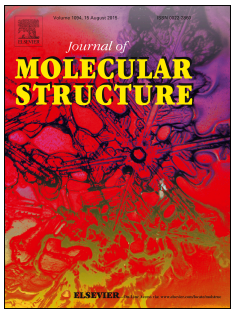
Accepted Manuscript



Thiazole fused thiosemicarbazones: Microwave-assisted synthesis, biological evaluation and molecular docking study

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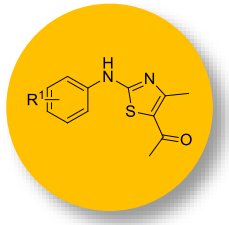
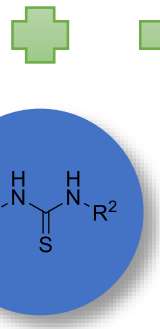
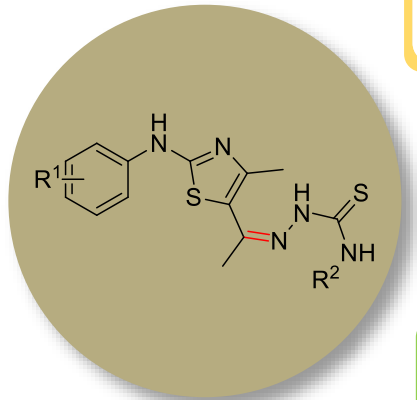
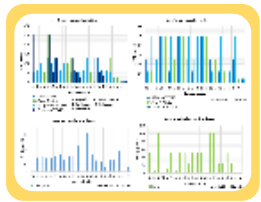
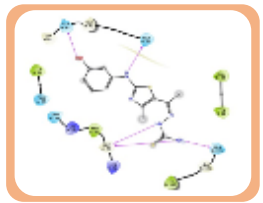




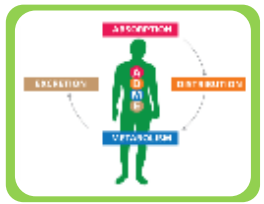




Biological Evaluation



Molecular Docking



In-silico ADME Study

Thiazole Fused Thiosemicarbazones: Microwave-Assisted Synthesis, Biological Evaluation and Molecular Docking Study

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- **Blue colour represents the changes made as per the reviewer's suggestions.**

Abstract:

In the present study, series of novel compounds (*E*)-2-(1-(4-methyl-2-(substituted-phenylamino)thiazol-5-yl)ethylidene)hydrazinecarbothio-amide (**5a-j**) and (*E*)-*N*-cyclohexyl-2-(1-(4-methyl-2-(substituted-phenylamino)thiazol-5-yl)ethylidene)hydrazinecarbothioamide (**6a-j**) have been synthesized using microwave irradiation method in high yields and characterized by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis. Synthesized compounds were evaluated for their *in-vitro* antimicrobial, antimalarial and anti-tuberculosis activity as well as *in-silico* study. The obtained results indicate that compound **6b** and **6g** were found to be the most potent against *S. aureus* bacterial strain. Further, the molecular docking study has been performed for all the new compounds with the immune system of *S. aureus* (PDB ID: 5D1Q) as a target enzyme, and this study validated the experimental results.

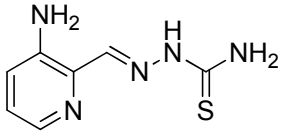
Keywords: 5D1Q, Biological activity, Microwave chemistry, Thiazole, Thiosemicarbazone

1. Introduction

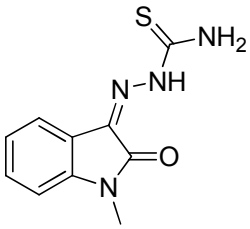
So far the heterocycles form the major divisions of organic chemistry due to their immense industrial as well as biological use [1]. From the last few decades, we have seen that researchers mainly focus in a heterocyclic compound containing sulfur and nitrogen, because

compound that possesses sulfur and nitrogen atoms are essential for living organisms. Hence, sulfur and nitrogen-containing small ring heterocyclic compounds have been under investigation for a long time on account of their therapeutic relevance and their synthetic diversity [2-6]. Among the wide range of heterocycles, thiazole occupies a vital role in medicinal chemistry [7-10]. Thiazole ring is an essential core scaffold found in a various natural compound such as thiamine (Vitamin B1), thiamine pyrophosphate and various synthetic medicinally important compounds [11-14]. Thiazole derivatives are having wide range of applications in various field such as agrochemicals, material science especially in the preparation of liquid crystals, cosmetic industry, sensors and pharmacological activities such as anticancer, antifungal, antiviral, antibacterial, anti-inflammatory etc. that can be represent by the large number of drugs in the market [15-16]. Thiazole is also used in the synthesis and applications of biomimicking and bioactive coordination compounds due to the versatile coordination ability of nitrogen and sulfur atoms toward various transition metal ions [17].

Thiosemicarbazones are very important class of compounds due to its pharmacological applications and are also belong to a large group of thiourea derivatives which show chemical functionality in biologically active molecules [18]. Literature revealed that from last few decades thiosemicarbazone derivatives had been evaluated in various biological activities such as antiviral, anticancer, antibacterial, antimalarial, antiHIV etc. [19-23]. Triapine (**1**) and Methisazone (**2**), synthetic analogs of thiosemicarbazone are easily available in the market. Triapine is a potent ribonucleotide reductase inhibitor and also used in cancer treatment while Methisazone is a good antiviral agent [18, 23].



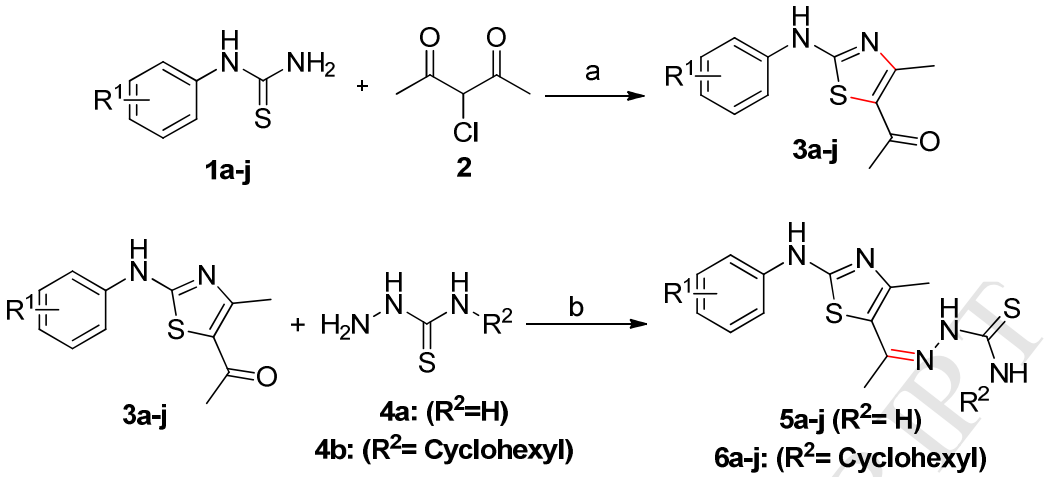
1



2

Microwave-assisted organic synthesis (MAOS) is an excellent tool of green chemistry where environment-friendly transformations have been carried out. In the last few decades, microwave technology has been used in synthetic organic chemistry [24-26]. For commercial use, the microwave oven has been designed especially for the organic synthesis which increased the number of microwave-based research articles [27-31]. Microwave-assisted organic synthesis is particularly crucial for industrial synthesis as it is cost-effective, efficient, time saver; leads to improve yields and lower waste generation compare to traditional synthetic protocols [32]. Five and six-membered heterocyclic compounds like thiazoles, pyrroles, pyrazoles, etc. have been synthesized in improved yields under solvent-free microwave as well as ultrasound assisted methods; because of it dramatic accelerations have been observed as compared to their traditional procedure [33-35].

Keeping in view, the advantages of microwave-assisted organic synthesis; the present work deals with the synthesis of an important class of organic compounds – Thiazole fused thiosemicarbazones – under microwave irradiation. All the newly synthesized compounds are well characterized and assessed for their *in-vitro* antimicrobial, antimalarial and anti-tuberculosis activity, which revealed that compounds **6b** and **6g** are more potent against *S. aureus* bacterial strain. So, molecular docking study has performed with *S. aureus* (PDB ID: 5D1Q) as a target enzyme, and the results lend weight to *in-vitro* biological study results. *In-silico* pharmacophoric study is explained in ADME study.



Reagents and conditions: (a) MWI 300 W (190 °C); (b) EtOH (5 mL), Gla. CH_3COOH (1.5 mL), MWI 300 W (190 °C)

Scheme 1. General reaction scheme

2. Results and Discussion

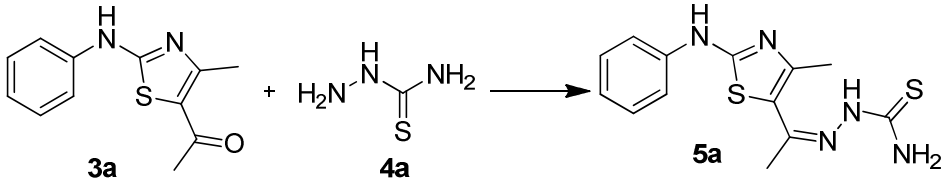
(*E*)-2-(1-(4-methyl-2-(substituted-phenylamino)thiazol-5-yl)ethylidene)hydrazinecarbothio-amide (**5a-j**) and (*E*)-*N*-cyclohexyl-2-(1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethylidene) hydrazinecarbothioamide (**6a-j**) were prepared by condensation reaction of substituted 1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethanone (**3a-j**) with hydrazinecarbothioamide (**4a**) and *N*-cyclohexylhydrazinecarbothioamide (**4b**), respectively via condensation reaction using microwave irradiation (MWI) in presence of a catalytic amount of glacial acetic acid (Gla. CH_3COOH). Substituted 1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethanone (**3a-j**) were prepared via Hantsch cyclization of substituted phenylthiourea (**1a-j**) and 3-chloropentane-2,4-dione (**2**) (**Scheme 1**) using microwave irradiation. This reaction of phenylthiourea and 3-chloropentane-2,4-dione proceeds well upon refluxing with ethanol for 2-4 h or with ultrasound conditions at room temperature for 60 min., but here we adapted this to microwave irradiation at 300W for 4-5 min without using any solvent. It resulted in good yields and shorter reaction time compared to the reflux or ultrasound protocol.

We initiated our screening by performing a model reaction of 1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethanone (**3a**) with hydrazinecarbothioamide (**4a**) using ethanol as solvent at room temperature. The reaction was incomplete even after 24 h, as unreacted starting materials were observed on TLC using (7:3) hexane: ethyl acetate (Table 1, Entry 1). Even no significant change observed in the reaction yield after refluxing the reaction mixture in ethanol for 12 h (Table 1, Entry 2). Generally, catalysts manifest themselves as asymmetric catalytic reactions and green chemical processes for forming novel structural motifs. Thus, in the model reaction, various catalysts were introduced, and their considerable effect on product (**5a**) yield was observed. Firstly, *p*-toluenesulphonic acid (*p*-TSA) was applied as a catalyst in the modal reaction under refluxing ethanol, which resulted in a 68% yield of product **5a** (Table 1, Entry 3). The reaction was also restrained using hydrochloric acid (HCl), formic acid (HCOOH) which resulted, peripheral to *p*-TSA in terms of product yield (Table 1 Entry 4, 5). Lewis acid catalyst like $Cu(OAc)_2$ was tested in refluxing ethanol which provided a trace amount of product (Table 1 Entry 6). It might have happened because of complex formation with metal Cu. Further, the reaction was explored using an increasing molar ratio of glacial acetic acid (5, 10, 15, 20 and 25 mol% gla. CH_3COOH) as a catalyst in refluxing ethanol for 5 h (Table 3, Entry 7-11). 20 mol% gla. CH_3COOH has worked best for this model reaction as the reaction was completed in 5 h with a 90% yield of product. We have also carried out this reaction using various solvents like methanol, isopropanol, and butanol with a fixed amount of gla. CH_3COOH (20 mol%) which forms 75, 84 and 80% of product yield, respectively (Table 1, Entry 12-14). However, ethanol was found as the best solvent for this transformation in terms of the yield of the product and the time of reaction. By seeing all the parameters, we found out that conventional method usually needs longer heating time, whereas microwave-assisted organic synthesis is fast, efficient, economical and environmentally friendly. Keeping this in mind, we performed this reaction under microwave

irradiation (MWI) of 100 W using 20 mol% gla. CH_3COOH as catalyst and ethanol as solvent, and surprisingly we obtained 73% of product yield in just 4 min of time (Table 1, Entry 16). Further, the reaction was conducted at a microwave irradiation power of 180 W with the same conditions found 85% of product yield increased compared to 100 W of power (Table 1, Entry 17). So, the reaction was carried out at increasing MWI of 300 W with same reaction conditions resulted in the excellent product yield of 94% (Table 1, Entry 18). Further increase in the MWI power did not affect the reaction. Also, we got good results in isopropanol in convention heating in terms of reaction time and product yield; isopropanol was also implemented in MWI of 300 W for 4 min., resulted in good yield (87%) of the product (Table 1, Entry 15).

From the optimization of reaction conditions, it can be inferred that the reaction of 1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethanone (**3a**) with hydrazinecarbothioamide (**4a**) in microwave irradiation at 300W using 20 mol% of gla. CH_3COOH as catalyst and ethanol as the solvent is optimum condition for this protocol of preparation of (*E*)-2-(1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethylidene)hydrazine carbothioamide (**5a**) (**Scheme 1**). Subsequently, reactions of differently substituted 1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethanone (**3a-j**) (1 mmol) with hydrazinecarbothioamide (**4a**) (1 mmol) or *N*-cyclohexylhydrazinecarbothioamide (**4b**) (1 mmol) in MWI (300 W) using 20 mol% gla. CH_3COOH and ethanol gave corresponding thiazole fused thiosemicarbazones (**5a-j** and **6a-j**) in good to high yields (Table 2). Moreover, highly pure products were obtained with no need of column purification using this protocol.

Table 1. Optimization of reaction conditions for the synthesis of thiazole fused thiosemicarbazones (**5a-j** and **6a-j**).[a]



Entry	Reaction conditions/ Temperature	Solvent	Catalyst	Time	Yield (%)[b]
1	R.T.	Ethanol	-	24h	-
2	Reflux	Ethanol	-	12h	-
3	Reflux	Ethanol	*p*-TSA	12h	68
4	Reflux	Ethanol	HCl	12h	54
5	Reflux	Ethanol	HCOOH	12h	47
6	Reflux	Ethanol	$Cu(OAc)_2$	12h	-
7	Reflux	Ethanol	Gla. CH_3COOH (5 mol%)	5h	65
8	Reflux	Ethanol	Gla. CH_3COOH (10 mol%)	5h	72
9	Reflux	Ethanol	Gla. CH_3COOH (15 mol%)	5h	80
10	Reflux	Ethanol	Gla. CH_3COOH (20 mol%)	5h	90

11	Reflux	Ethanol	Gla. CH_3COOH (25 mol%)	5h	89
12	Reflux	Methanol	Gla. CH_3COOH (20 mol%)	5h	75
13	Reflux	Isopropanol	Gla. CH_3COOH (20 mol%)	5h	84
14	Reflux	Butanol	Gla. CH_3COOH (20 mol%)	5h	80
15	MWI, 300 W	Isopropanol	Gla. CH_3COOH (20 mol%)	4 min.	87
16	MWI, 100 W	Ethanol	Gla. CH_3COOH (20 mol%)	4 min.	73
17	MWI, 180 W	Ethanol	Gla. CH_3COOH (20 mol%)	4 min.	85
18	MWI, 300 W	Ethanol	Gla. CH_3COOH (20 mol%)	4 min.	94

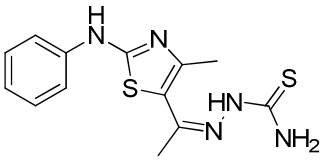
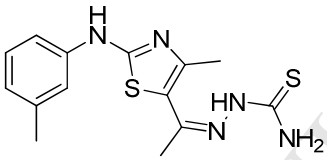
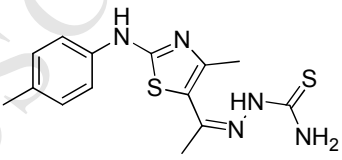
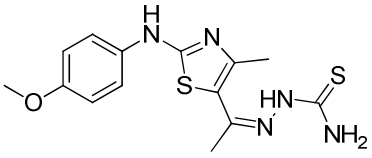
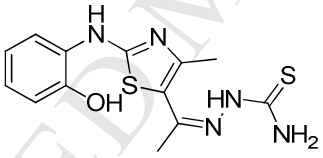
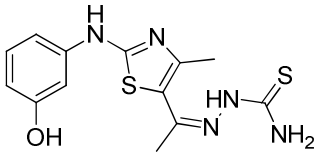
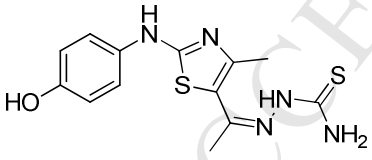
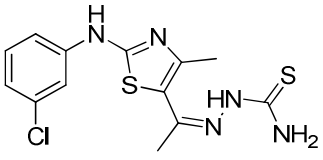
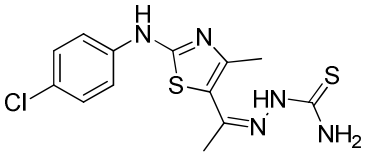
[a] = 1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethanone (**3a**; 1 mmol), hydrazinecarbothioamide (**4a**; 1 mmol).

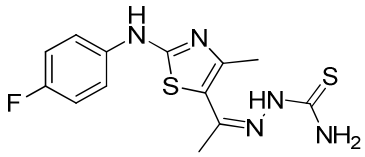
[b] = Yield of isolated product

The phenyl ring of 1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethanone (**3**) substituted with electron donating groups like $–CH_3$, -OH at *para*-position form high yield compared to those

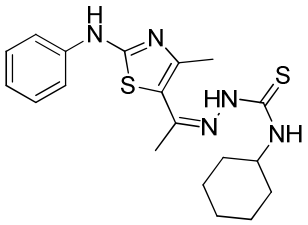
substituted with electron withdrawing –Cl, -F groups or unsubstituted. The scope of the present protocol was extended by investigating reactions of substituted thiazole with cyclohexylthiosemicarbazone under identical conditions. The cyclohexyl substituted thiosemicarbazones (**6a-j**) gave low yield as compared to simple thiosemicarbazones (**5a-j**). The higher yield obtained in the case of **5d** was attributed due to the presence of strong electron donating group like –OCH_3 (Table 2).

Table 2. Prepared derivatives of thiazole fused thiosemicarbazone.[a]

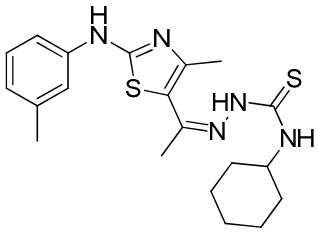
		
5a	**5b**	**5c**
(4 min.; 94%)	(4 min.; 92%)	(4 min.; 96%)
MP: 203-210 °C	MP: 170-175 °C	MP: 227-229 °C
		
5d	**5e**	**5f**
(4 min.; 97%)	(4 min.; 90%)	(4 min.; 88%)
MP: 193-197 °C	MP: 188-192 °C	MP: 172-176 °C
		
5g	**5h**	**5i**
(4 min.; 91%)	(4 min.; 92%)	(4 min.; 94%)
MP: 192-196 °C	MP: 180-186 °C	MP: 153-156 °C



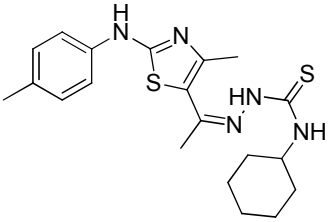
5j
(4 min.; 90%)
MP: 204-207 °C



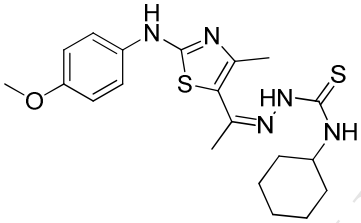
6a
(5 min.; 90%)
MP: 212-215 °C



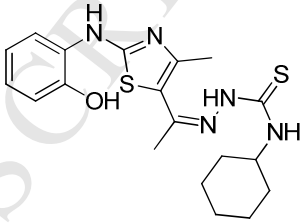
6b
(5 min.; 89%)
MP: 173-177 °C



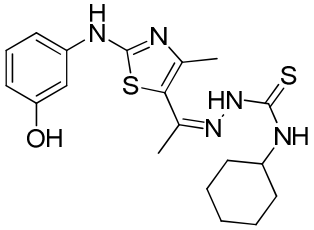
6c
(5 min.; 93%)
MP: 235-238 °C



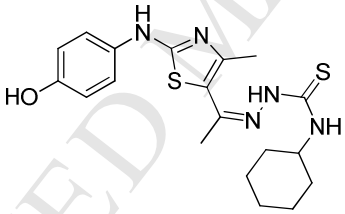
6d
(5 min.; 92%)
MP: 175-179 °C



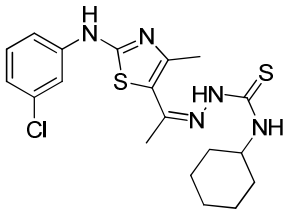
6e
(5 min.; 87%)
MP: 204-208 °C



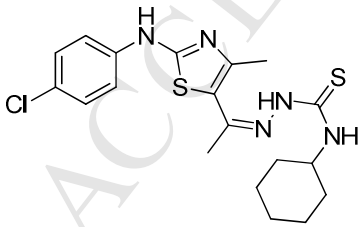
6f
(5 min.; 85%)
MP: 195-197 °C



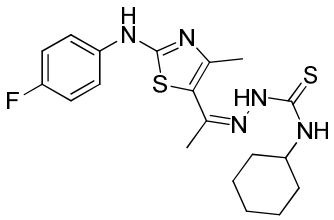
6g
(5 min.; 89%)
MP: 178-181 °C



6h
(5 min.; 90%)
MP: 180-184 °C



6i
(5 min.; 91%)
MP: 170-174 °C



6j
(5 min.; 92%)
MP: 171-176 °C

[a] Reaction conditions: 1-(4-methyl-2-(substituted phenylamino)thiazole-5-yl)ethanone **3a-j** (1 mmol), Thiosemicarbazide or *N*-cyclohexyl hydrazinecarbothioamide **4a-c** (1 mmol), Gla. CH_3COOH (20 mol%), EtOH (5 mL), MWI (300W, 190 °C) for 4-5 min.

The reaction mechanism shows that thiazole derivatives form via Hantzsch synthetic approach, which involves cyclization and condensation of haloketones with thioamide. The reaction initiates with a nucleophilic attack of sulphur atom of substituted phenyl thiourea on the α-carbon atom of α-haloketone, which form α-thioketone intermediate. The dehydration of α-thioketone intermediate generates the corresponding thiazoles (1-(4-thiazolyl)ethanone).

Now, in the second step the addition reaction between substituted isothiocyanates and hydrazine hydrate produced substituted thiosemicarbazide in excellent yield. We proposed that mechanistically a lone pair of electrons present on the primary amine of thiosemicarbazide attacks on the carbocation of the acetyl group of 1-(4-thiazolyl)ethanone due to the presence of acetic acid as the catalyst. Acetic acid pulls the electron from the carbonyl group of 1-(4-thiazolyl)ethanone to form the reactive intermediate carbocation. At that time proton transfer occurs from nitrogen atom to oxygen atom. In the next step, removal of water molecule occurs from the proton of nitrogen atom and hydroxyl group at carbon atom, situated at the adjacent position, to form the thiazole substituted thiosemicarbazone derivatives.

3. Experimental

3.1 Chemistry

3.1.1 Materials and instruments

All chemicals and solvents were obtained from commercial sources and were used without further purification. The progress of the reactions was monitored by thin layer chromatography (TLC) on Macherey-Nagel (Germany) Alugram Sil G/UV254 TLC plates, visualized under UV light. Melting points were determined on an Optimelt MPA 100 melting point apparatus and are uncorrected. Yields refer to isolated compounds after crystallization

in ethanol, estimated to be >95% pure as determined by ^{1}H-NMR. FT-IR spectra were recorded on a Perkin Elmer FT-IR 377spectrometer using KBr as reference. Mass spectra were recorded on Water's SQD detector with single quardropole analyzer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AV 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as the internal reference. The chemical shifts were reported in ppm (δ) relative to TMS and coupling constants (*J*) in Hertz (*Hz*), whereas s, d, t and m refer to singlet, doublet, triplet and multiplet, respectively. All the ^{1}H-NMR and ^{13}C-NMR spectral data are refined using Mnova.

*3.1.2 General procedure for the synthesis of Thiazole derivatives (**3a-j**)*

Substituted thiourea (1 mmol) and 3-chloropentane-2,4-dione (1 mmol) were placed in an open vessel microwave (Discover CEM, 300 W and temperature control set at 190 °C measured with an IR sensor) for 4-5 min. Reaction progress was checked by TLC using hexane: ethyl acetate (7: 3) as mobile phase. After completion of the reaction, 10 mL of ethanol was added to the reaction mixture and the resulting mixture was further added to ice cold water to form desired product (**3a-j**). The precipitates were filtered and purified by recrystallization from ethanol.

*3.1.3 General procedure for the synthesis of Thiosemicarbazone derivatives (**5a-j, 6a-j**)*

A mixture of 1-(4-methyl-2-(substituted phenylamino)thiazole-5-yl)ethanone (1 mmol), thiosemicarbazide or *N*-cyclohexyl hydrazinecarbothioamide (1 mmol) and glacial acetic acid (20 mol%) in ethanol (5 mL) was irradiated in an open vessel microwave (Discover CEM, 300 W and temperature control set at 190 °C measured with an IR sensor) for appropriate time shown in Table 1. The reaction was monitored by TLC using hexane: ethyl acetate (7: 3) as mobile phase, during the interval of 1 minute. After cooling the reaction mixture at ambient temperature, ethanol was removed by rotary evaporator. The product was

precipitated out by addition of cold water. The product was collected by filtration and purified by crystallization from ethanol. The representative procedure was followed for the synthesis of all the thiazole fused thiosemicarbazones **5a-j**, **6a-j**.

3.1.4 Physical and Spectral data of representative compounds

*(E)-2-(1-(4-Methyl-2-(phenylamino)thiazol-5-yl)ethylidene)hydrazinecarbothioamide (**5a**)*

^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 165.0, 159.0, 156.6, 152.7, 149.3, 140.0, 129.1, 118.1, 117.1, 18.4, 15.7; IR (KBr): ν (cm^{-1}) = 3429, 3215, 3059, 2854, 1600, 1496, 1446, 1324, 1123; MS: *m/z* (%) = $[M+1]^+$ = 306.4271

*(E)-2-(1-(4-Methyl-2-(m-tolylamino)thiazol-5-yl)ethylidene)hydrazinecarbothioamide (**5b**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.35 (1H, s, NH), 10.28 (2H, s, NH_2), 8.26 (1H, s, NH), 7.44-7.42 (1H, d, Ar, *J* = 8 Hz), 7.34 (1H, s, Ar), 7.22-7.18 (1H, t, Ar, *J* = 7.8 Hz), 6.801-6.79 (1H, d, Ar, *J* = 7.2 Hz), 2.43 (3H, s, CH_3), 2.31 (6H, s, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 178.2, 161.6, 148.7, 145.6, 140.7, 138.4, 129.1, 122.4, 119.4, 117.8, 114.4, 21.4, 18.4, 16.7; IR (KBr): ν (cm^{-1}) = 3407, 3259, 3064, 2953, 2805, 1598, 1490, 1347, 1283, 1118, 772, 697; MS: *m/z* (%) = $[M+1]^+$ = 320.4575

*(E)-2-(1-(4-Methyl-2-(p-tolylamino)thiazol-5-yl)ethylidene)hydrazinecarbothioamide (**5c**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.52 (3H, s, NH & NH_2), 8.33 (1H, s, NH), 7.44-7.41 (2H, d, Ar, *J* = 12.4 Hz), 7.06-7.04 (2H, d, Ar, *J* = 8.8 Hz), 2.45 (3H, s, CH_3), 2.32 (6H, s, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 178.5, 165.2, 157.3, 143.7, 139.7, 130.7, 123.4, 118.4, 115.1, 24.4, 16.1, 15.1; IR (KBr): ν (cm^{-1}) = 3413, 3303, 3266, 2963, 2806, 1597, 1511, 1444, 1289, 1116, 832; MS: *m/z* (%) = $[M+1]^+$ = 320.4575

*(E)-2-(1-(2-((4-Methoxyphenyl)amino)-4-methylthiazol-5-yl)ethylidene)hydrazinecarbo-thioamide (**5d**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.49 (1H, s, NH), 9.99 (2H, s, NH_2), 8.32 (1H, s, NH), 7.44-7.42 (2H, d, Ar, *J* = 8.8 Hz), 7.04-7.02 (2H, d, Ar, *J* = 8.8 Hz), 3.78 (3H, s, OCH_3), 2.44 (3H, s, CH_3), 2.31 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO): (δ ppm) = 178.2, 165.0, 157.0, 143.7, 140.8, 131.0, 123.0, 118.4, 114.8, 55.3, 16.1, 15.4; IR (KBr): ν (cm^{-1}) = 3450, 3311, 3181, 3059, 2972, 1716, 1632, 1550, 1379, 1288, 1120, 834; MS: *m/z* (%) = $[M+1]^+$ = 336.4576

*(E)-2-(1-(2-((2-Hydroxyphenyl)amino)-4-methylthiazol-5-yl)ethylidene)hydrazinecarbothioamide (**5e**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.35 (1H, s, NH), 9.94 (1H, s, NH_2), 9.51 (1H, s, NH_2), 8.25 (1H, s, NH), 7.96-7.97 (1H, d, Ar, *J* = 7.6 Hz), 7.15 (1H, s, OH), 6.48-6.91 (3H, m, Ar), 2.40 (3H, s, CH_3), 2.28 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 177.9, 163.0, 148.3, 147.0, 145.6, 128.3, 123.4, 120.4, 119.4, 119.1, 115.4, 18.4, 17.1; IR (KBr): ν (cm^{-1}) = 3421, 3243, 3051, 2808, 1602, 1454, 1341, 1237, 1111, 760; MS: *m/z* (%) = $[M+1]^+$ = 322.4270

*(E)-2-(1-(2-((3-Hydroxyphenyl)amino)-4-methylthiazol-5-yl)ethylidene)hydrazinecarbothioamide (**5f**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.37 (1H, s, NH), 9.50 (2H, s, NH_2), 8.27 (1H, s, NH), 7.22 (1H, s, OH), 7.16-7.08 (2H, m, Ar), 6.96 (1H, s, Ar), 6.42-6.40 (1H, d, Ar, *J* = 8 Hz), 2.44 (3H, s, CH_3), 2.31 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 178.1, 161.6, 158.0, 148.3, 145.3, 141.4, 129.7, 119.4, 109.1, 108.4, 104.7, 18.4, 16.7; IR (KBr): ν (cm^{-1}) = 3403, 3261, 3089, 2967, 1599, 1425, 1345, 1286, 1114, 843, 766; MS: *m/z* (%) = $[M+1]^+$ = 322.4270

*(E)-2-(1-(2-((4-Hydroxyphenyl)amino)-4-methylthiazol-5-yl)ethylidene)hydrazinecarbothioamide (**5g**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.31 (1H, s, NH), 9.99 (2H, s, NH_2), 8.21 (1H, s, NH), 7.35-7.33 (2H, d, Ar, *J* = 8.8 Hz), 7.16 (1H, s, OH), 6.75-6.73 (2H, d, Ar, *J* = 8.8 Hz), 2.40 (3H, s, CH_3), 2.28 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 177.9, 163.0, 153.0, 149.0, 145.7, 132.4, 120.0, 118.4, 115.5, 18.4, 16.8; IR (KBr): ν (cm^{-1}) = 3421, 3243, 3051, 2808, 2722, 1602, 1509, 1454, 1280, 1111, 848; MS: *m/z* (%) = $[M+1]^+$ = 322.4270

*(E)-2-(1-(2-((3-Chlorophenyl)amino)-4-methylthiazol-5-yl)ethylidene)hydrazinecarbothio-amide (**5h**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.42 (1H, s, NH), 9.72 (2H, s, NH_2), 8.30 (1H, s, NH), 7.81 (1H, s, Ar), 7.50-7.48 (1H, d, Ar, *J* = 8 Hz), 7.40-7.35 (1H, t, Ar, *J* = 8.2 Hz), 7.09-7.07 (1H, d, Ar, *J* = 8 Hz), 2.46 (3H, s, CH_3), 2.33 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 178.3, 161.6, 146.6, 144.8, 141.4, 133.5, 130.7, 122.0, 120.4, 117.4, 116.5, 17.7, 16.6; IR (KBr): ν (cm^{-1}) = 3435, 3261, 3129, 2959, 1634, 1558, 1431, 1260, 1105, 833, 777, 690; MS: *m/z* (%) = $[M+1]^+$ = 340.7811, $[M+3]^+$ = 342.1832

*(E)-2-(1-(2-((4-Chlorophenyl)amino)-4-methylthiazol-5-yl)ethylidene)hydrazinecarbothio-amide (**5i**)*

IR (KBr): ν (cm^{-1}) = 3417, 3237, 2806, 1595, 1499, 1348, 1122, 764, 818; MS: *m/z* (%) = $[M+1]^+$ = 340.7811, $[M+3]^+$ = 342.1832

*(E)-2-(1-(2-((4-Fluorophenyl)amino)-4-methylthiazol-5-yl)ethylidene)hydrazinecarbothio-amide (**5j**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.48 (1H, s, NH), 9.61 (2H, s, NH_2), 8.33 (1H, s, NH), 7.61-7.57 (2H, d, Ar, *J* = 14 Hz), 7.28-7.26 (2H, d, Ar, *J* = 6.8 Hz), 2.45 (3H, s, CH_3), 2.32 (3H, s, CH_3); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 178.2, 164.0, 160.0, 157.6,

144.0, 135.4, 122.0, 119.4, 116.1, 16.3; IR (KBr): ν (cm^{-1}) = 3451, 3317, 3187, 2950, 1650, 1504, 1233, 1125, 1033, 837; MS: *m/z* (%) = $[M+1]^+$ = 324.4175

*(E)-N-Cyclohexyl-2-(1-(4-methyl-2-(phenylamino)thiazol-5-yl)ethylidene)hydrazinecarbo-thioamide (**6a**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.48 (1H, s, NH), 7.74 (1H, s, NH), 7.60-7.58 (2H, d, Ar, *J* = 7.6 Hz), 7.38-7.34 (2H, t, Ar, *J* = 8 Hz), 7.07-7.04 (1H, t, Ar, *J* = 7.2 Hz), 4.15 (1H, s, NH), 2.44 (3H, s, CH_3), 2.32 (3H, s, CH_3), 1.91-1.31 (11H, m, Cy); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 176.2, 162.1, 146.3, 144.4, 139.9, 129.2, 122.9, 119.5, 118.3, 51.3, 31.8, 25.1, 24.2, 17.5, 16.6; IR (KBr): ν (cm^{-1}) = 3339, 3191, 2928, 2850, 1619, 1495, 1441, 1309, 1127; MS: *m/z* (%) = $[M+1]^+$ = 388.5771

*(E)-N-Cyclohexyl-2-(1-(4-methyl-2-(m-tolylamino)thiazol-5-yl)ethylidene)hydrazinecarbo-thioamide (**6b**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.35 (1H, S, NH), 7.55-7.53 (1H, d, Ar, *J* = 8.4 Hz), 7.39-7.25 (3H, m, (1H) NH & (2H) Ar), 6.95-6.93 (1H, d, Ar, *J* = 7.6 Hz), 4.15 (1H, s, NH), 2.44 (3H, s, CH_3), 2.32 (6H, s, $2CH_3$), 1.90-1.22 (11H, m, Cy); IR (KBr): ν (cm^{-1}) = 3326, 3206, 2929, 2853, 1633, 1524, 1378, 1257, 1128, 785, 698; MS: *m/z* (%) = $[M+1]^+$ = 402.5970

*(E)-N-Cyclohexyl-2-(1-(4-methyl-2-(p-tolylamino)thiazol-5-yl)ethylidene)hydrazinecarbo-thioamide (**6c**)*

^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 176.4, 173.1, 143.0, 139.0, 132.2, 134.4, 129.9, 128.1, 118.3, 52.0, 31.7, 25.1, 24.2, 20.9, 17.2, 14.8; IR (KBr): ν (cm^{-1}) = 3354, 3053, 2928, 2851, 1605, 1513, 1470, 1303, 1125, 822; MS: *m/z* (%) = $[M+1]^+$ = 402.5970

*(E)-N-Cyclohexyl-2-(1-(2-((4-methoxyphenyl)amino)-4-methylthiazol-5-yl)ethylidene)-hydrazinecarbothioamide (**6d**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.54 (1H,s, NH), 7.57 (1H, s, NH), 7.49-7.47 (2H, d, Ar, *J* = 7.2 Hz), 6.99-6.97 (2H, d, Ar, *J* = 8.8 Hz), 4.16 (1H, s, NH), 3.76 (3H, s, OCH_3), 2.44 (3H, s, CH_3), 2.30 (3H, s, CH_3), 1.92-1.23 (11H, m, Cy); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 176.4, 172.2, 164.0, 156.4, 143.6, 132.3, 122.0, 118.9, 114.6, 55.3, 51.2, 31.5, 25.0, 23.9, 21.1, 16.3; IR (KBr): ν (cm^{-1}) = 3338, 3221, 2928, 2849, 1618, 1520, 1385, 1251, 1115, 828; MS: *m/z* (%) = $[M+1]^+$ = 418.5972

*(E)-N-Cyclohexyl-2-(1-(2-((2-hydroxyphenyl)amino)-4-methylthiazol-5-yl)ethylidene)-hydrazinecarbothioamide (**6e**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.59 (1H, s, NH), 9.23 (1H, s, NH), 7.87-7.85 (2H, d, Ar, , *J* = 7.2 Hz), 7.47-7.37 (2H, dd, Ar, *J* = 8 Hz), 5.75 (1H, s, OH), 4.17 (1H, s, NH), 2.37 (3H, s, CH_3), 2.17 (3H, s, CH_3), 1.92-1.24 (11H, m, Cy); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 176.4, 173.1, 167.3, 143.3, 139.0, 136.3, 134.4, 131.7, 129.8, 128.1, 118.5, 51.9, 31.8, 24.2, 20.9, 17.2, 14.7; IR (KBr): ν (cm^{-1}) = 3438, 3255, 3023, 2801, 1615, 1478, 1335, 1218, 1108, 771; MS: *m/z* (%) = $[M+1]^+$ = 404.5672

*(E)-N-Cyclohexyl-2-(1-(2-((3-hydroxyphenyl)amino)-4-methylthiazol-5-yl)ethylidene)-hydrazinecarbothioamide (**6f**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.49 (1H, s, NH), 7.91 (1H, s, NH), 7.55-7.53 (1H, d, Ar, *J* = 8.4 Hz), 7.16-7.12 (1H, t, Ar, *J* = 8.2 Hz), 7.05 (1H, s, Ar), 6.96-6.94 (1H, d, Ar, *J* = 9.2 Hz), 6.51 (1H, s, OH), 4.16 (1H, s, NH), 2.44 (3H, s, CH_3), 2.32 (3H, s, CH_3), 1.90-1.32 (11H, m, Cy); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 176.2, 162.5, 158.2, 144.2, 140.6, 129.9, 119.5, 110.7, 109.4, 105.8, 5.3, 31.8, 25.1, 24.2, 17.2, 16.6; IR (KBr): ν (cm^{-1})

= 3333, 3190, 2929, 2851, 1581, 1524, 1308, 1201, 1129, 862, 782; MS: *m/z* (%) = $[M+1]^+$ = 404.5672

*(E)-N-Cyclohexyl-2-(1-(2-((4-hydroxyphenyl)amino)-4-methylthiazol-5-yl)ethylidene)-hydrazinecarbothioamide (**6g**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.59 (1H, s, NH), 7.55-7.53 (2H, d, Ar, *J* = 8 Hz), 7.29 (2H, s, OH, NH), 6.85-6.82 (2H, d, Ar, *J* = 8.8 Hz), 4.14 (1H, s, NH), 2.42 (3H, s, CH_3), 2.30 (3H, s, CH_3), 1.83-1.23 (11H, m, Cy); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 176.4, 165.1, 155.7, 143.2, 129.8, 123.5, 11.3, 116.1, 52.9, 31.4, 25.1, 23.8, 15.9, 15.3; IR (KBr): ν (cm^{-1}) = 3443, 3354, 3196, 2938, 2853, 1595, 1522, 1451, 1266, 1126, 834; MS: *m/z* (%) = $[M+1]^+$ = 404.5672

*(E)-2-(1-(2-((3-Chlorophenyl)amino)-4-methylthiazol-5-yl)ethylidene)-N-cyclohexyl-hydrazinecarbothioamide (**6h**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.47 (1H, s, NH),7.86 (1H, s, Ar), 7.54-7.52 (2H, d, (1H) NH & 1H (Ar), *J* = 8.4 Hz), 7.34-7.32 (1H, d, Ar, *J* = 8 Hz), 7.04-7.02 (1H, d, Ar, *J* = 8 Hz), 4.15 (1H, s, NH), 2.46 (3H, s, CH_3), 2.32 (3H, s, CH_3), 1.93-1.23 (11H, m, Cy); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 176.2, 160.9, 147.5, 144.4, 141.8, 133.2, 130.5, 121.3, 120.7, 116.8, 115.9, 51.3, 31.8, 25.1, 24.2, 18.1, 16.6; IR (KBr): ν (cm^{-1}) = 3330, 3179, 2931, 2852, 1633, 1571, 1449, 1202, 1124, 892, 783, 671; MS: *m/z* (%) = = $[M+1]^+$ = 423.1123, $[M+3]^+$= 425.6321

*(E)-2-(1-(2-((4-Chlorophenyl)amino)-4-methylthiazol-5-yl)ethylidene)-N-cyclohexyl-hydrazinecarbothioamide (**6i**)*

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.49 (1H, s, NH), 9.03 (1H, s, NH), 7.66-7.64 (2H, d, Ar, *J* = 8.8 Hz), 7.41-7.39 (2H, d, Ar, *J* = 8.8 Hz), 4.14 (1H, s, NH), 2.45 (3H, s, CH_3), 2.32 (3H, s, CH_3), 1.91-1.31 (11H, m, Cy); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) =

176.2, 161.9, 146.3, 144.2, 139.0, 129.0, 125.9, 120.1, 51.3, 31.8, 25.1, 24.1, 17.5, 16.6; IR (KBr): ν (cm^{-1}) = 3339, 3191, 2928, 2850, 1619, 1583, 1525, 1495, 1200, 1127, 774, 615; MS: *m/z* (%) = $[M+1]^+$ = 423.1123, $[M+3]^+$= 425.6321

(E)-N-Cyclohexyl-2-(1-(2-((4-fluorophenyl)amino)-4-methylthiazol-5-yl)ethylidene)-hydrazinecarbothioamide ***(6j)***

^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm) = 10.53 (1H, s, NH), 7.64-7.61 (3H, d, (1H) NH & (2H) Ar, *J* = 9.2 Hz), 7.27-7.25 (2H, d, Ar, *J* = 8.4 Hz), 4.15 (1H, s, NH), 2.44 (3H, s, CH_3), 2.32 (3H, s, CH_3), 1.88-1.23 (11H, m, Cy); ^{13}C-NMR (100 MHz, DMSO): δ (ppm) = 176.2, 163.1, 159.4, 157.3, 143.9, 135.6, 121.3, 119.5, 116.1, 51.3, 31.5, 25.0, 23.9, 16.8, 16.3; IR (KBr): ν (cm^{-1}) = 3348, 3193, 2929, 2853, 1587, 1521, 1314, 1207, 1123, 1041, 845; MS: *m/z* (%) = $[M+1]^+$ = 406.5673

3.2 Biological assay

This work has been done in Microcare Laboratory and TRC, Surat, India as per the reported method. (Hawkey and Lewis, 2003)

4. Biological Evaluation

All newly prepared thiazole hybrid thiosemicarbazone derivatives were inspected for antimicrobial activity against two Gram-positive bacterial strains, two Gram-negative bacterial strains and three fungal strains using the agar dilution method [36]. Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamycin and Norfloxacin were used as standard control drugs for antibacterial activity, whereas Griseofulvin and Nystatin were used as standard control drugs for antifungal activity.

The *in-vitro* antimalarial activity was carried out in 96 well microtiter plates according to the microassay protocol of Reickmann and co-workers [37] with minor modifications. Chloroquine and Quinine were used as standard control drugs for antimalarial activity as well

as Isoniazid and Rifampicin were used as standard control drugs for the anti-tuberculosis activity. The zone of inhibition and minimum inhibitory concentrations (MIC) were noted (Table 3).

Table 3. *In-vitro* antibacterial, antifungal, antimalarial and anti-tuberculosis activities of compounds **5a-j** and **6a-j**.

Entry	Compound code	*In-vitro* Antibacterial activity MIC[a] (µg/mL)				*In-vitro* Antifungal activity MFC[b] (µg/mL)			*In-vitro* Antimalarial activity IC_{50}[c] (µg/mL)	*In-vitro* Anti-tuberculosis activity MIC[a] (µg/mL)
		EC[d]	SA[e]	PA[f]	SP[g]	CA[h]	AN[i]	AC[j]	PF[k]	H37Rv[l]
1	5a	**100**	500	**100**	500	**500**	250	250	0.82	500
2	5b	125	**250**	125	250	1000	250	250	0.97	50
3	5c	250	**125**	200	125	**500**	500	500	1.04	1000
4	5d	200	**100**	250	200	1000	1000	1000	0.86	25
5	5e	250	500	200	500	**500**	1000	1000	0.90	100
6	5f	200	**100**	250	**100**	**250**	>1000	>1000	1.06	500
7	5g	250	**200**	250	250	**500**	500	500	1.11	62.5
8	5h	**100**	**200**	125	200	1000	1000	1000	0.84	500
9	5i	200	**200**	200	200	1000	>1000	>1000	1.02	500
10	5j	125	**200**	125	200	**500**	1000	1000	0.99	250

11	6a	**100**	**250**	250	250	**250**	500	500	1.70	500
12	6b	125	**62.5**	**100**	**100**	**500**	500	1000	2.18	500
13	6c	**100**	**250**	**100**	**50**	1000	500	>1000	2.57	500
14	6d	125	500	125	250	**500**	1000	1000	1.15	250
15	6e	250	**100**	250	250	**500**	250	250	0.73	1000
16	6f	125	**100**	**125**	125	1000	500	500	0.65	1000
17	6g	250	**12.5**	**100**	**100**	**250**	500	>1000	0.32	250
18	6h	250	**125**	500	**100**	**250**	250	250	0.84	250
19	6i	125	**100**	125	**62.5**	1000	250	250	0.82	500
20	6j	**100**	**125**	125	**100**	**500**	500	1000	1.40	250
21	Ampicillin[m]	100	250	100	100	-	-	-	-	-
22	Chloramphenicol[m]	50	50	50	50	-	-	-	-	-
23	Ciprofloxacin[m]	25	50	25	50	-	-	-	-	-
24	Gentamycin[m]	0.05	0.25	1	0.5	-	-	-	-	-
25	Norfloxacin[m]	10	10	10	10	-	-	-	-	-
26	Griseofulvin[m]	-	-	-	-	500	100	100	-	-

27	Nystatin[m]	-	-	-	-	100	100	100	-		-
28	Chloroquine[m]	-	-	-	-	-	-	-	0.020		-
29	Quinine[m]	-	-	-	-	-	-	-	0.268		-
30	Isoniazid[m]	-	-	-	-	-	-	-	-		0.20
31	Rifampicin[m]	-	-	-	-	-	-	-	-		0.25

[a] MIC= Minimum Inhibitory Concentration

[b] MFC= Minimum Fungicidal Concentation

[c] IC_{50}= Half maximal inhibitory concentration

[d] EC= *Escherichia coli* (*E. coli*) MTCC 443, [e] SA= *Staphylococcus aureus* (*S. aureus*) MTCC 96, [f] PA= *Pseudomonas aeruginosa* (*P. aeruginosa*) MTCC 1688, [g] SP= *Streptococcus pyogenes* (*S. pyogenes*) MTCC 442, [h] CA= *Candida albicans* (*C. albicans*) MTCC 227, [i] AN= *Aspergillus niger* (*A. niger*) MTCC 282, [j] AC= *Aspergillus clavatus* (*A. clavatus*) MTCC 1323, [k] PF= *Plasmodium falciparum 3D7* (*P. falciparum*), [l] H37Rv= *Mycobacterium tuberculosis* strain

[m] = Standard control drug

4.1. In-vitro antibacterial activity

The *in-vitro* antibacterial activities of compounds **5a–j** and **6a-j** were evaluated against Gram-positive and Gram-negative bacteria using the cultures of four different standard microorganisms: *Escherichia coli* (*E. coli*) MTCC 443 and *Pseudomonas aeruginosa* (*P. aeruginosa*) MTCC 1688 as Gram-negative models, and *Staphylococcus aureus* (*S. aureus*) MTCC 96 and *Streptococcus pyogenes* (*S. pyogenes*) MTCC 442 as a Gram-positive model. The results of this study are given in Table 3.

Bioassay results of series of **5a-j** and **6a-j** compounds revealed that almost all derivatives (**5b-d**, **5f-j**, **6a-c, 6e-j**) were found to exhibit excellent activity against specific *S. aureus* microbial strain compared to standard control drug Ampicillin. Compound **5a** having unsubstituted phenyl ring was found to be equally effective with 100 μg/mL MIC value against *E. coli* and *P. aeruginosa* bacteria compared to Ampicillin. Compound **5h** with chloro group on *meta*-position of the phenyl ring showed equal potency with 100 μg/mL MIC value against *E. coli* compared to Ampicillin. Compound **5f** having electron donating hydroxyl group on the *meta*-position of the phenyl ring found 100 μg/mL MIC value against *S. pyogenes* equivalent to Ampicillin. Compound **6a**, **6c** and **6j** showed MIC values of 100 μg/mL and are equally active as compared to Ampicillin against *E. coli* microorganism. Compound **6b**, **6c** and **6g** (MIC 100 μg/mL) are equally potent against *P. aeruginosa* strain whereas compound **6b**, **6g**, **6h** and **6j** (MIC 100 μg/mL) are equally potent against *S. pyogenes* strain. Compound **6b** having methyl group on *meta*-position of phenyl ring has presented excellent activity (MIC 62.5 μg/mL) against *S. aureus* compared to Ampicillin whereas compound **6g** having hydroxyl group on *para*-position of phenyl ring exhibited excellent activity against the same microorganism compared to standard drugs Ampicillin, Chloramphenicol and Ciprofloxacin with MIC value 12.5 μg/mL. Compound **6c** having a

methyl group on *para*-position of the phenyl ring is equally potent (MIC 50 μg/mL) against *S. pyogenes* compared to Chloramphenicol and Ciprofloxacin, however 50% more active (MIC 50 μg/mL) against the same microorganism compared to Ampicillin. Compound **6i** having chloro group on *para*-position of the phenyl ring has shown excellent activity (MIC 62.5 μg/mL) against the same microorganism compared to Ampicillin.

4.2. In-vitro antifungal activity

The *in-vitro* antifungal activity of compound **5a-j** and **6a-j** were evaluated against the cultures of three different standard fungal strains: *Candida albicans* (*C. albicans*) MTCC 227, *Aspergillus niger* (*A. niger*) MTCC 282 and *Aspergillus clavatus* (*A. clavatus*) MTCC 1323. The results of this study are given in Table 3.

The antifungal activities of thiazole fused thiosemicarbazone derivatives (**5a-j** and **6a-j**) indicate that, thirteen out of twenty novel compounds (**5a**, **5c**, **5e**, **5f**, **5g**, **5j**, **6a**, **6b**, **6d**, **6e**, **6g**, **6h** and **6j**) tested in the present study were found to have good to excellent activity against the *C. albicans* microorganism compared to standard control drug Griseofulvin, whereas none of them found active against *A. niger* and *A. clavatus* fungal strains compared to both the standard control drugs Griseofulvin and Nystatin. The best results were encountered with compounds **5f**, **6a**, **6g** and **6h** with the MIC value of 250 μg/mL against *C. albicans* compared to Griseofulvin. Results of the remaining five compounds **5a**, **5c**, **5e**, **5g**, **5j**, **6b**, **6d**, **6e** and **6j** showed MIC values 500 μg/mL, and were equally effective as compared to Griseofulvin against *C. albicans* microorganisms.

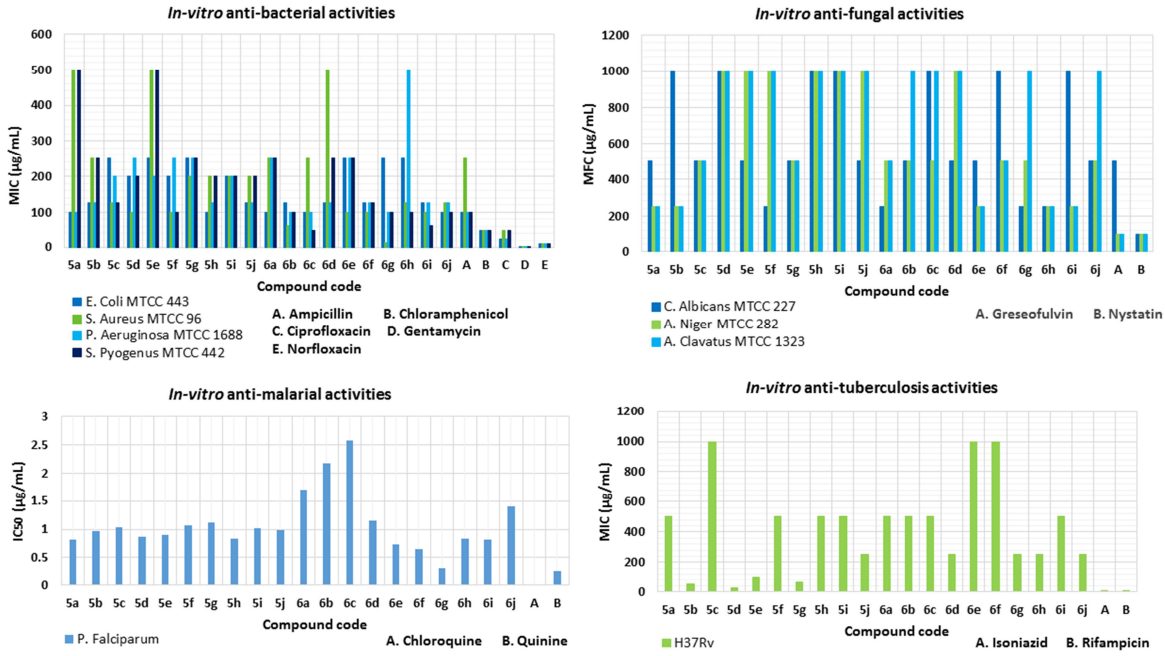


Figure 1. Graphical presentation of biological assay

4.3 In-vitro antimalarial and anti-tuberculosis activity

All the newly synthesized compounds (**5a-j** and **6a-j**) were also screened for their *in-vitro* antimalarial activity against *Plasmodium falciparum 3D7* (*P. falciparum*) strain by measuring the mean Half Maximal Inhibitory Concentration (IC_{50} µg/mL) values. All experiments were performed in duplicate. As shown in Table 3, neither of the compounds had shown satisfactory results against *P. falciparum* as good as standard control drug Chloroquine and Quinine.

The *in-vitro* anti-tuberculosis activity of series of compounds (**5a-j** and **6a-j**) was performed against *Mycobacterium tuberculosis* H37Rv microorganism compared to standard drugs Isoniazid and Rifampicin. None of the synthesized compounds match up to the MIC (µg/mL) values of the standard control drugs Isoniazid (0.20 µg/mL) and Rifampicin (0.25 µg/mL) against the H37Rv strain of tuberculosis.

5. ADME Study

ADME properties were used to evaluate *in-silico* pharmacophoric properties leading to drug-likeness (Schrodinger, LLC, New York, NY, 2015). ADME properties of the title molecules were analyzed using QikProp incorporated in Maestro 11. Jorgensen's method was utilized to compute pharmacophoric properties and descriptors in QikProp tool [38].

Pharmacophoric properties of the title molecules were described in table 4 with their acceptable limits. Most of the compounds have polar surface area in the acceptable range of limit assures good impact on bioavailability of the molecule (Lu *et al.*, 2004). Caco-2 cell permeability (QPPCaco) for the predicted molecules shows excellent intestinal absorption. Water solubility parameter (QPlogS) of all the tested molecules except **5e** shows results out of the permissible range. Further, octanol/water partition coefficient (QPlogPo/w) of all the tested molecules except **6h** and **6i**; and percentage human oral absorption (PHOA) of all the compounds showed outcome in the acceptable range. Moreover, the results vary in the permissible range for blood/brain partition coefficient (QPPlogBB). We consider that this survey may help in broadening the scope for further evaluation of deserving molecules.

Table 4. ADME results.

Comp. Code	**PSA[a] (70-200 Ao)**	**QPPCaco[b] (<25 poor, >500 great)**	**QPlogBB[c] (-1.5 to -3.0)**	**QPlogPo/w[d] (<5)**	**QPlogS[e] (>-4)**	**PHOA[f] (<25% poor, >80% high)**
1	79.61	723.086	-0.705	2.306	-4.155	91.62
2	76.378	1004.546	-0.543	2.813	-4.76	100
3	79.637	723.667	-0.739	2.627	-4.671	93.508
4	84.589	1003.703	-0.6	2.649	-4.527	96.178
5	99.977	301.96	-1.173	1.711	-3.845	81.348

6	102.201	219.469	-1.455	1.76	-4.422	79.158
7	98.904	305.069	-1.133	1.735	-3.987	81.571
8	79.632	722.245	-0.557	2.816	-4.849	94.599
9	79.625	723.509	-0.555	2.814	-4.844	94.602
10	79.617	722.948	-0.601	2.569	-4.5	93.16
11	64.441	2408.535	-0.341	4.694	-6.733	100
12	64.469	2382.896	-0.367	4.992	-7.281	100
13	64.456	2400.401	-0.361	4.991	-7.278	100
14	72.751	2378.109	-0.413	4.76	-6.783	100
15	85.749	873.86	-0.934	3.98	-6.378	100
16	87.009	729.378	-1.029	3.919	-6.391	100
17	86.968	731.825	-1.026	3.918	-6.386	100
18	64.422	2406.793	-0.181	5.18	-7.453	100
19	64.434	2401.204	-0.182	5.178	-7.456	100
20	64.446	2399.984	-0.234	4.921	-7.081	100

[a] PSA= Polar Surface Area

[b] QPPCaco= Caco-2 cell permeability

[c] QPLogBB= Blood/brain partition co-efficient

[d] QPLogPo/w= Patition co-efficient

[e] QPLogS= Water Solubitity

[f] PHOA= Percentage Human Oral Absorption

6. Molecular Docking Study

Molecular Docking was performed using Glide tool in maestro 11. Protein (PDB id: 5D1Q) was downloaded from the protein data bank (RCSB PDB) and prepared with the help of a protein preparation wizard by using default parameters and saved. All the ligands were

prepared by LigPrep tool. Sitemaps were defined by using SiteMap tool, sitemap used for receptor grid generation has a site score of 1.032. Receptor grid was generated in Glide utilizing the Receptor Grid Generation tool using default parameters, which was further used for molecular docking of prepared ligands using Ligand Docking tool. Docking was performed in XP (extra precision) mode. Generated docking results were described in table 5. Poses of the docked ligands were captured using Maestro 11.

Table 5. Molecular Docking.

Sr. No.	Compound Code	GScore	Sr. No.	Compound Code	GScore
1	5a	-3.897	11	6a	-2.977
2	5b	-3.836	12	6b	-4.586
3	5c	-4.263	13	6c	-3.091
4	5d	-4.488	14	6d	-1.87
5	5e	-4.123	15	6e	-3.054
6	5f	-4.626	16	6f	-4.232
7	5g	-3.166	17	6g	-5.14
8	5h	-3.892	18	6h	-2.274
9	5i	-4.071	19	6i	-2.867
10	5j	-4.326	20	6j	-2.364

Protein used for this study is classified as an immune system of *S. aureus* in *Homo sapiens* expression system. X-ray diffraction method was used, and resolution of protein is 3.22 Å. Molecular docking score correlates with of *S. aureus* strain *in-vitro* activity in maximum extent. There is no hydrogen bond between ligand **6g** and protein molecule, so the lowest energy of **6g** is due to van der Waal's force of attraction (Fig. 2, 6g). Description of poses is explained in table 6 and poses are presented in figure 2.

Table 6. Ligand-Protein interactions.

Compound Code	**Atom**[a]	**H-Bond**	
		Amino acid[b]	**Bond-Length (Å)**
5f	H of OH	O of GLN 43	2.00
	H of NH	O of GLN 39	2.74
	H of NH	O of GLY 41	2.62
	H of NH_2	O of GLY 41	1.94
	H of NH_2	O of GLN 105	2.00
6b	H of NH	O of GLU 152	2.16

[a] =Bonded atom of the ligand

[b] =Interactive group or atom of the amino acid

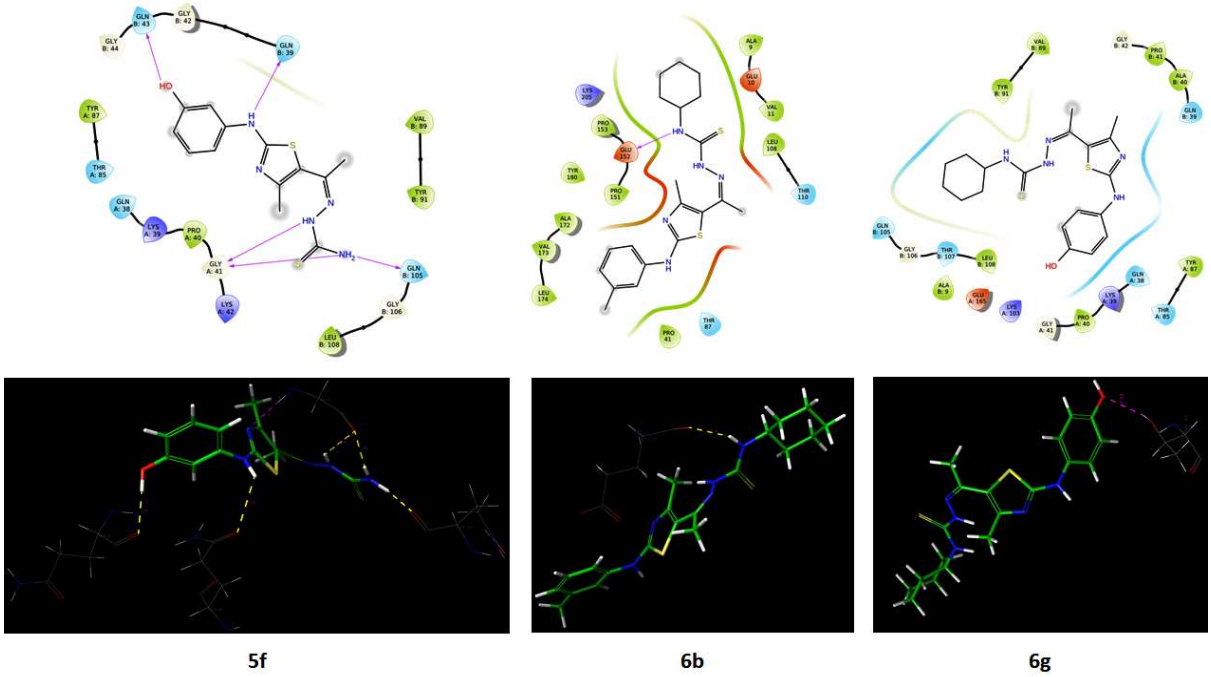


Figure 2. Docking poses

7. Conclusion

In conclusion, some new thiazole hybrid thiosemicarbazide (**5a-j** and **6a-j**) were synthesized using a mild and efficient microwave irradiation method at 300W power with 20 mol% gla. acetic acid as catalyst and ethanol as solvent. This protocol proceeds smoothly in case of thiazole substituted with electron releasing groups on the phenyl ring. The major advantages of the present protocol are excellent product yield, short reaction time and a simple work-up procedure. Bioassay study of the newly synthesized compounds reveals that thirteen out of twenty compounds presented good *in-vitro* antibacterial activity against *S. aureus*. Compound **6b** and **6g** exhibited highly potent activity against most of the tested bacterial strains and docking study culminate in the identification of a new class of potent inhibitors of 5D1Q.

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Supplementary Data:

Supporting information contains the spectral data (Mass, IR and Proton and Carbon NMR spectra) of all compounds.

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Highlights:

- Series of novel Thiazole fused thiosemicarbazones was synthesized using microwave irradiation (MWI) method.
- The newly synthesizes compounds were characterized by Mass, IR, ^{1}H- and ^{13}C-NMR spectroscopy.
- *In-vitro* antimicrobial, antimalarial and anti-tuberculosis activities were evaluated.
- Molecular docking and ADME study of all derivatives was performed.