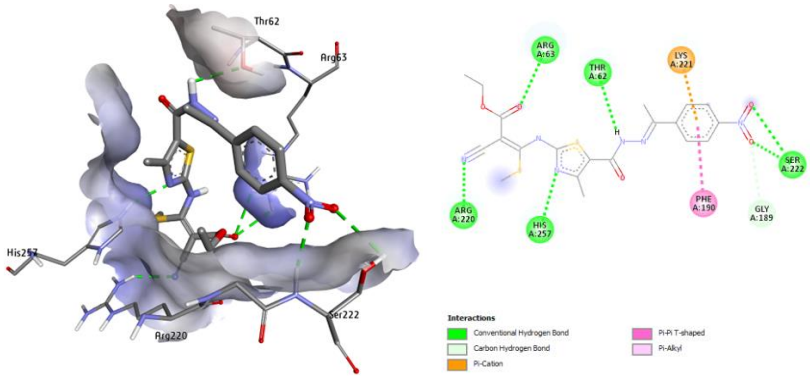


Fig. 2: Representative 2D &3D molecular docking data of compound **JTAE-11**

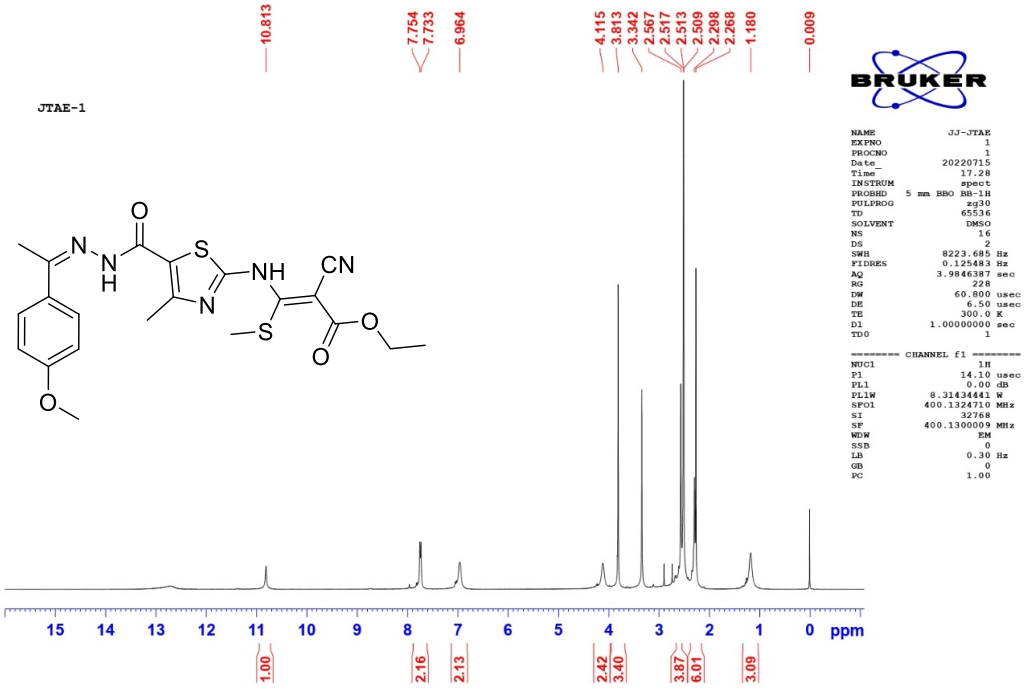


Fig. 3: Representative ^{1}H NMR spectrum of compound **JTAE-1**

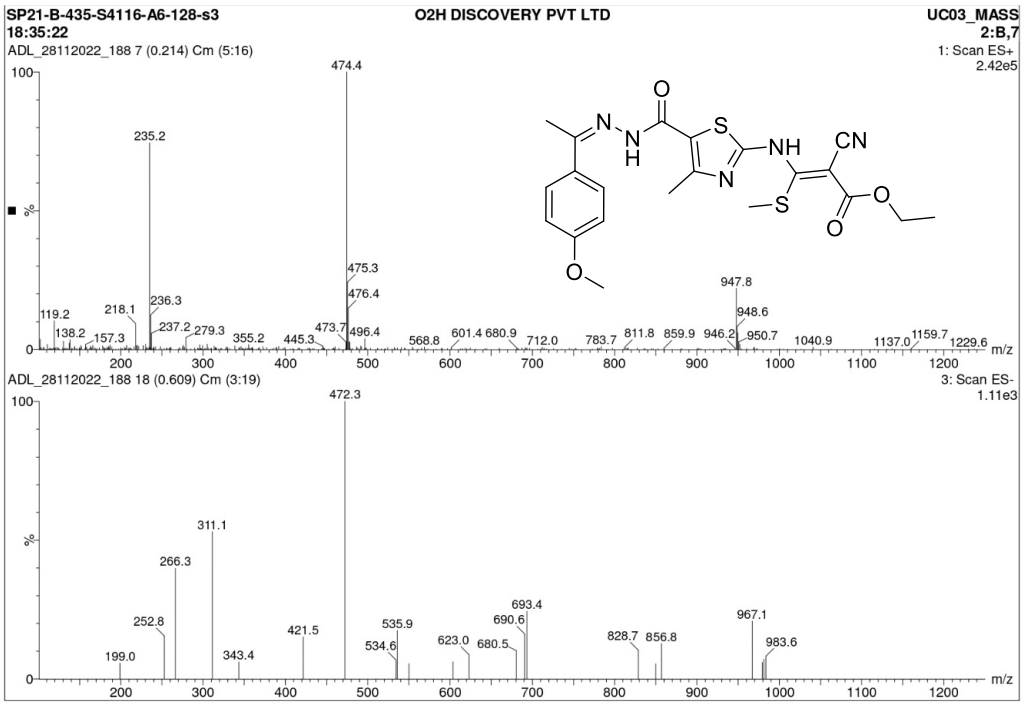


Fig. 4: Representative mass spectrum of compound **JTAE-1**

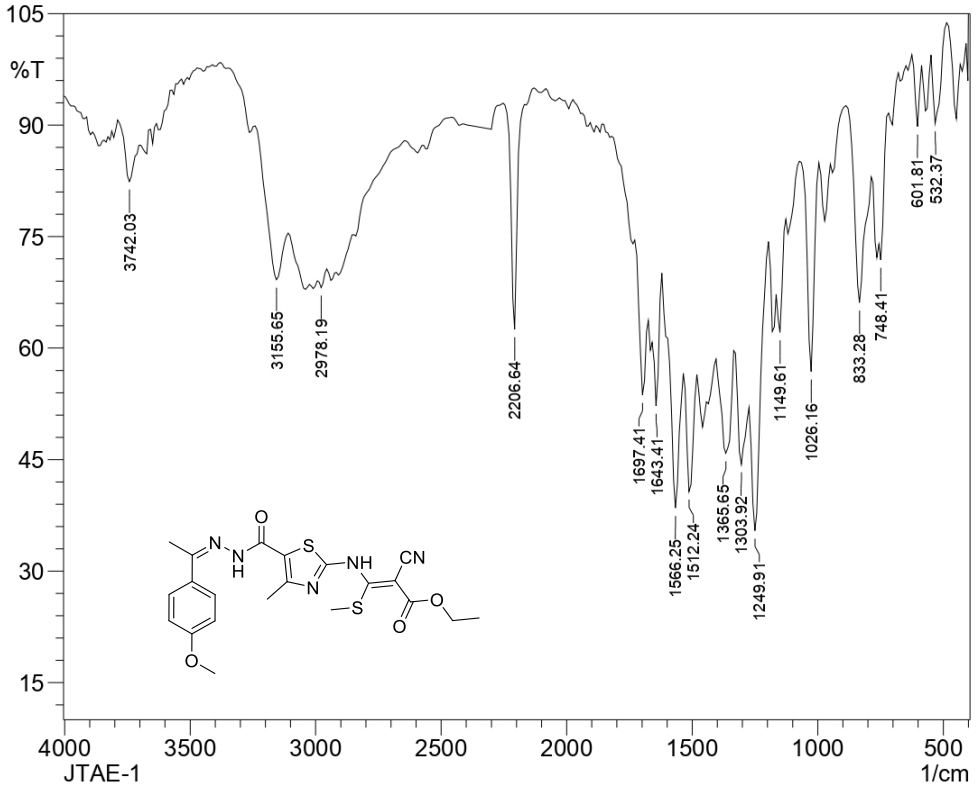


Fig. 5: Representative IR spectrum of compound **JTAE-1**

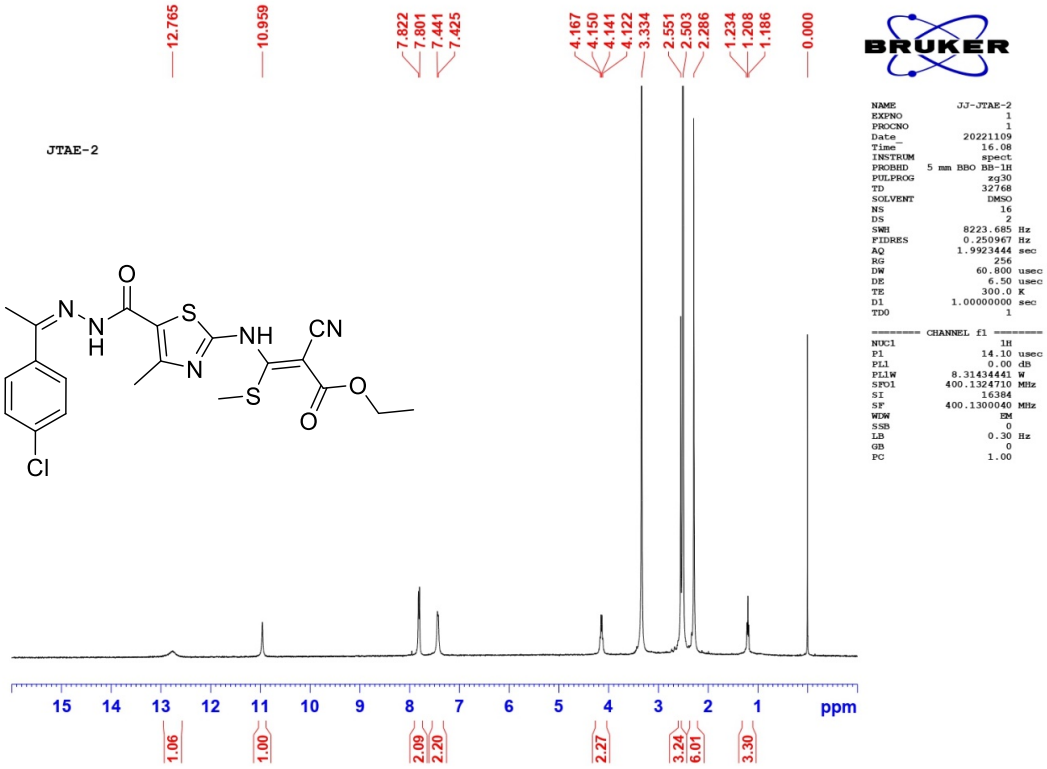


Fig. 6: Representative ^{1}H NMR spectrum of compound **JTAE-2**

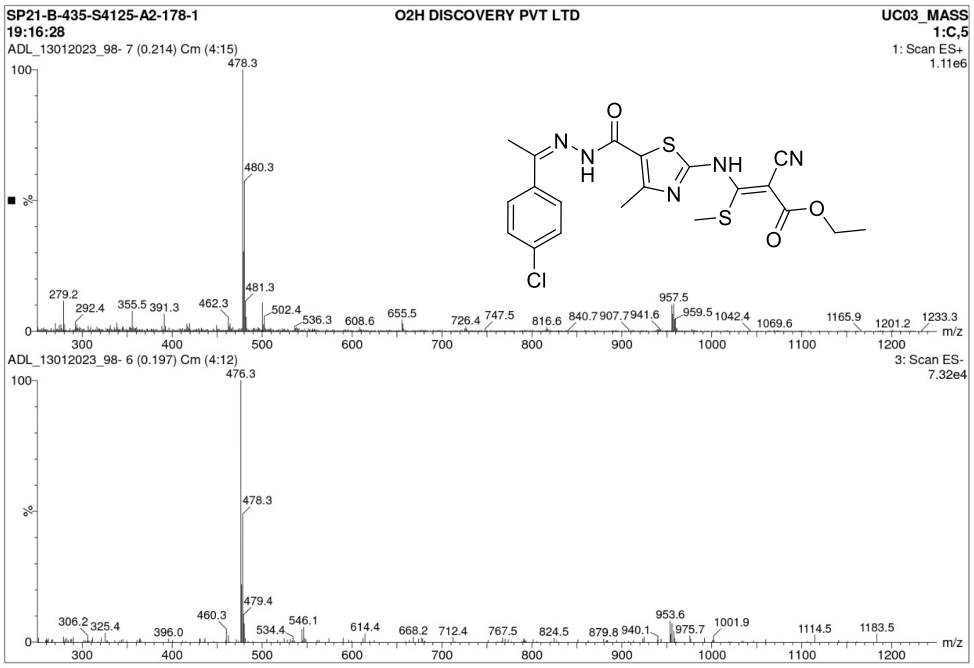


Fig.7: Representative mass spectrum of compound **JTAE-2**

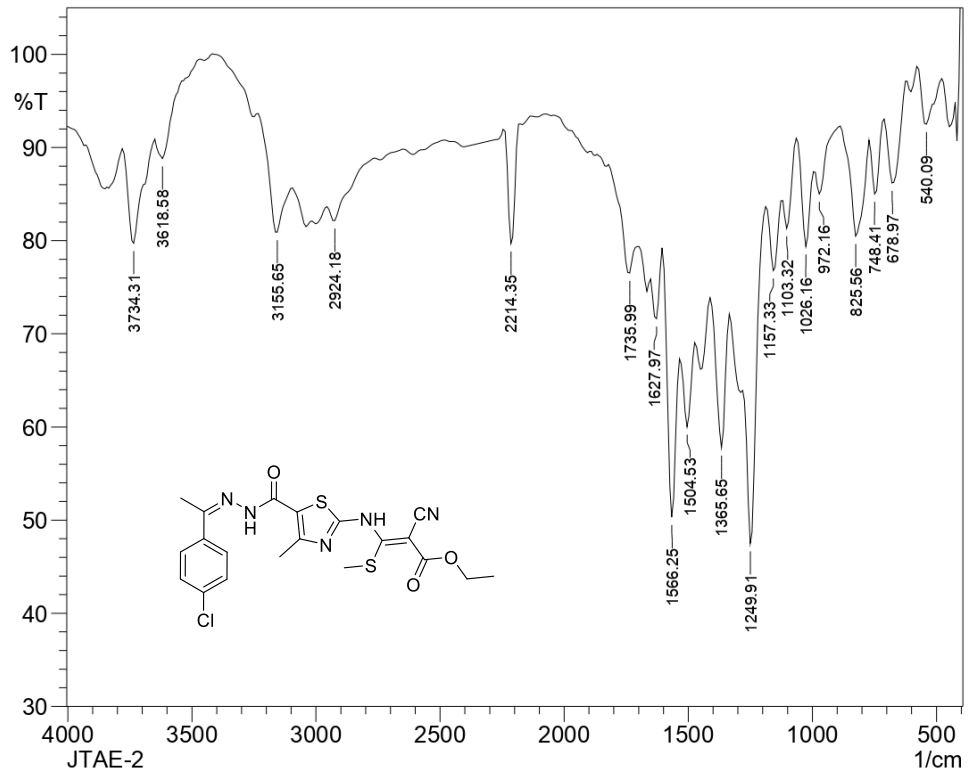


Fig. 8: Representative IR spectrum of compound **JTAE-2**

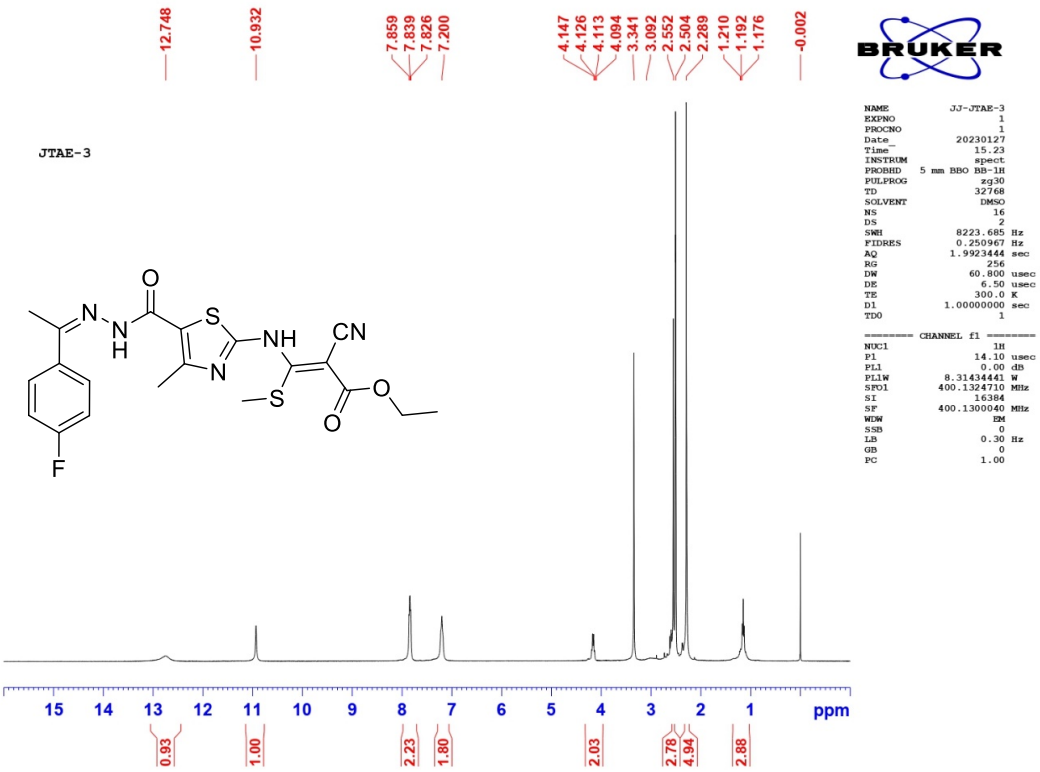


Fig. 9: Representative ^{1}H NMR spectrum of compound **JTAE-3**

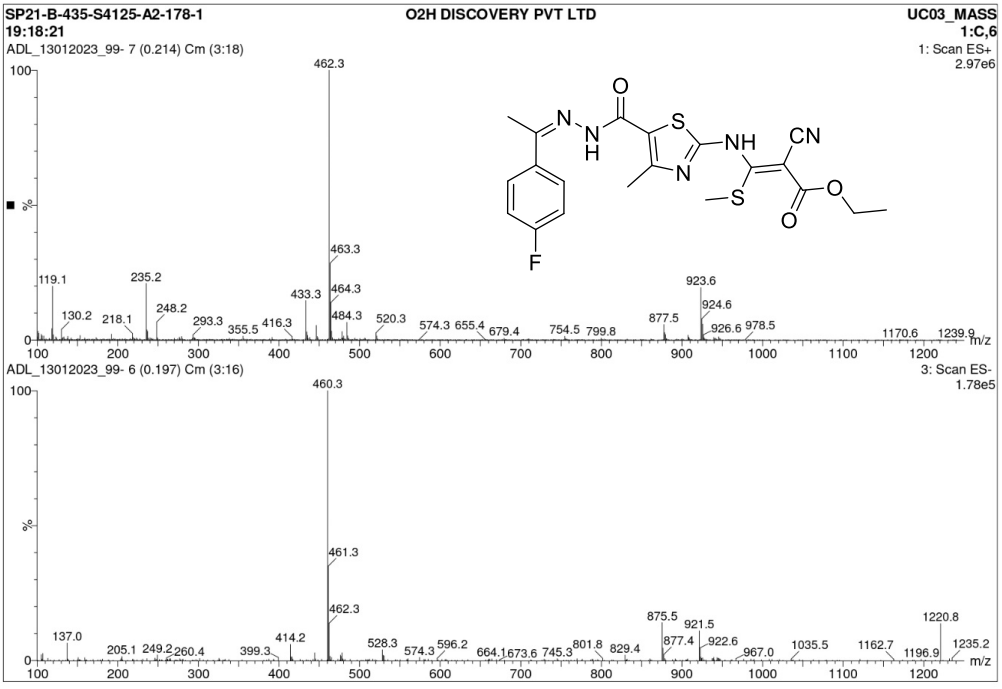


Fig. 10: Representative mass spectrum of compound **JTAE-3**

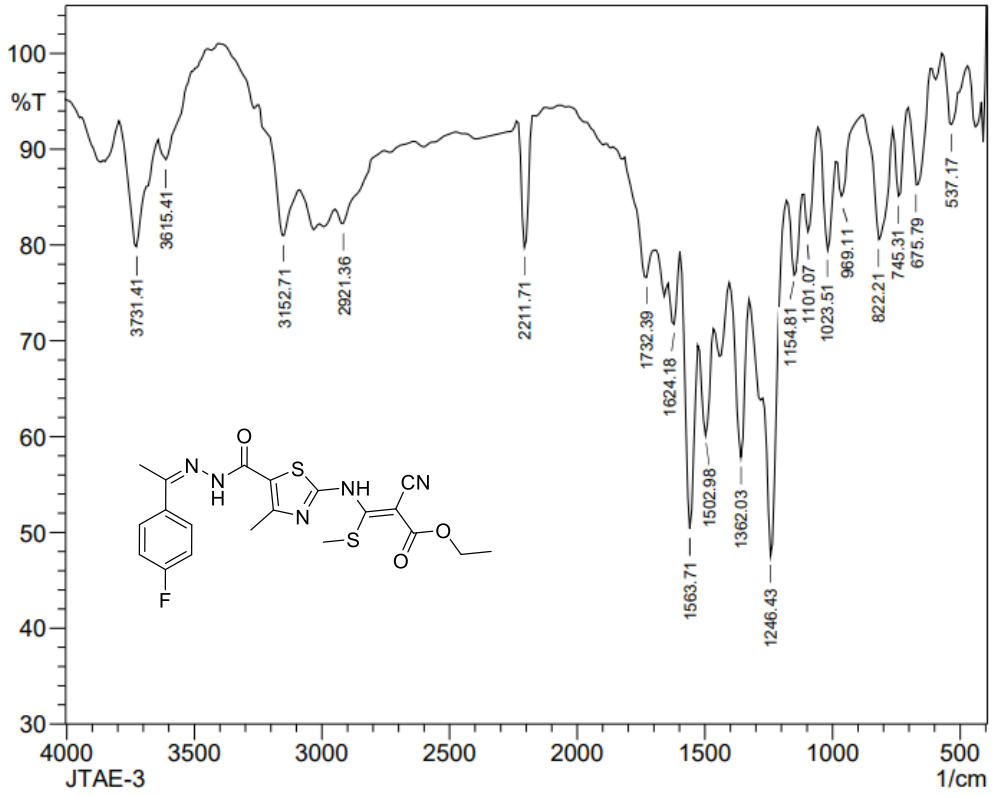


Fig. 11: Representative IR spectrum of compound **JTAE-3**

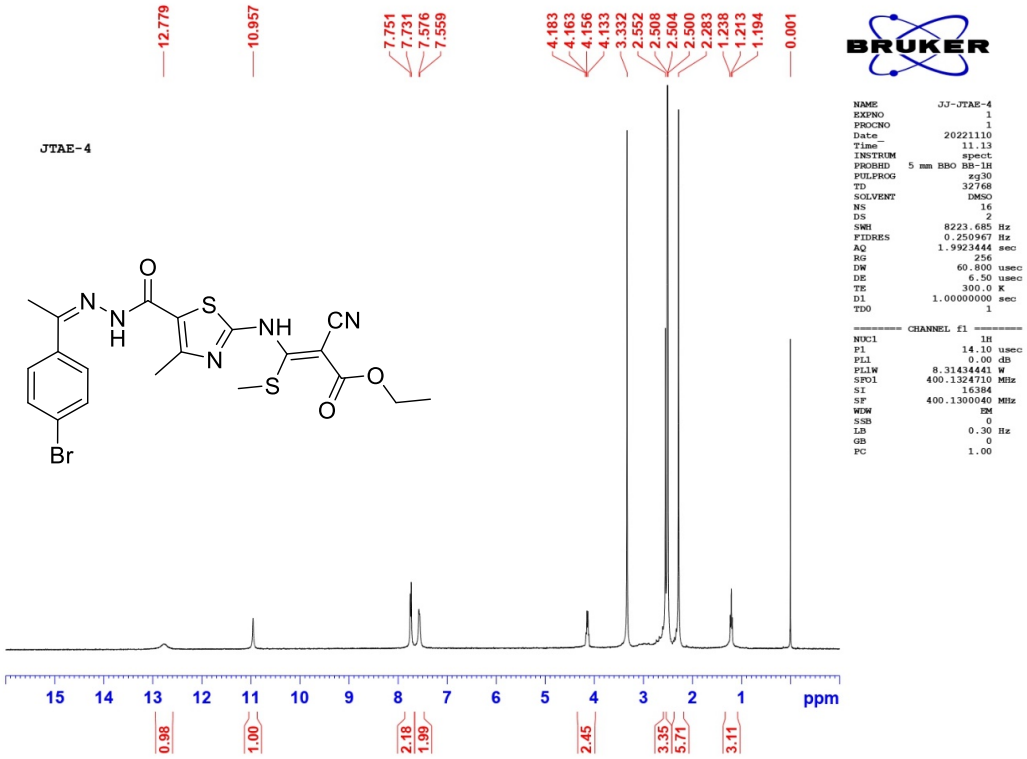


Fig. 12: Representative ^{1}H NMR spectrum of compound **JTAE-4**

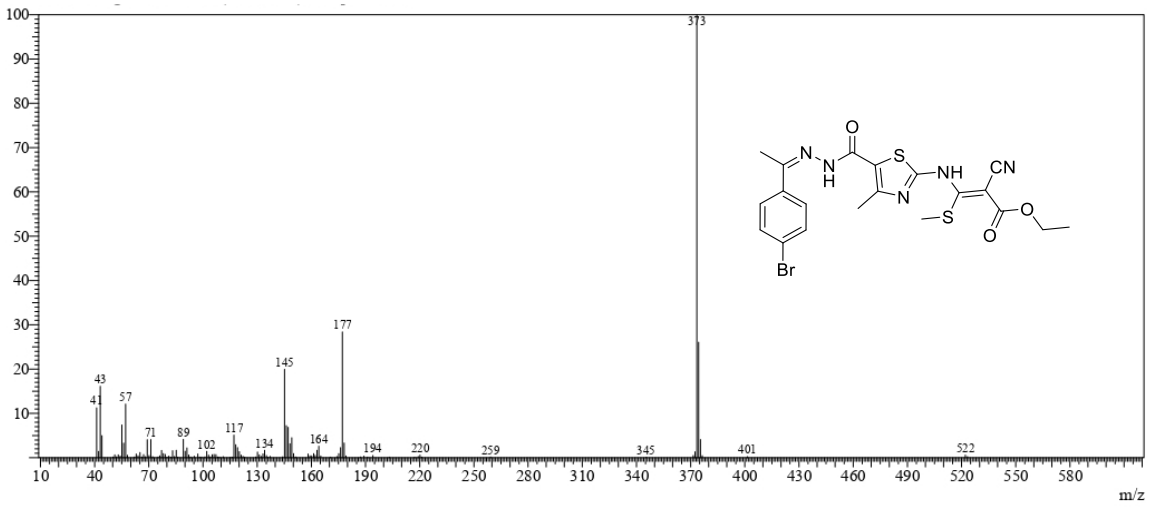


Fig. 13: Representative mass spectrum of compound **JTAE-4**

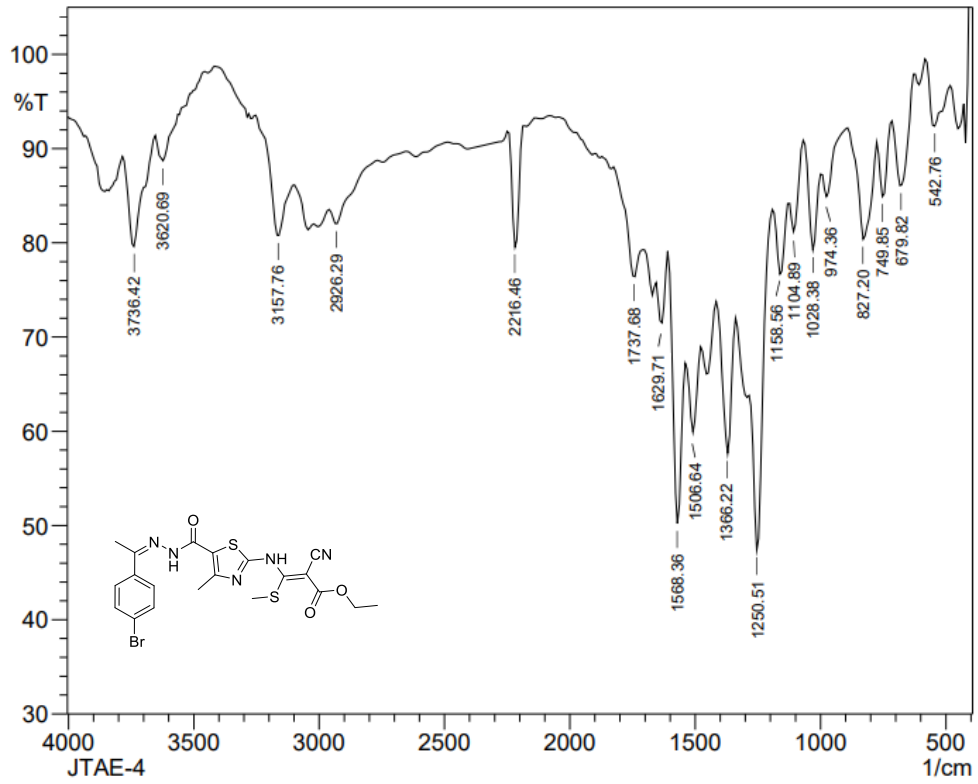


Fig. 14: Representative IR spectrum of compound **JTAE-4**

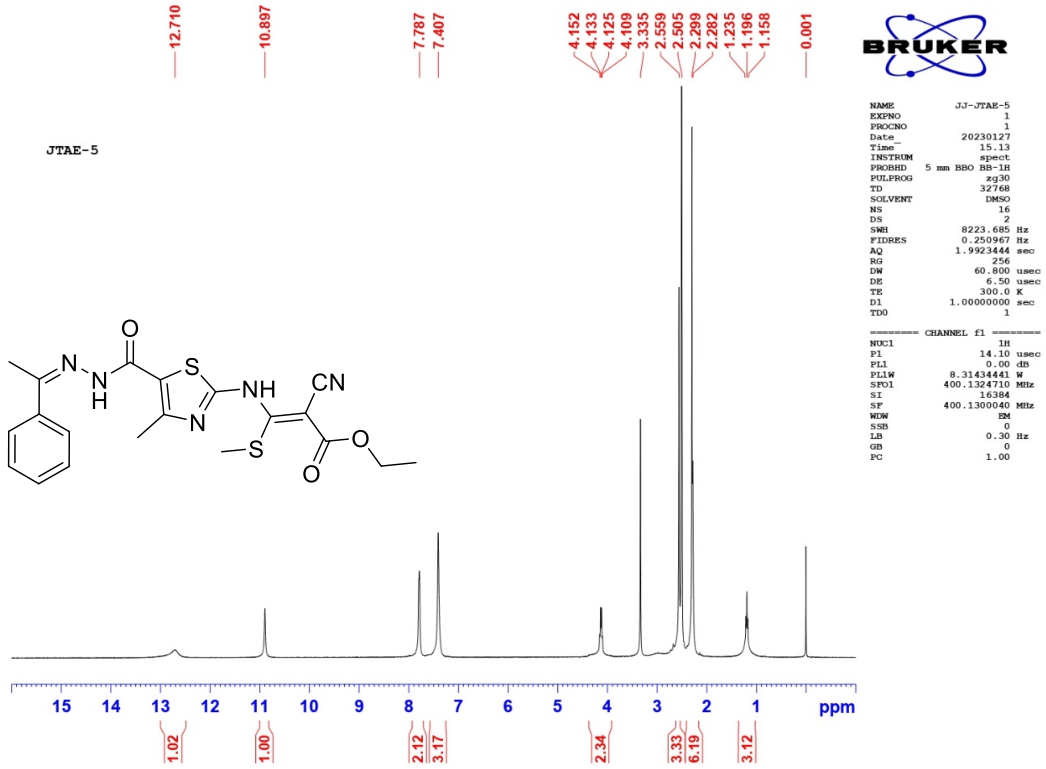


Fig. 15: Representative ^{1}H NMR spectrum of compound **JTAE-5**

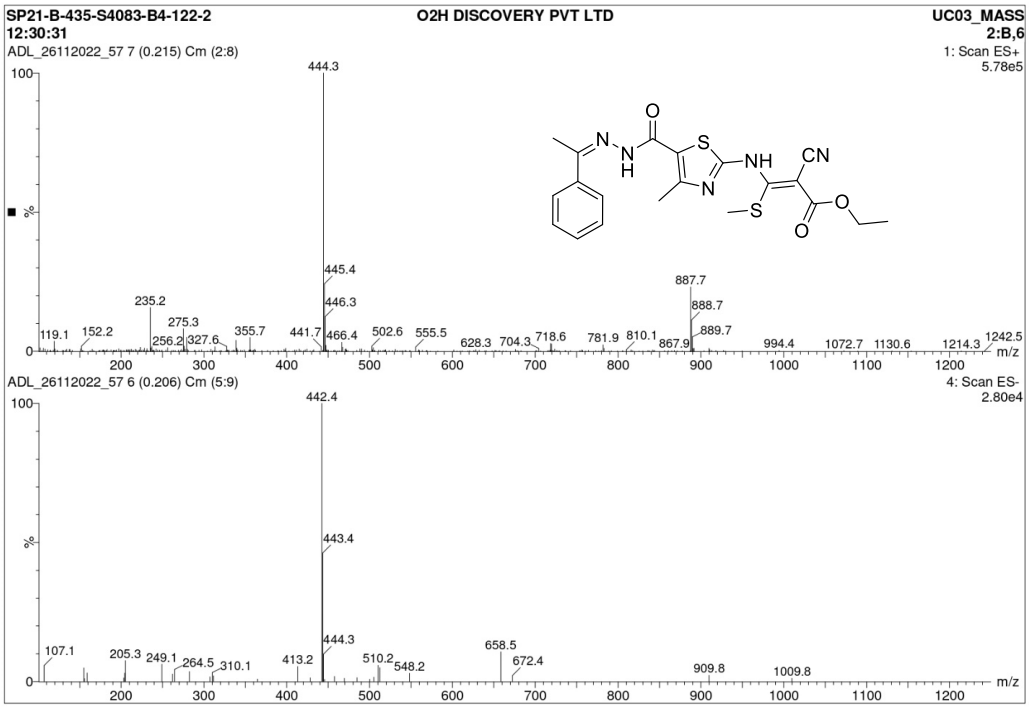


Fig. 16: Representative mass spectrum of compound **JTAE-5**

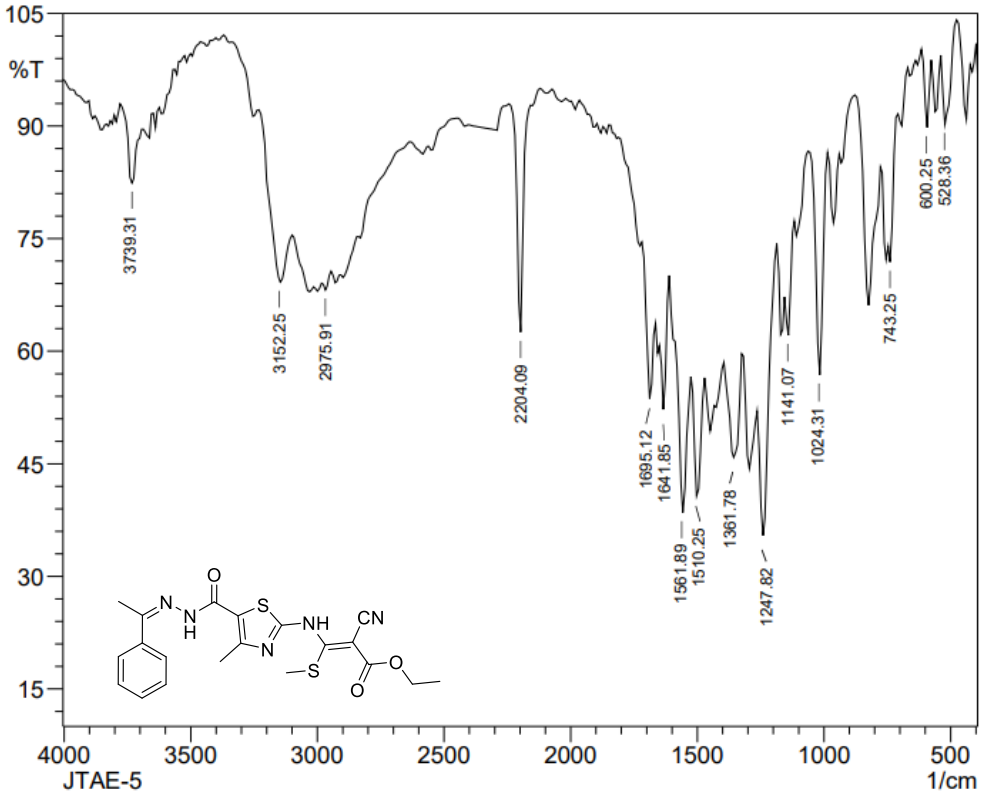


Fig. 17: Representative IR spectrum of compound **JTAE-5**

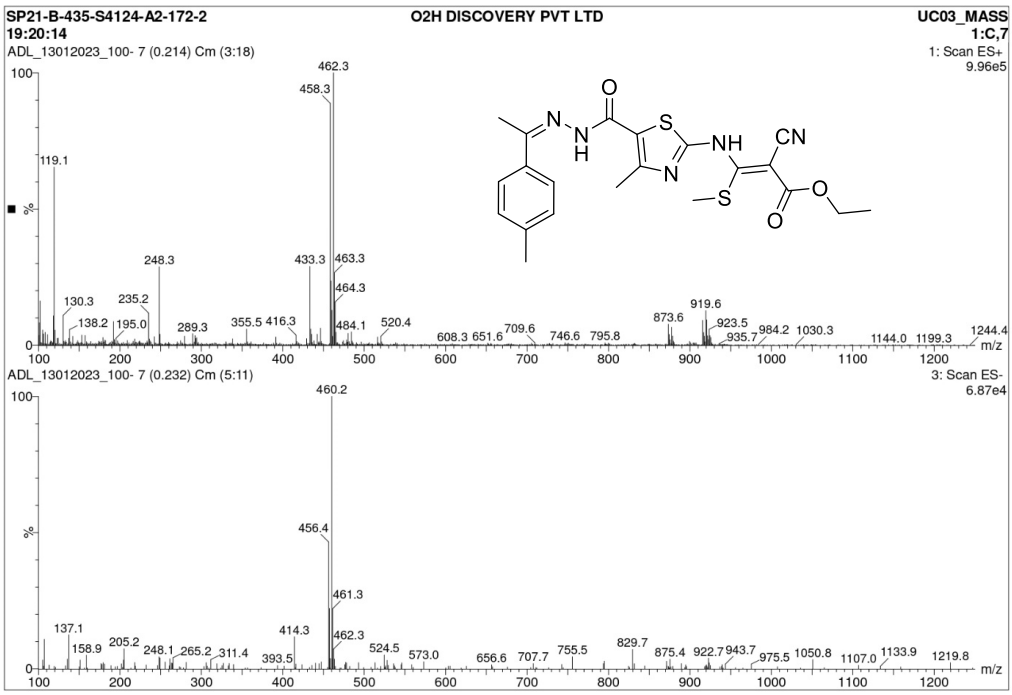


Fig. 18: Representative mass spectrum of compound **JTAE-6**

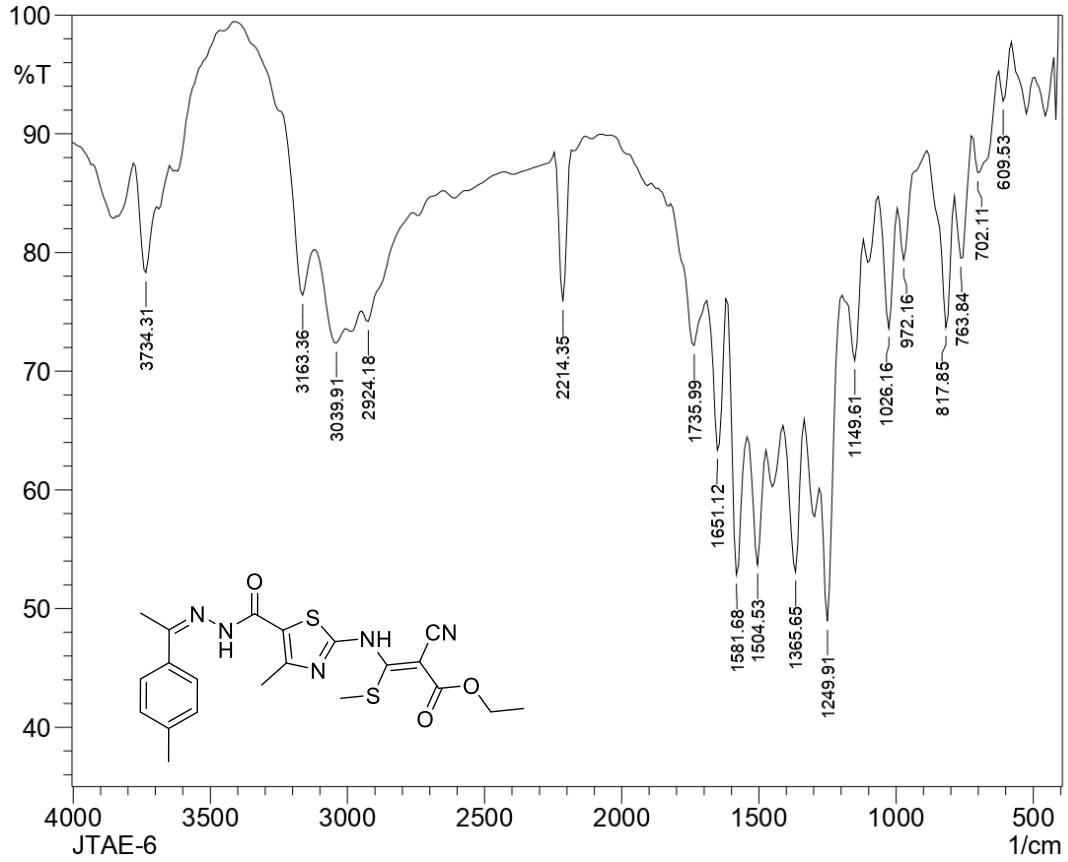


Fig. 19: Representative IR spectrum of compound **JTAE-6**

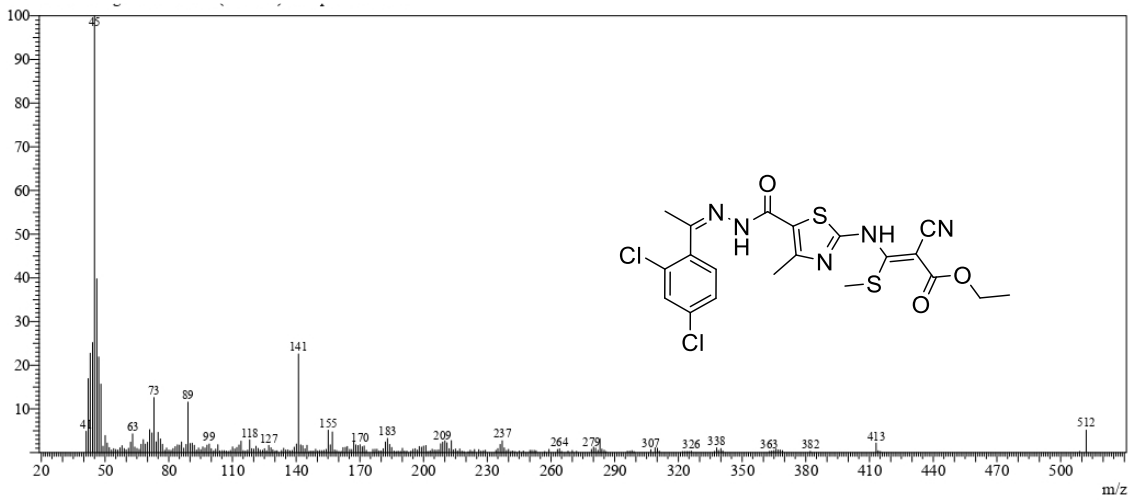


Fig. 20: Representative mass spectrum of compound **JTAE-7**

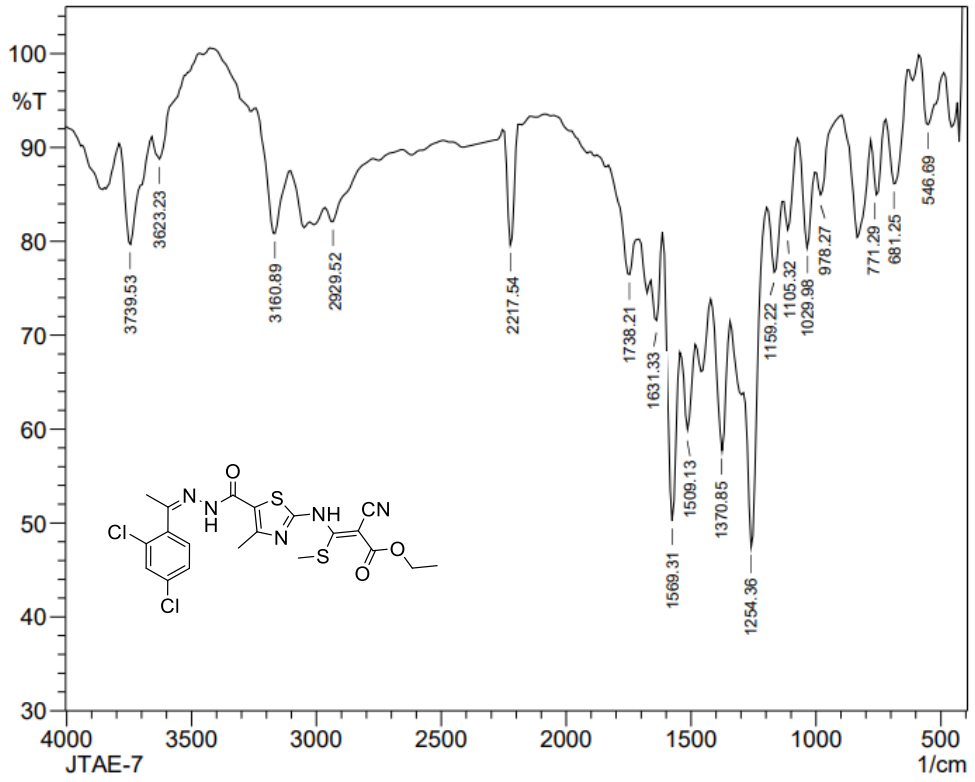


Fig. 21: Representative IR spectrum of compound **JTAE-7**

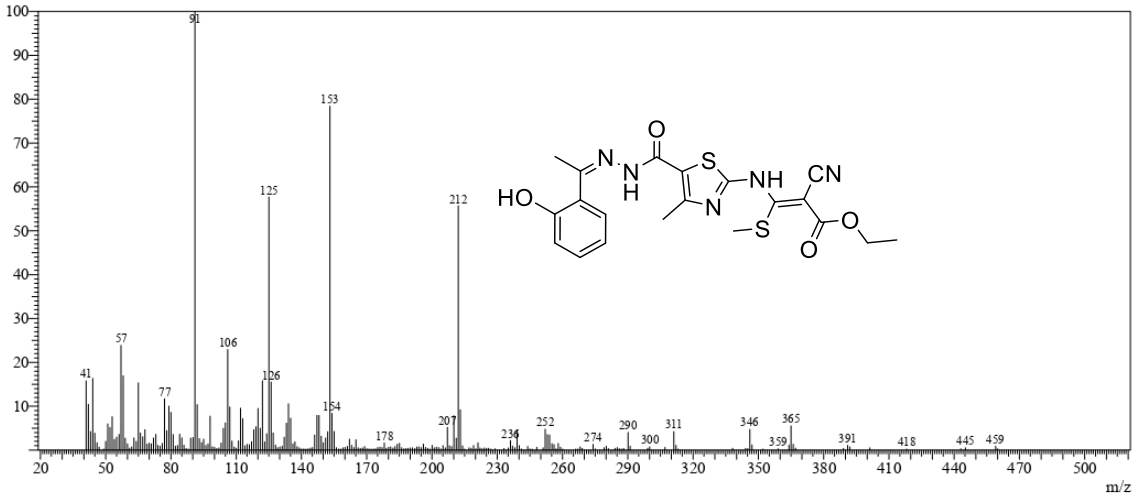


Fig. 22: Representative mass spectrum of compound **JTAE-8**

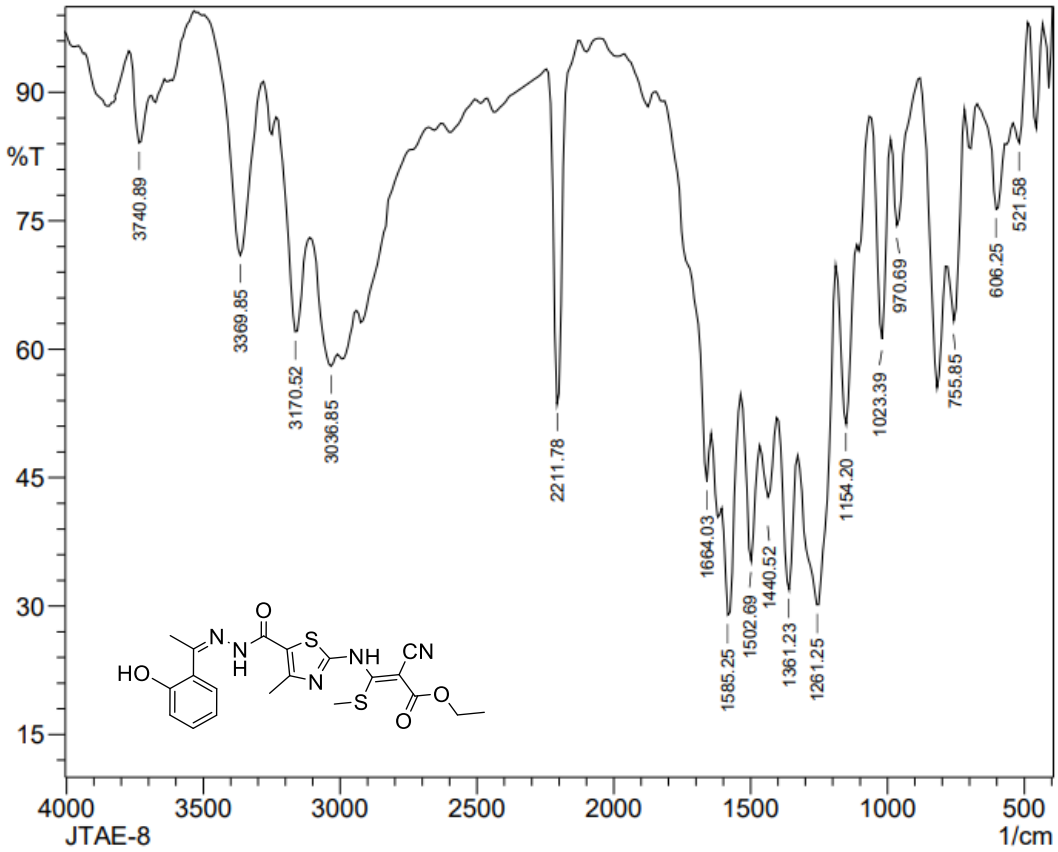


Fig. 23: Representative IR spectrum of compound **JTAE-8**

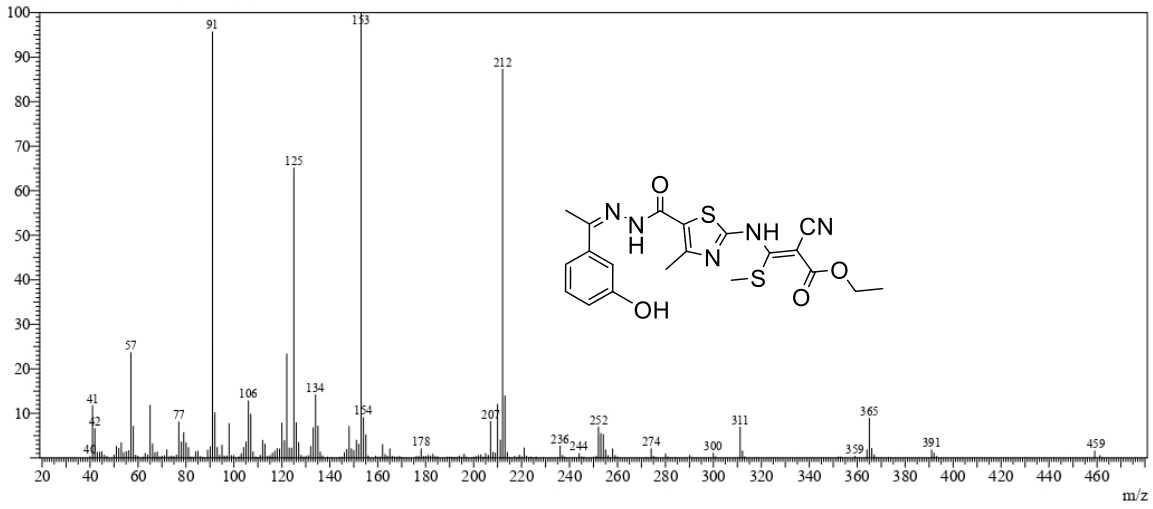


Fig. 24: Representative mass spectrum of compound **JTAE-9**

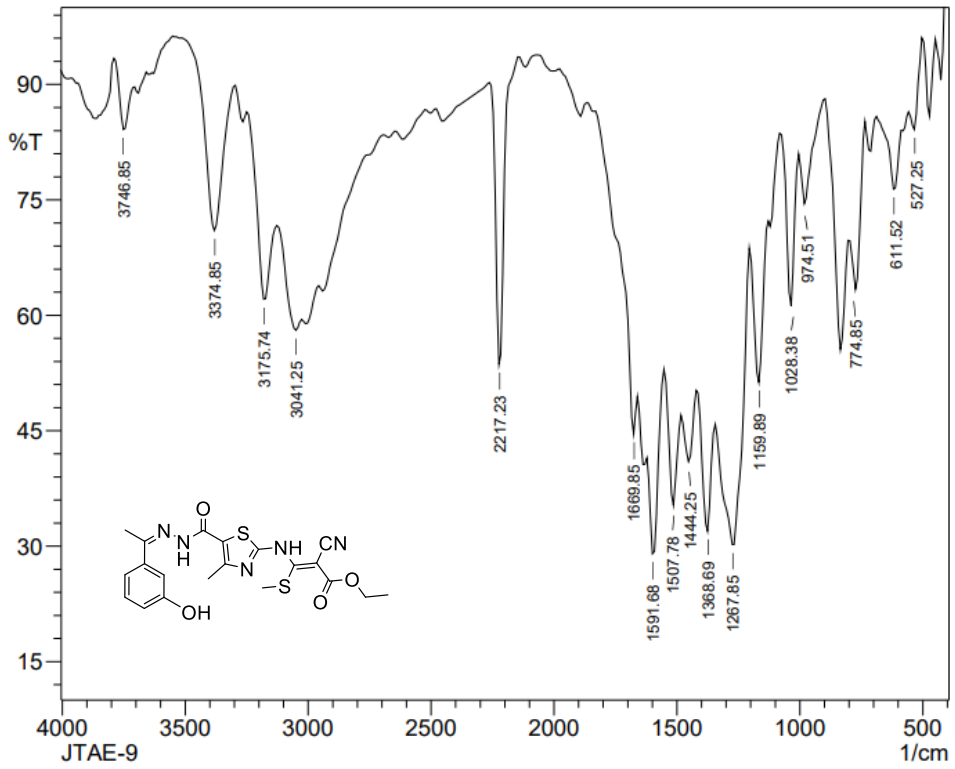


Fig. 25: Representative IR spectrum of compound **JTAE-9**

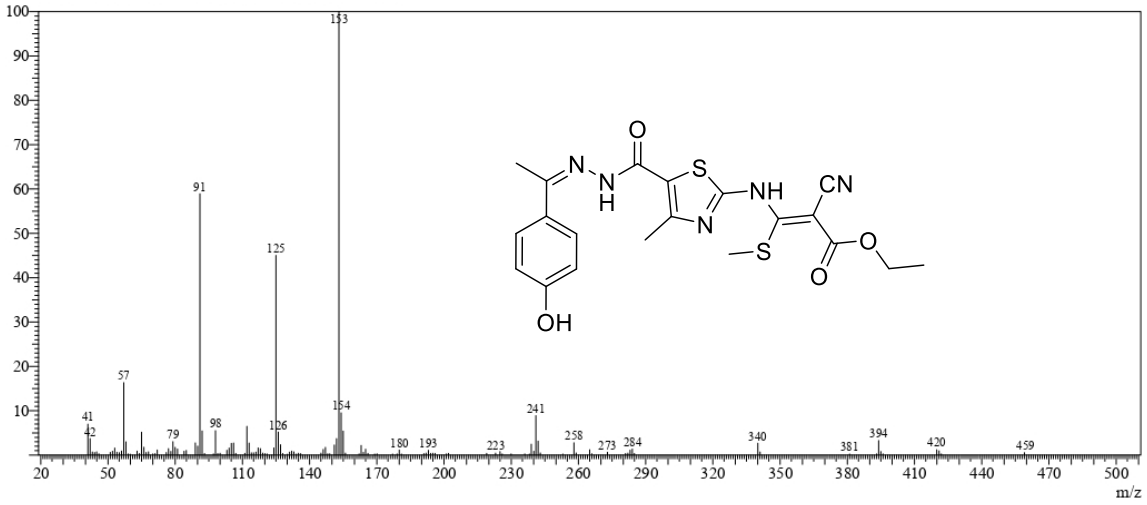


Fig. 26: Representative mass spectrum of compound **JTAE-10**

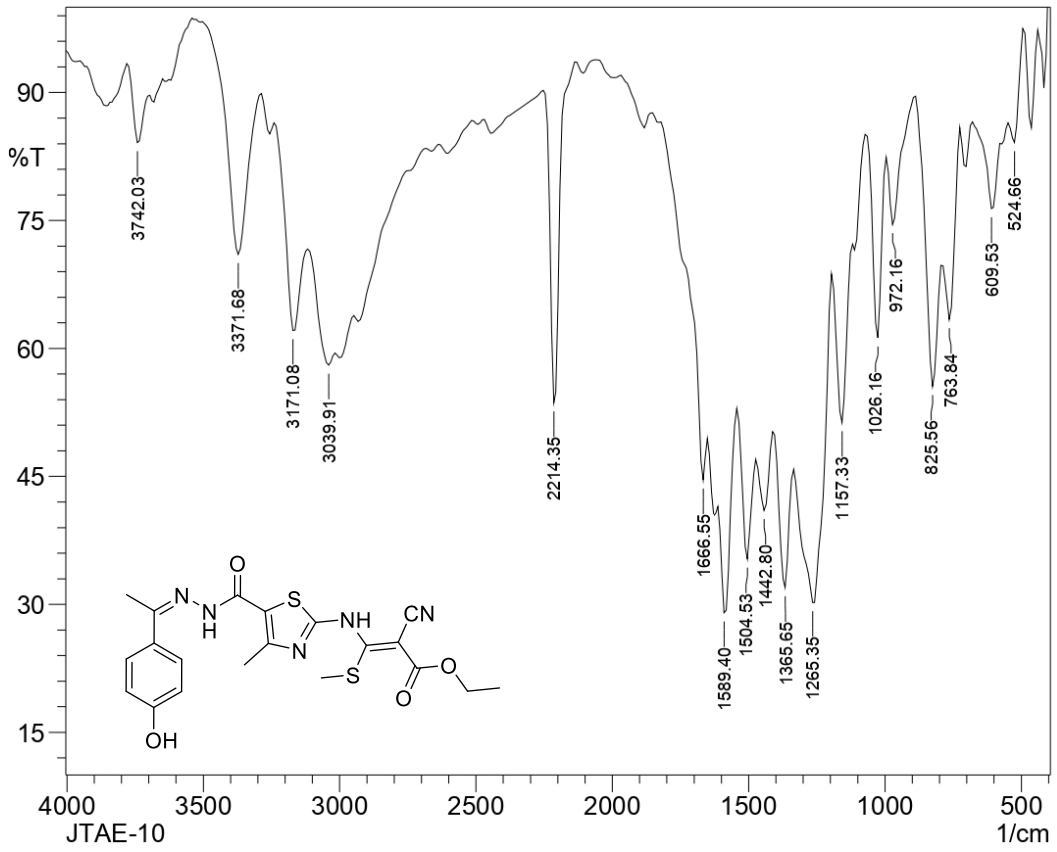


Fig. 27: Representative IR spectrum of compound **JTAE-10**

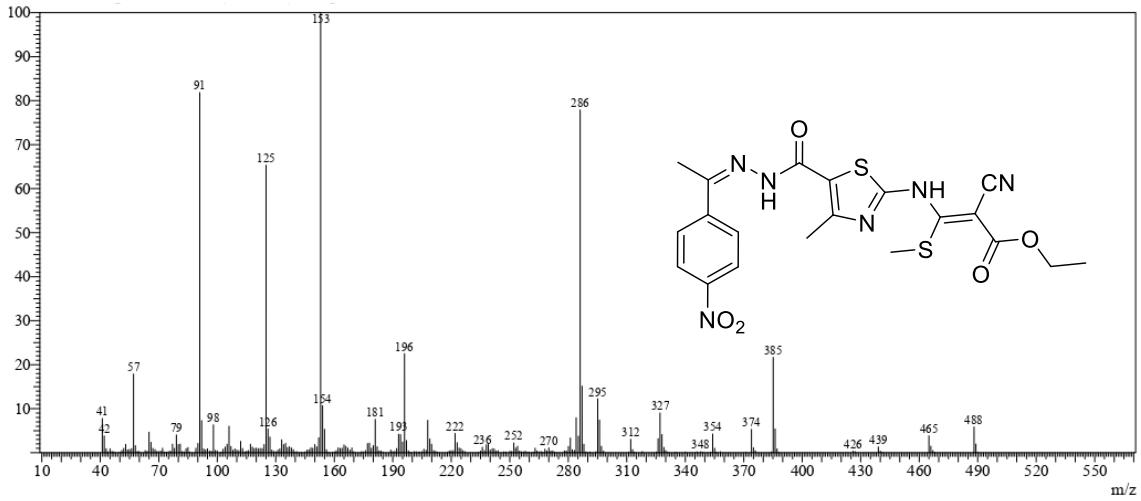


Fig. 28: Representative mass spectrum of compound **JTAE-11**

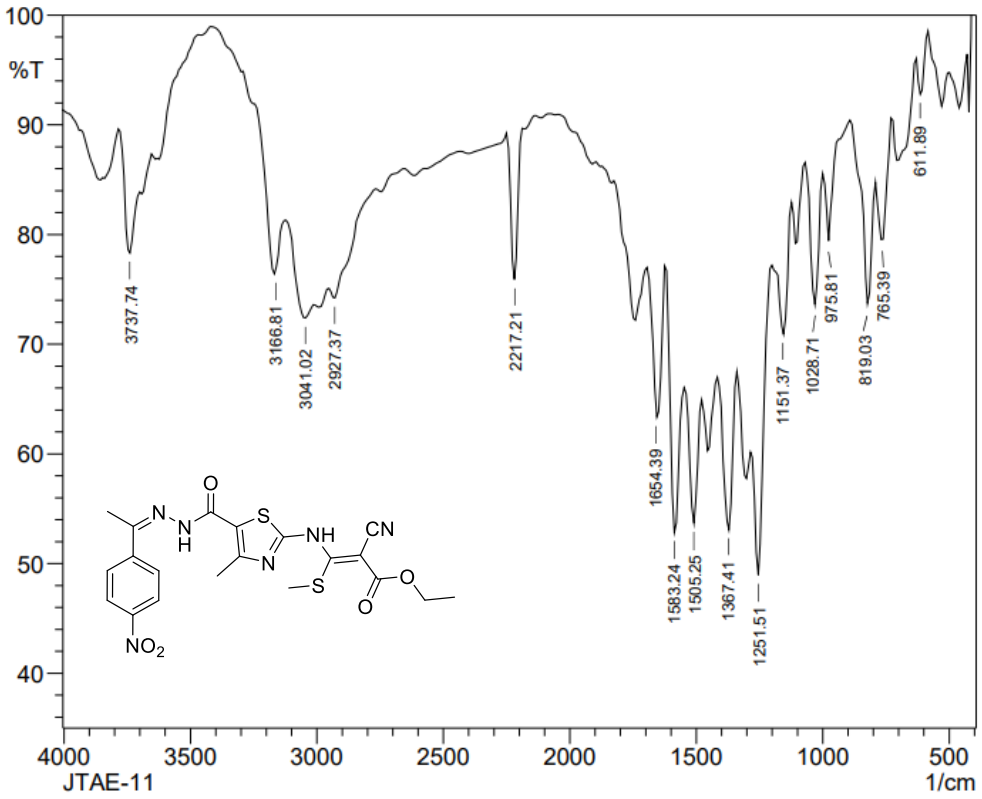


Fig. 29: Representative IR spectrum of compound **JTAE-11**