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2-(2-Hydrazinyl)thiazole derivatives: Design, synthesis and *in vitro* antimycobacterial studies

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ABSTRACT

In an attempt to discover new potent inhibitors for *Mycobacterium tuberculosis* (*Mtb*), a series of 2-(2-hydrazinyl)thiazole derivatives with a wide range of substitutions at 2-, 4- and 5-positions were designed by considering Lipinski rule. The designed compounds were synthesized, characterized and evaluated for their inhibitory potential against *Mtb*, $H_{37}Rv$, by *in vitro* assay. The compounds, ethyl-4-methyl-2-[(*E*)-2-[1-(pyridin-2-yl)ethylidene]hydrazin-1-yl]-1,3-thiazole-5-carboxylate, **4d**, and ethyl-2-[(*E*)-2-[(2-hydroxyphenyl)methylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate, **2i** showed noticeable inhibitory activity against *Mtb*, $H_{37}Rv$ with minimum inhibitory concentration (MIC) of 12.5 μM and 25 μM respectively. An attempt has been made to understand the mechanism of action by binding interactions of these molecules with β-ketoacyl-ACP synthase protein through docking studies. The inhibition constants for compounds **4d** and **2i** were found to be 1.46 μM and 0.177 μM respectively.

1. Introduction

Tuberculosis (TB) is an airborne and extremely contagious ancient disease which is caused by the *Mycobacterium tuberculosis* (*Mtb*) [1]. Although the causative agent for this disease was discovered by Robert Koch in 1882, the chemotherapy was started in 1944 with streptomycin, an antibiotic derived from *Streptomyces griseus* [2]. Besides persistent effort to eradicate this disease, it continues to be one of the major global health threat. According to the sixteenth global report of the World Health Organization (WHO), closely 2 million people died among the 8.8 million incident cases of TB in 2010 and fatalities may reach up to 36 million by 2020 [3]. The progress in eradicating TB is stagnant in high burden countries due to poverty, synergy with the HIV/AIDS pandemic [4–7] and slow progress in developing novel and target effective drugs [8,9]. The progress in developing new therapeutics and their clinical uses to treat TB are apprehensive due to the disease complexity, prolonged and poor administration of treatment. Though streptomycin cured TB initially, *Mtb* developed resistance towards streptomycin, due to this, other drugs such as *para*-aminosalicylic acid (1948), isoniazid (1951), pyrazinamide (1952), cycloserine (1952), ethionamide (1956), rifampin (1957), kanamycin (1957) and ethambutol (1962) *etc*. have been developed and the multidrug therapy, a combination of two or three or four drugs has also been introduced in the 1970s [10]. The progress in chemotherapy to treat TB was overshadowed by drug susceptibility to *Mtb*, as a result, evolution of multi-drug resistant (MDR), extensively drug-resistant (XDR) strains took place and very recently, totally drug-resistant TB (TDR) has also been reported [11]. It is very disappointing to note that the practice of using a combination of four drugs chemotherapy for the duration of six months is still followed in clinical practice and no approval of new class of drugs for curing drug susceptible TB has been given so far. Multidrug therapies and the alarming increase of MDR-TB and XDR-TB strains of *Mtb* pose the biggest challenge in treating TB [12–15]. Hence, an inevitable progress is mandatory in developing novel, effective and safe anti-TB drugs which can target MDR-TB and XDR-TB strains through new biochemical pathways.

In an attempt to generate novel anti-TB drugs, during the last decade, a wide spectrum of known and novel scaffolds has been tested for their ability to inhibit *Mtb*. Fluoroquinolones, rifamycins, oxazolidinones and riminophenazines are some of the known drugs which have been repurposed to treat TB [15]. On the other hand, the discovery and development of new chemical entities such

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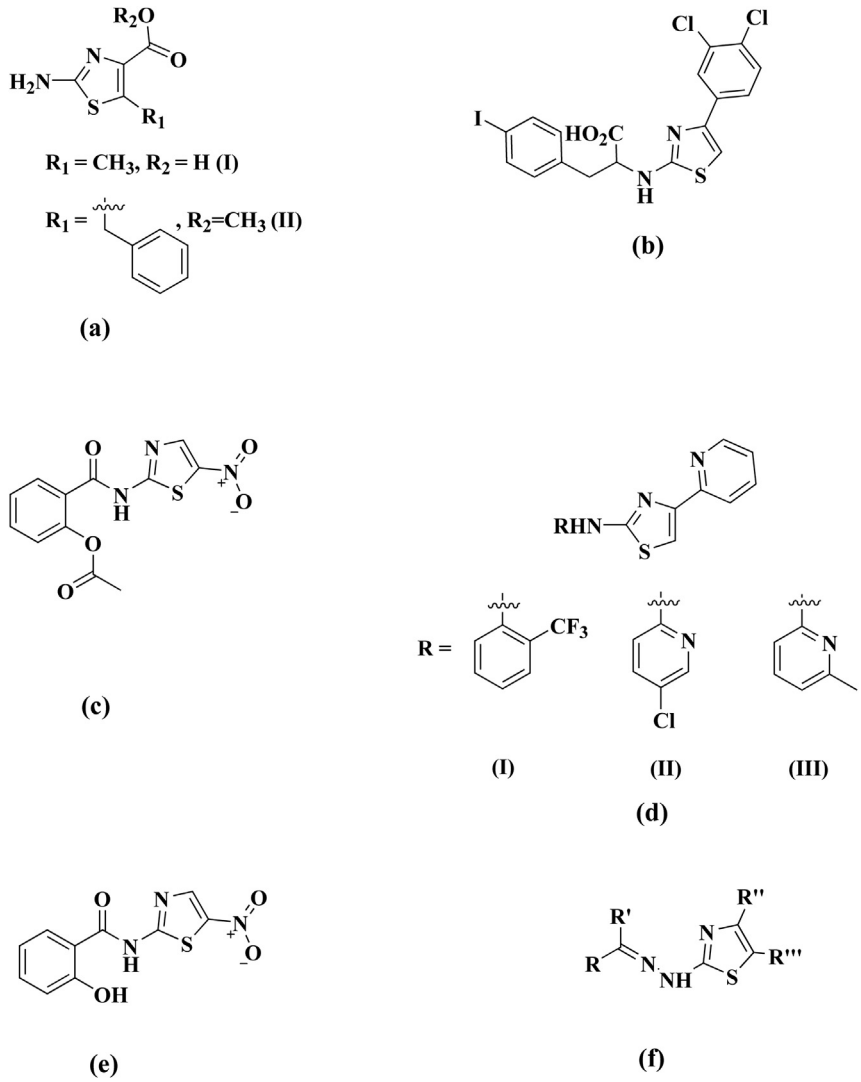


Fig. 1. Potent aminothiazole derivatives against *Mtb*. (a) 2-Aminothiazole-4-carboxylates, (b) 2-((4-(3,4-dichlorophenyl)thiazol-2-yl)amino)-3-(4-iodophenyl)propanoic acid, (c) NTZ, (d) thiazolamines, (e) TIZ, (f) 2-(2-hydrazinyl)thiazole derivatives designed for the present investigation.

as nitroimidazoles, dipiperidine, capuramycin, benzothiazinone and caprazene nucleoside have also been successfully tested against TB. Both the repurposed and novel molecules are currently in the pool of clinical trials [16]. Bedaquiline and diarylquinoline derivatives, which primarily targets ATP synthase have been recently approved by the FDA to use as part of the combination therapy for adults with MDR pulmonary TB [17]. In addition to these successful candidates, 2-aminothiazoles, benzopyranones, phenoxyalkylimidazoles, thieno[2,3-*d*]pyrimidines, thiophene-1,1-dioxides, piperidinamines, thioamides, ureas, thioureas, adamantyl amides and amines, 5-nitrofuran-2-carboxamides and diazolylthio derivatives were also screened and found to have good inhibition against *Mtb*, $H_{37}Rv$ strain [18]. Among the promising scaffolds, 2-aminothiazole scaffold (See Fig. 1) is of particular interest due to its target specificity and its enhanced inhibition of *Mtb*. Moreover, thiazole derivatives have been constantly evaluated for their diverse biological activities as anticancer [19] and antitumor [20,21], antimalarial [22], antimicrobial [23], anti-inflammatory [24] and anti hypolipidemic [25] agents. The synthesis of thiazole scaffold can be easily attained by classical Hantzsch thiazole methodologies and the scope for generating novel thiazole derivatives is also high [26].

2-Aminothiazole scaffold is structurally similar to thiolactomycin (TLM), a naturally occurring and synthetically challenging antibiotic [27]. TLM inhibits β-ketoacyl-ACP synthase (KasA) in mtFabH fatty acid synthesis and consequently inhibits the cell wall biosynthesis leading to the death of *Mtb* [28]. Interestingly, 2-aminothiazole-4-carboxylate derivatives (see a in Fig. 1) are potent inhibitors of KasA protein [29]. Similarly, 2-aminothiazole derivative (see b in Fig. 1) has shown superior biological activity over thiazolidinones in inhibiting UDP-galactopyranose mutase thwart biochemical pathway to inhibit *Mtb* [30]. Nitazoxanide (NTZ) (see c in Fig. 1), a drug approved by FDA to treat protozoal infections [31], 2-aminothiazole-4-carboxylates (see a in Fig. 1) and 2-amino-4-(2-pyridyl) thiazoles (see d in Fig. 1) are currently in clinical trials [32]. Very few recent reports suggest that 2-(2-hydrazinyl)thiazole derivatives comprising 2-aminothiazole scaffold have exhibited good activity against *Mtb* strain, $H_{37}Rv$ [33–35]. As 2-aminothiazole scaffold shows promising anti-TB activity, in the present investigation, 2-(2-hydrazinyl) thiazole derivatives with a wide range of substitutions at 2-, 4- and 5-positions by considering Lipinski rule of five [36] are designed, synthesized and evaluated for their inhibitory activity against *Mtb*, $H_{37}Rv$.

Table 1
Data calculated[a] using Lipinski rule of five for 2-(2-hydrazinyl) thiazole derivatives along with antimycobacterial activity against MTB.

Entry	Compound	Lipinski rule of five					MIC (μM) against MTB
		Log *P*	MW	H acceptors	H donors	No. of violations	
1	**2a**	2.953	289.360	5	1	0	>200
2	**2b**	3.400	303.387	5	1	0	>200
3	**2c**	3.563	321.377	5	1	0	200
4	**2d**	3.583	323.805	5	1	0	>200
5	**2e**	4.054	337.832	5	1	0	>200
6	**2f**	3.631	323.805	5	1	0	>200
7	**2g**	4.237	358.250	5	1	0	>200
8	**2h**	3.738	368.256	5	1	0	>200
9	**2i**	2.893	305.359	6	2	0	25
10	**2j**	2.450	305.359	6	2	0	>200
11	**2k**	3.226	303.387	5	2	0	>200
12	**2l**	3.010	319.386	6	1	0	>200
13	**2m**	2.279	335.385	7	2	0	>200
14	**2n**	2.668	349.412	7	2	0	>200
15	**2o**	2.292	335.385	7	2	0	>200
16	**2p**	2.600	349.412	7	1	0	>200
17	**2q**	2.843	333.369	7	1	0	>200
18	**2r**	2.584	379.438	8	1	0	>200
19	**2s**	3.402	303.387	5	1	0	>200
20	**2t**	4.465	331.441	5	1	0	>200
21	**2u**	3.055	332.429	6	1	0	200
22	**2v**	2.081	288.352	5	1	0	>200
23	**2w**	2.893	305.359	6	2	0	200
24	**2x**	3.928	328.224	4	1	0	>200
25	**2y**	2.275	349.412	7	1	0	>200
26	**2z**	2.510	303.343	6	1	0	>200
27	**4a**	2.210	279.321	6	1	0	200
28	**4b**	3.299	309.416	5	1	0	100
29	**4c**	3.103	328.397	6	2	0	200
30	**4d**	2.229	304.375	6	1	0	12.50
31	**4e**	2.915	302.403	5	1	0	>200
32	**4f**	2.110	304.375	6	1	0	200
33	**5a**	1.901	249.295	5	1	0	200
34	**5b**	2.989	279.39	4	1	0	200
35	**5c**	2.794	298.371	5	2	0	>200
36	**5d**	1.920	274.349	5	1	0	100
37	**6b**	2.151	304.375	6	1	0	200
38	**6a**	2.217	304.375	6	1	0	200
39	Isoniazide	−0.969	137.142	4	3	0	0.45
40	TLM	3.455	210.298	2	1	0	–

[a] Calculated from online server http://www.molinspiration.com/cgi-bin/properties.

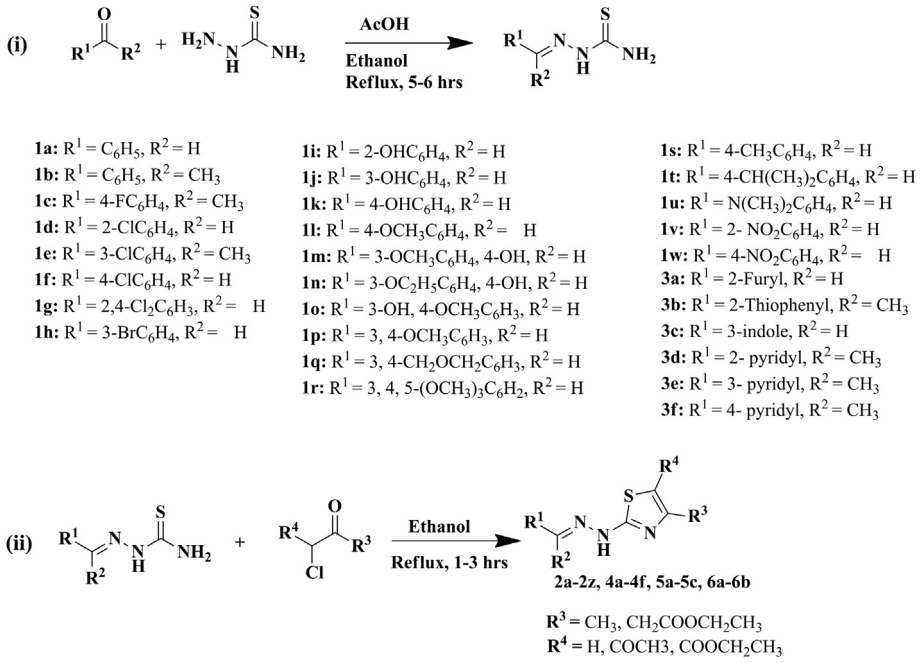
2. Results and discussion

To identify new lead molecules to treat *Mtb*, initially, 2-(2-hydrazinyl)thiazole derivatives with both hydrophilic and lipophilic inducing groups have been designed as shown in Fig. 1 (see f). It is interesting to note that the NTZ (see c in Fig. 1) and its hydrolyzed active metabolite tizoxanide (TIZ) (see e in Fig. 1) have shown the inhibition of replicating *Mtb* with MIC value of 52.12 μM and 60.38 μM respectively. It is suggested that the time-dependent anti-TB effect of NTZ [37] can be enhanced with the modification of head groups [38]. Similarly, by considering the structural features of other compounds shown in Fig. 1, the rational design of the prototypic modified analogs are proposed as shown in Fig. 1(f). The designed hybrid 2-(2-hydrazinyl)thiazole scaffold derivatives are retained with core 2-aminothiazole scaffold and additionally both lipophilic and hydrophilic inducing groups are introduced proportionally to maintain the cell wall permeability and the solubility.

2.1. Lipinski rule analysis

Before the synthesis of designed 2-(2-hydrazinyl)thiazole derivatives, the suitability of these compounds as drugs has been evaluated using Lipinski rule of five. The Lipinski rule of five, while designing the drug, takes the account of the correlation between the physico-chemical properties of the chemical entities and the drug pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (ADME) [36]. According to this rule, the chemical molecule is more likely to exhibit poor absorption or permeation when the molecular weight is more than 500, Log *P* (Octanol/Water partition coefficient), a measure of lipophilicity, is over 5, H-bond donors exceeds 5, H-bond acceptors, the sum of N and O atoms is over 10. Regardless of some exceptions to this rule, it is a simple and widely accepted method to screen all patterns of drugs. Molinspiration server is used to deduce the data based on Lipinski rule of five for all the 2-(2-hydrazinyl)thiazole derivatives and the results are summarized in Table 1.

The data reveal that the molecular weights of the designed compounds are below 500, and are in the range of 249.295–379.438. The Log *P* values are found to be in the range of 1.901–4.465. These values are found to be in the range recommended by Lipinski rule. Similarly, all the compounds have shown 4–7 hydrogen bond acceptors except compound **2r** which has shown the highest (8) hydrogen bond acceptors. Similarly, all the compounds are possessing less than 2 hydrogen bond donors which are well below the value recommended by Lipinski. Hence, all these compounds have shown no violations of Lipinski rule and found to be possessing drug like molecule (DLM) features.



Scheme 1. Synthesis of (i) thiosemicarbazone derivatives (ii) 2-(2-hydrazinyl)thiazole derivatives.

2.2. Synthesis of 2-(2-hydrazinyl)thiazole derivatives

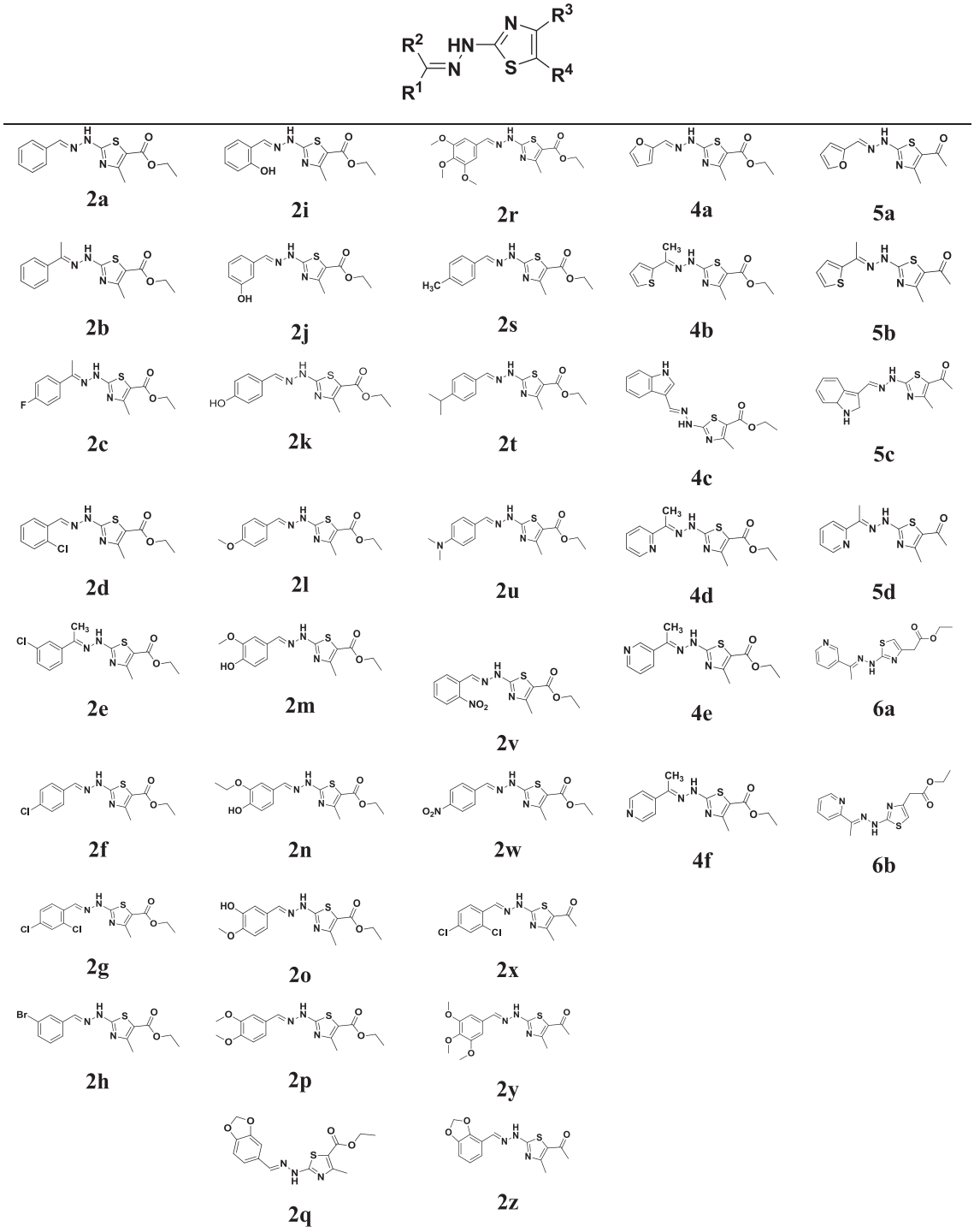
As all the molecules have DLM features, synthesis of targeted 2-(2-hydrazinyl)thiazole derivatives has been carried out according to the synthetic pathways described in Scheme 1. To synthesize 2-(2-hydrazinyl)thiazole derivatives, as shown in Scheme 1(i), first thiosemicarbazones (**1a**–**w** and **3a**–**f**) are synthesized from thiosemicarbazide and wide range of substituted aromatic and heteroatom containing carbonyl compounds using the literature procedure [39–42]. The ^{1}H Nuclear Magnetic Resonance (NMR) spectra of these thiosemicarbazones show the characteristic peak due to imine methyl protons between 2.5 ppm and 3.0 ppm. Proton present in the Schiff base resonates at 7.0 ppm and the aromatic phenyl ring protons resonate between 7.00 ppm and 8.6 ppm. The characteristic peak due to =NH proton appears between 8.5 ppm and 9.0 ppm as a singlet and the peak corresponding to –NH proton resonate between 10 ppm and 11.5 ppm. Due to the exchange of H between the terminal NH and S, the characteristic peak due to this proton appears approximately at 11.5 ppm. The carbon present in C=N group resonates around 150 ppm in ^{13}C NMR spectra of thiosemicarbazones. These results confirm that the condensation between carbonyl group and thiosemicarbazide is successful. In addition, disappearance of the broad peak due to carbonyl (C=O) functional group at 1600–1700 cm^{-1} and the appearance of the characteristic peak of N=CH group in the range of 1542–1579 cm^{-1} in the Infrared (IR) spectra of compounds further confirm the absence of carbonyl reactant and formation of desired thiosemicarbazones. Apart from the synthesis of reported thiosemicarbazones, three new thiosemicarbazones have also been synthesized and the structures of these three compounds are also confirmed using the same way used for the reported compounds.

In the next step, the desired 2-(2-hydrazinyl)thiazole derivatives with aryl (**2a**–**z**) and hetero aryl (**4a**–**f**, **5a**–**d** and **6a**–**f**) substitutions at 2nd position have been synthesized from corresponding thiosemicarbazones and aliphatic α-halo ketones as shown in Scheme 1(ii). All the synthesized compounds have been characterized using NMR, IR and mass spectroscopic techniques. Except compound **6a** and **6b**, R_3 in all 2-(2-hydrazinyl)thiazole derivatives is CH_3. The protons present in this CH_3 group are observed at 2.25 ppm for α-chloro ketones. After cyclization, these CH_3 protons are shifted downfield at 2.40–2.60 ppm due to the aromatic nature of the newly formed thiazole ring. This result confirms the formation of thiazole ring. It is interesting to note that the NH protons present in thiosemicarbazones appear at 9.0–9.5 ppm whereas, due to the formation of aromatic thiazole ring, these protons appear downfield at 10.5–11.5 ppm in 2-(2-hydrazinyl)thiazole derivatives. Similarly, appearance of peaks corresponding to aliphatic protons in the region 13–65 ppm and three characteristic peaks of the thiazole ring at approximately 90–110, 135–150 and 165–175 ppm confirm the formation of the 2-(2-hydrazinyl)thiazole derivatives. The precise matching of the experimental mass and CHNS values with the calculated values confirms the formation of analytically pure desired products. All the synthesized 2-(2-hydrazinyl)thiazole derivatives with structural variations at 2-, 4- and 5-positions are listed in Table 2.

2.3. Single crystal X-ray study

The 2-aminothiazole ring and aromatic rings attached to C=N group may exist in either *E* configuration or *Z* configuration. As the configuration of these compounds may decide the drug properties, it is important to understand the configuration of newly synthesized 2-(2-hydrazinyl)thiazole derivatives. For this, two compounds, **2i** and **2l** have been selected as the representative of 2-(2-hydrazinyl) thiazole derivatives and single crystal of these two compounds have been grown in acetonitrile by slow evaporation at 25–30 °C. The solved single crystal structures of **2i** and **2l** are shown in Fig. 2. The results reveal that the molecules are planar and 2-aminothiazole ring and aromatic ring at C=N are in *E*-configuration. The crystal structure of **2i** preferred to stabilize with strong O–H···N intra molecular hydrogen bonds (2.01 Å, 137.73°) between N of C=N and *ortho*-O–H of the phenyl ring. In addition to this, weak C–H···O hydrogen bond interaction (2.13 Å, 129.10°) [43] also stabilizes this compound. The molecules are packed in head to head dimer fashion with N–H···O (1.91 Å, 169.56°) intermolecular hydrogen bond interactions leading to the stabilization of the three dimensional assembly. Similarly, **2l** stabilizes by the weak intra molecular C–H···O

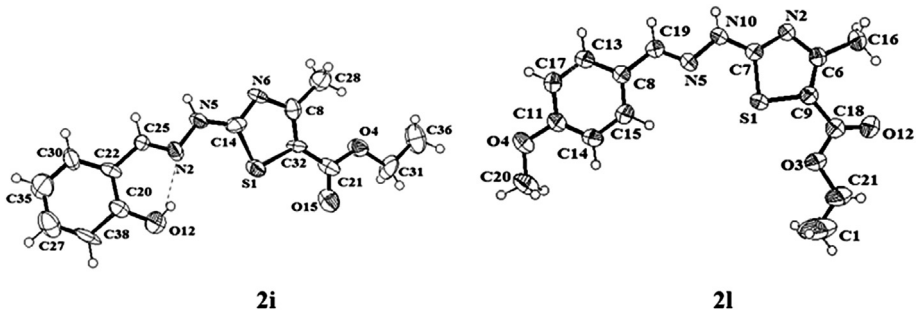
Table 2
Library of synthesized 2-(2-hydrazinyl)thiazole derivatives.



interactions (2.29 Å, 129.10°) and two strong intermolecular N–H···N (2.17 Å, 171.43°) hydrogen bond interactions between N–H at 2-position of thiazole ring and N of thiazole ring.

2.4. Antimycobacterial activity

After the successful design, synthesis and structural confirmation, the antimicrobial activities of the newly synthesized 2-(2-hydrazinyl)thiazole derivatives (**2a**–**6b**) have been tested against *Mtb*, H_{37}Rv. In this study, the minimum inhibitory concentration (MIC) of 2-(2-hydrazinyl)thiazole derivatives that yield ≥80% inhibition using Resazurin microtiter assay (REMA) [44] is considered as positive. Isoniazide, the first line TB drug in clinical practice is used as a reference compound. The MIC values of *in vitro* test results are given in Table 1 along with Lipinski rule results. It is interesting to note that anti-TB activity results are varied based on the



2i 2l

Fig. 2. ORTEP diagram of compound **2i** and **2l**.

substitutions at 4th and 5th positions of thiazole ring and substitution at hydrazinyl group.

Compound **2a**, the primary molecule of thiozole derivatives **2a**–**2w**, shows no activity with MIC value of more than 200 μM. Compound **2b**, imine form of **2a** also shows similar activity. When **2b** is substituted with fluorine at 4th position, it shows the moderate activity with MIC value of 200 μM. This indicates that Schiff base and imine systems are not influencing the activity significantly, but it is the functional groups substituted on the phenyl ring decide the anti-TB activity. To further explore, single and multiple substitutions of chlorine at 2nd, 3rd and 4th positions (**2d**–**g**) are changed. But, none of them show MIC value less than 200 μM. Similarly, when Br is introduced at 3rd position (**2h**), the MIC value is more than 200 μM. In addition, the effect of OH and alkoxy group on the phenyl ring has been studied extensively. In this series, the OH group is attached at 2nd, 3rd and 4th positions. The 2-hydroxyphenyl system (**2i**) shows significant activity with MIC value of 25 μM whereas its counterparts (**2j**, **2k**) do not show MIC values less than 200 μM. To further explore the activity of OH and its methyl and ethyl derivatives, the OH is replaced with OCH_3 at 4th position (**2l**), new OCH_3 and OC_2H_5 groups are introduced at 3rd positions by keeping OH groups constant at 4th positions (**2m**, **2n**). The OH and OCH_3 groups are also exchanged (**2o**). Apart from this, di and tri OCH_3 (**2p**, **2r**) and 1, 3-benzodioxo systems (**2q**) are also studied. Unfortunately, all of these compounds are not active as the MIC values are more than 200 μM. To further explore, compounds with alkyl substitutions such as CH_3 and $-C(CH_3)_2$ groups at 4th position (**2s**, **2t**), NO_2 substitutions at 2nd and 4th position (**2w**, **2v**) and $-N(CH_3)_2$ substitution at 4th position (**2u**) are also studied. In this series, $-NO_2$ and $-N(CH_3)_2$ substitutions at 4th position show moderate activity with MIC value of 200 μM. Efforts have also been made to introduce and identify the effect of $-COCH_3$ group in place of $-COOC_2H_5$ at 5th position of the thiazole ring (**2x**, **2y**, **2z**) as these compounds have keto–enol tautomerism to influence activity [45,46]. But, there is no significant difference in the activity.

In continuation of this study, aromatic heterocyclic systems which include both five membered and six membered rings are introduced in place of phenyl ring. The five membered heterocyclic systems such as thiophene (**4b**, **5b**), furan (**4a**, **5a**) and 3-indole (**4c**, **5c**) are introduced at 2nd position of thiazole ring and 5th position of thiazole ring is varied with $COOC_2H_5$ and $COCH_3$ groups. It is interesting to note that thiophene with $COOC_2H_5$ at 5th position (**4b**) shows good inhibition against *Mtb* with MIC value of 100 μM. Replacement of $COOC_2H_5$ with $COCH_3$ (**5b**) decreases the activity to the MIC value of 200 μM. In contrast, furan (**4a**, **5a**) and 3-indole (**4c**, **5c**) systems show moderate activity with MIC value of 200 μM. Similarly, 2-, 3- and 4-pyridyl systems with variations at 4th and 5th positions using aliphatic ketone and ester groups have also been tested. In this series, 2-pyridyl system with $COOC_2H_5$ group at 5th position (**4d**) shows significant activity with MIC value of 12.50 μM whereas replacement of $-COOC_2H_5$ group with $-COCH_3$ group at 5th position (**5d**) decreases the activity to more than six folds with MIC value of 100 μM. Similarly, a compound with H atom at 5th position and $CH_2COOC_2H_5$ group at 4th position (**6b**) shows moderate activity with MIC value of 200 μM. 3-Pyridyl and 4-pyridyl systems with CH_3 at 4th position and $COOC_2H_5$ at 5th position (**4e**, **4f**) show moderate activity with MIC value of 200 μM. Further variations of 3-pyridyl ring system with H at 5th position and $CH_2COOC_2H_5$ at 4th position (**6a**) show no difference in the activity. In conclusion, 2-pyridyl and 2-hydroxyphenyl hydrazinyl substitutions at the 2nd position of thiazole ring and CH_3 at 4th and $COOC_2H_5$ at 5th positions of thiazole ring (**2i**, **4d**) show significant inhibition of *Mtb* with MIC value of 12.50 μM and 25.50 μM respectively which is comparable with the literature [33,34] results. The structural similarity between these two compounds is four membered and five membered coordination systems. This could be the possible structural feature to enhance the activity. It may also

Table 3
Cytotoxicity studies of 2-(2-hydrazinyl)thiazole derivatives against human lung carcinoma type II epithelial cells (A549).

Entry	Compound	Anti proliferation analysis: human lung carcinoma type II epithelial cells (A549) (IC_{50})
1	**5d**	~50
2	**4d**	~140
3	**2b**	~150
4	**6b**	~200

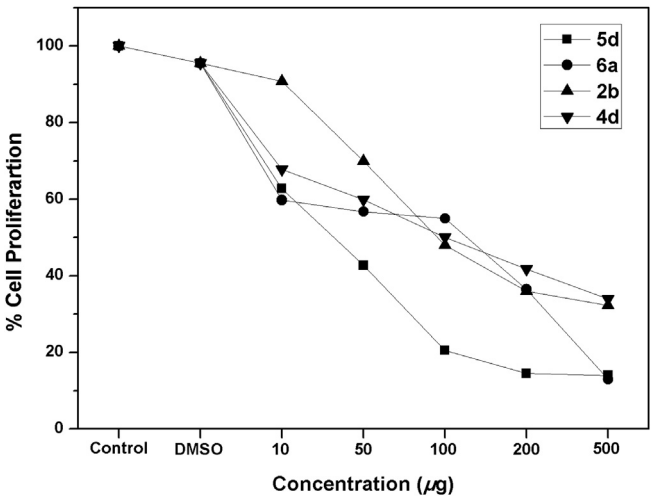


Fig. 3. Effect of **2b**, **4d**, **5d** and **6b** on the proliferation of human lung carcinoma type II epithelial cells.

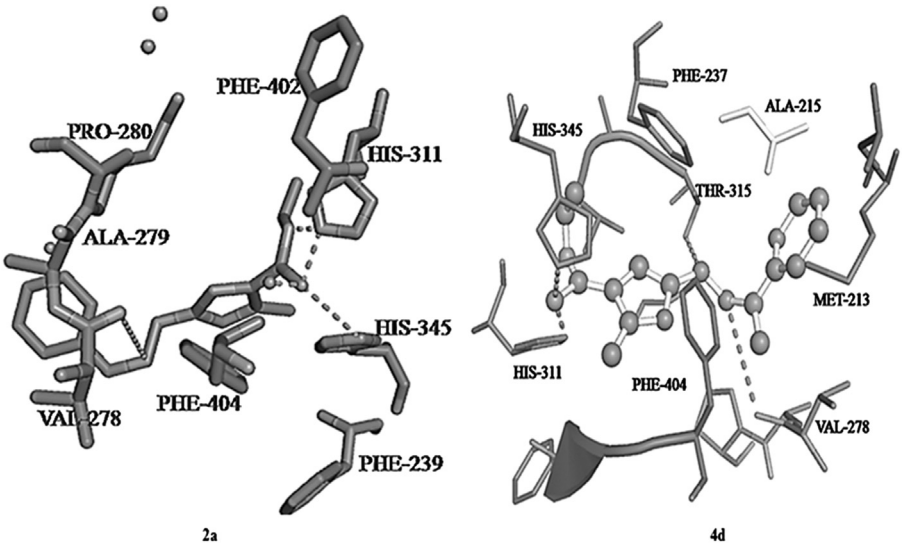


Fig. 4. Hydrogen bond interactions of compounds **2a** and **4d** with β-ketoacyl-ACP synthase (KasA) of *Mtb*.

be concluded that the $COOC_2H_5$ at 5th position of thiazole ring is an activity enhancing group.

2.5. Cytotoxicity studies

The *in vitro* cytotoxicity was tested against human lung carcinoma type II epithelial cells (A549) [47]. A549 cells were treated with **2b**, **4d**, **5d** and **6b** at different concentration in a 96 wells plate for 48 h and the cell growths were analyzed by MTT assay. The cytotoxicity of the compounds was calculated from their IC_{50} values and the results are summarized in Table 3. The cytotoxicity order of these compounds is **5d** > **4d** > **2b** > **6b** with IC_{50} values of 50, 140, 150 and 200 respectively. The effect of **2b**, **4d**, **5d** and **6b** on the proliferation of cells with concentration variations is depicted in Fig. 3. All these results suggest that the compounds with 2-pyridyl substitution with an ester group attached to the thiazole ring at 4th and 5th position, **6b** and **4d**, have shown the low cytotoxicity with IC_{50} values 200 and 140. Whereas 2-pyridyl substitution with the keto group at the 5th position, **5d**, has shown high cytotoxicity with IC_{50} value of 50. The aromatic ring system, **2b** has shown IC_{50} value of 150. The low toxicity values and the high antimycobacterial values of the 2-pyridyl system at 2-position suggest that these compounds could be efficient therapeutic candidates to treat TB.

2.6. Docking studies with KasA protein

After successful finding of DLMs, it is important to understand how these DLMs interact with *Mtb*. Docking studies give a fair idea related to drug–receptor interactions. For this study, KasA protein has been chosen as it is assumed that the interaction of drug with this protein is the important step during the deactivation of *Mtb*. Two representative compounds, **2a** and **4d**, have been chosen for this study. The compound **2a** has been selected as it is the basic structure of all compounds presented in this study and **4d** has been selected as it shows the highest activity among the compounds reported in this report.

Compounds **2a** and **4d** show the inhibition constant (Ki) values of 3.29 μM and 1.46 μM with binding energy of −7.48 kcal/mol and −7.96 kcal/mol respectively. The inhibition of the protein by **4d** is through hydrogen bonding interactions of NH in **4d** with a COO^- group of THR − 315, C═N of **4d** with a COO^- group of VAL − 278 and C═O in **4d** with an NH group present in HIS − 311, and HIS − 345 protein residues as shown in Fig. 4. Similarly, in case of **2a**, C═N and C═O functional groups form hydrogen bonding interactions with COO^- group of VAL − 278 and NH group of HIS − 311, and HIS − 345 respectively as shown in Fig. 4. The hydrogen bonding interactions of these compounds with KasA protein residues are similar to that of TLM, the known TB drug which inhibits KasA protein. These results suggest that the present molecules could be the possible candidates to inhibit mycolic acid synthesis, an essential step in cell wall biosynthesis, by inhibiting the KasA protein of mtFabH. The differences in Ki and binding energy values of **2a** and **4d** and their patterns of binding interactions with the KasA protein suggests that **4d** could be better candidate for inhibition of KasA protein of mtFabH.

3. Conclusion

2-(2-Hydrazinyl)thiazole derivatives have been synthesized from corresponding thiosemicarbazones with aliphatic α-halo ketones. Among the compounds, **4d** and **2i** are found to be potential molecules against *Mtb*, $H_{37}Rv$ with MIC values 12.5 μM and 25 μM respectively. The activity result suggests that molecules with structural features of either hydrogen bond acceptor or hydrogen bond donor at 2-position of the aromatic substituents induce activity. Among these, the 2-pydidyl system proves to be the promising substituent. In the cytotoxicity studies against human lung carcinoma type II epithelial cells, compound **6b** exhibits low cytotoxicity. The protein–ligand interaction studies reveal that the hydrogen bonding interactions of **2a** and **4d** with KasA protein are similar to that of TLM. The aim to explore the 2-(2-hydrazinyl) thiazole derivatives with special focus on the present lead compounds to achieve improved antimycobacterial activities and further validation of them to use as potential drugs are under progress in our laboratory.

4. Experimental

4.1. Materials and methods

Thiosemicarbazide and carbonyl compounds were purchased either from Sigma–Aldrich, USA or Himedia Biosciences or Spectrochem Pvt. Ltd (Mumbai, India). α-Halo ketones were purchased from Himedia Biosciences. All the chemicals were used without

further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded with 400 MHz and 101 MHz respectively using Bruker Avance-II NMR instrument. IR spectra were recorded on a Thermo Nicolet 6700 FT-IR spectrometer and only major peaks are reported in cm^{-1}. Elemental analysis of all the compounds was performed on Elementar Vario EL-II CHNS analyzer. Mass spectra (MS) were recorded on a Thermo Scientific High Resolution Magnetic Sector MS DFS by chemical ionization (CI) or negative-ion electrospray ionization (ESI) method. The single crystals were analyzed using Oxford Diffractometer and data collection, cell refinement and data reduction were carried out using CrysAlis PRO, Oxford Diffraction Ltd., Version 1.171.34.44 (release 25-10-2010 CrysAlis171. NET) software. The programs used to solve the structures and to refine structures are olex2.solve (compiled Oct 25, 2010,18:11:34) and SHELXL respectively. All the IUPAC names for the synthesized compounds were deduced using MarvinSketch software version 5.11.5.

4.2. General procedures for the synthesis of thiosemicarbazone derivatives

The appropriate thiosemicarbazide (1.0 mmol), aldehyde or ketone (1.1 mmol) were dissolved in ethanol (10 ml) and acetic acid (0.2 mmol) was added to the above solution. The reaction mixture was refluxed for 5–6 h and then cooled to room temperature. The resulting precipitate was filtered, washed by ether and recrystallized from ethanol to obtain the corresponding thiosemicarbazones, **1a**–**w** and **3a**–**f**. All the compounds were characterized by spectral techniques and were found to be identical with the reported data in the literature [39–42]. The unreported data for the thiosemicarbazones are given below.

4.2.1. [(E)-[(3-Bromophenyl)methylidene]amino]thiourea (**1h**)

White solid from EtOH (81% yield). m.p. 224–226 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): δ 11.52 (s, 1H), 8.27 (s, 1H), 8.21 (s, 1H), 8.19 (t, J = 1.7 Hz, 1H), 8.02 (s, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.57 (m, 1H), 7.36 (t, J = 7.9 Hz, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): δ 178.19, 140.46, 136.68, 132.27, 130.68, 128.88, 126.95, 122.34.

4.2.2. [(E)-[(4-Methylphenyl)methylidene]amino]thiourea (**1s**)

White solid from EtOH (77% yield). m.p. 194–196 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): δ 10.22 (s, 1H), 7.92 (s, 1H), 7.53 (d, J = 8.2 Hz, 2H), 7.23 (s, 1H), 7.19 (d, J = 7.9 Hz, 2H), 6.60 (s, 1H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): δ 178.27, 144.51, 141.39, 130.40, 129.72, 127.62, 77.48, 77.16, 76.85, 21.67.

4.2.3. [(E)-{[4-(Propan-2-yl)phenyl]methylidene}amino]thiourea (**1t**)

White solid from EtOH (85% yield). m.p. 245–246 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): δ 11.38 (s, 1H), 8.16 (s, 1H), 8.02 (s, 1H), 7.92 (s, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.26 (d, J = 8.2 Hz, 1H), 2.89 (hept, J = 6.9 Hz, 1H), 1.19 (d, J = 6.9 Hz, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): δ 178.10 (s), 152.20 (s), 144.60 (s), 130.78 (s), 127.73 (s), 127.07 (s), 77.48 (s), 77.16 (s), 76.84 (s), 34.23 (s), 23.83 (s).

4.3. General procedures for the synthesis of 2-(2-hydrazinyl) thiazole derivatives

The appropriate thiosemicarbazone (1.0 mmol) and aliphatic α-halo ketones (1.1 mmol) were dissolved in ethanol (10 ml). The reaction mixture was refluxed for 1–3 h and then was cooled to room temperature. The resulting precipitate was filtered, washed with cold ethanol and recrystallized from ethanol to obtain the corresponding 2-(2-hydrazinyl)thiazole derivatives, **2a**–**z**, **4a**–**f**, **5a**–**f** and **6a**, **6b** in analytically pure form. All the compounds were characterized by spectral techniques and the data confirm the proposed structures.

4.3.1. Ethyl 4-methyl-2-[(E)-2-(phenylmethylidene)hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**2a**)

Light brown solid from EtOH (78% yield). m.p. 196–197 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.33 (t, J = 6.8 Hz, 3H), 2.58(3H, s), 4.23 (q, J = 6.8, 2H), 7.38–7.40 (m, 3H), 7.63–7.65 (m, 2H), 7.87 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 14.44, 17.04, 60.61, 111.45, 127.02, 128.80, 130.05, 133.89, 143.84, 157.17, 162.47, 170.96; IR (KBr, ν_{max}, cm^{-1}): 3199, 3084, 2997, 2932, 1670, 1575, 1370, 1320, 1280, 1087; Anal. Calcd. for $C_{14}H_{15}N_3O_2S$: C, 58.11; H, 5.23; N, 14.52; S, 11.08; Found: C, 58.09; H, 5.28; N, 14.51; S, 10.97; HR-MS (ESI+): Calcd. m/z for $C_{14}H_{15}N_3O_2S$: 289.08850; Found m/z $[M + H]^+$: 289.08854.

4.3.2. Ethyl 4-methyl-2-[(E)-2-(1-phenylethylidene)hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**2b**)

White solid from EtOH (75% yield). m.p. 182–183 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.37 (t, J = 6.8 Hz, 3H), 2.40 (s, 3H), 2.78(3H, s), 4.33 (q, J = 6.8, 2H), 7.24–7.40 (m, 3H), 7.63–7.65 (m, 2H), 7.87 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 14.52, 15.42, 17.08, 60.77, 112.15, 126.72, 129.40, 131.05, 133.79, 144.14, 158.17, 162.67, 171.06; IR (KBr, ν_{max}, cm^{-1}): 3199, 3084, 2997, 2932, 1670, 1575, 1370, 1320, 1280, 1087; Anal. Calcd. for $C_{15}H_{17}N_3O_2S$: C, 59.38; H, 5.65; N, 13.85; S, 10.57; Found: C, 59.37; H, 5.63; N, 13.88; S, 10.56; HR-MS (ESI+): Calcd. m/z for $C_{15}H_{17}N_3O_2S$: 303.10414; Found m/z $[M + H]^+$: 303.10426.

4.3.3. Ethyl 2-[(E)-2-[1-(4-fluorophenyl)ethylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2c**)

Brown solid from EtOH (80% yield). m.p. 177–178 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.24 (t, J = 7.20 Hz, 3H), 2.30 (s, 3H), 2.46 (s, 3H), 4.18 (q, J = 7.20 Hz, 2H), 7.23 (d, J = 8.50 Hz, 2H), 7.81 (d, J = 8.53, 2H), 11.82 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.23, 14.33, 16.53, 21.04, 60.10, 115.21, 128.03, 128.11, 134.16, 161.94, 163.978, 169.82, 172.02; IR (KBr, ν_{max}, cm^{-1}): 3200, 3089, 2983, 1681, 1556, 1423, 1372, 1271, 1090; Anal. Calcd. for $C_{15}H_{16}FN_3O_2S$: C, 56.06; H, 5.02; N, 13.08; S, 9.98; Found: C, 56.10; H, 5.05; N, 13.12; S, 9.94; HR-MS (ESI+): Calcd. m/z for $C_{15}H_{16}FN_3O_2S$: 323.04952; Found m/z $[M + H]^+$: 323.04958.

4.3.4. Ethyl 2-[(E)-2-[(2-chlorophenyl)methylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2d**)

Antique white solid from EtOH (83% yield). m.p. 172–180 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.26 (t, J = 7.1, 3H), 2.48 (s, 3H), 4.20 (q, J = 7.1, 2H), 7.45–7.39 (m, 2H), 7.53–7.48 (m, 1H), 7.98–7.91 (m, 1H), 8.43 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.27, 16.74, 60.17, 116.48, 126.55, 127.66, 129.92, 131.13, 132.56, 134.05, 144.24, 156.53, 162.15, 169.09; IR (KBr, ν_{max}, cm^{-1}): 3142, 3038, 2985, 2936, 1706, 1558, 1426, 1372, 1313, 1271, 1215, 1088; Anal. Calcd. for $C_{14}H_{14}ClN_3O_2S$: C, 51.93; H, 4.36; N, 12.98; S, 9.90; Found: C, 51.96; H, 4.31; N, 13.01; S, 9.95; HR-MS (ESI+): Calcd. m/z for $C_{14}H_{14}ClN_3O_2S$: 323.04953; Found m/z $[M + H]^+$: 323.04958.

4.3.5. Ethyl 2-[(E)-2-[1-(3-chlorophenyl)ethylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2e**)

Antique white solid from EtOH (80% yield). m.p. 192–194 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.26 (t, J = 7.2 Hz, 3H), 2.47 (s, 3H), 4.20 (q, J = 7.2 Hz, 2H), 7.45 (t, J = 3.5 Hz, 2H), 7.64 (t, J = 3.5 Hz, 1H), 7.70 (s, 1H), 8.06 (s, 1H), 12.53 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.29, 17.11, 18.50, 60.17, 116.88, 125.26, 125.95, 129.37, 130.73, 133.64, 136.24, 154.79, 161.81, 167.10, 169.19; IR (KBr, ν_{max}, cm^{-1}): 3143, 3038, 2986, 2905, 1705, 1573, 1470, 1432, 1373, 1317, 1275, 1214, 1128, 1091, 973; Anal. Calcd. for $C_{15}H_{16}ClN_3O_2S$: C,

53.33; H, 4.77; N, 12.44; S, 9.49; Found: C, 53.36; H, 4.82; N, 12.48; S, 9.50; HR-MS (ESI+): Calcd. *m/z* for $C_{15}H_{16}ClN_3O_2S$: 337.06518; Found *m/z* $[M + H]^+$: 337.06522.

*4.3.6. Ethyl 2-[(E)-2-[(4-chlorophenyl)methylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2f**)*

Antique white solid from EtOH (77% yield). m.p. 206–208 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.34 (t, *J* = 7.14 Hz, 3H), 2.57 (s, 3H), 4.24 (q, *J* = 7.14 Hz, 2H), 7.36 (d, *J* = 8.53 Hz, 2H), 7.58 (d, *J* = 8.53 Hz, 2H), 7.80 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.26, 16.98, 60.08, 109.22, 128.18, 128.85, 132.95, 134.15, 143.00, 158.00, 161.83, 169.11; IR (KBr, max, cm^{-1}): 3200, 3089, 2983, 1681, 1556, 1423, 1372, 1271, 1090; Anal. Calcd. for $C_{14}H_{14}ClN_3O_2S$: C, 51.93; H, 4.36; N, 12.98; S, 9.91; Found: C, 52.01; H, 4.31; N, 12.91; S, 9.89; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{14}ClN_3O_2S$: 323.04952; Found *m/z* $[M + H]^+$: 323.04958.

*4.3.7. Ethyl 2-[(E)-2-[(2,4-dichlorophenyl)methylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2g**)*

Antique white solid from EtOH (75% yield). m.p. 223–224 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.36 (t, *J* = 7.1, 1H), 2.61 (s, 1H), 4.27 (q, *J* = 7.1, 1H), 4.78 (s, 1H), 7.28 (dd, *J* = 8.6, 1.7, 1H), 7.39 (d, *J* = 1.9, 1H), 7.99 (d, *J* = 8.6, 1H), 8.24 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.36, 17.19, 60.04, 110.96, 115.76, 120.97, 125.39, 145.08, 147.10, 149.07, 161.98, 169.02; IR (KBr, ν_{max}, cm^{-1}): 3459, 3221, 3092, 2976, 2933, 1676, 1591, 1565, 1422, 1472, 1334, 1100, 876; Anal. Calcd. for $C_{14}H_{13}Cl_2N_3O_2S$: C, 46.94; H, 3.66; N, 11.73; S, 8.95; Found: C, 46.97; H, 3.68; N, 11.76; S, 8.99; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{13}Cl_2N_3O_2S$: 357.0105; Found *m/z* $[M + H]^+$: 357.0117.

*4.3.8. Ethyl 2-[(E)-2-[(3-bromophenyl)methylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2h**)*

Yellow solid from EtOH (82% yield). m.p. 209–210 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.37 (t, *J* = 7.13 Hz, 3H), 2.60 (s, 3H), 4.31 (q, *J* = 7.13 Hz, 2H), 7.27 (t, *J* = 8.00 Hz, 2H), 7.51 (d, *J* = 8.00 Hz, 1H), 7.55 (d, *J* = 7.70 Hz, 1H), 7.83 (s, 1H), 7.78 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 14.56, 16.66, 60.24,117.48, 123.09, 128.37, 130.47, 133.27, 134.68, 137.51, 143.46, 156.82, 160.99, 170.51; IR (KBr, ν_{max}, cm^{-1}): 3141, 3037, 2985, 2904, 1701, 1558, 1433, 1274, 1091; Anal. Calcd. for $C_{14}H_{14}BrN_3O_2S$: C, 45.66; H, 3.83; N, 11.41; S, 8.71 Found: C, 45.62; H, 3.79; N, 11.49; S, 8.65; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{14}BrN_3O_2S$: 366.99901; Found *m/z* $[M + H]^+$: 366.99907.

*4.3.9. Ethyl 2-[(E)-2-[(2-hydroxyphenyl)methylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2i**)*

Antique white solid from EtOH (88% yield). m.p. 197–200 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.25 (t, *J* = 6.47 Hz, 3H), 2.45 (s, 3H), 4.16 (q, *J* = 6.47 Hz, 3H), 6.98–6.79 (m, 2H), 7.24 (d, *J* = 8.01 Hz, 1H), 7.62 (d, *J* = 8.01 Hz, 1H), 10.31 (s, 1H), 8.40 (s, 1H), 12.36 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.12, 16.54, 60.17, 116.20, 119.51, 119.65, 127.30, 131.09, 144.15, 156.62, 161.77, 168.39; IR (KBr, ν_{max}, cm^{-1}): 3307, 3154, 3027, 2960, 2825, 1678, 1611, 1513, 1372, 1223, 1165, 1093, 968; Anal. Calcd. for $C_{14}H_{15}N_3O_3S$: C, 55.07; H, 4.95; N, 13.76; S, 10.5; Found: C, 55.10; H, 4.96; N, 13.78; S, 10.56; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{15}N_3O_3S$: 305.08341; Found *m/z* $[M + H]^+$: 305.08346.

*4.3.10. Ethyl 2-[(E)-2-[(3-hydroxyphenyl)methylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2j**)*

White solid from EtOH (72% yield). m.p. 199–201 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.34 (t, *J* = 7.2, 3H), 2.38 (s, 3H), 4.11 (q, *J* = 7.2, 2H), 6.85 (dd, *J* = 6.0 Hz, 3.2 Hz, 2H), 7.58 (dd, *J* = 6.0, 3.2 Hz, 2H), 7.88 (d, *J* = 6.8, 1H), 9.92 (s, 1H), 12.12 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.75, 17.33, 61.20, 116.45, 12.03, 128.48, 143.94, 156.93, 160.31, 162.09, 168.16; IR (KBr, ν_{max}, cm^{-1}): 3307, 3154, 3027, 2960, 2825, 1678, 1611, 1513, 1372, 1223, 1165, 1093, 968; Anal. Calcd. for $C_{14}H_{15}N_3O_3S$: C, 55.07; H, 4.95; N, 13.76; S, 10.50; Found: C, 55.13; H, 4.94; N, 13.78; S, 10.54; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{15}N_3O_3S$: 305.08341; Found *m/z* $[M + H]^+$: 305.08353.

*4.3.11. Ethyl 2-[(E)-2-[(4-hydroxyphenyl) methylidene] hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2k**)*

Antique white from EtOH (76% yield). m.p. 179–180 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.24 (t, *J* = 7.2, 3H), 2.44 (s, 3H), 4.16 (q, *J* = 7.2, 2H), 6.80 (dd, *J* = 6.0 Hz, 3.2 Hz, 2H), 7.48 (dd, *J* = 6.0, 3.2 Hz, 2H), 7.97 (d, *J* = 6.8, 1H), 9.88 (s, 1H), 12.17 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.35, 17.16, 60.04, 115.79, 125.03, 128.48, 143.94, 156.93, 159.31, 161.99, 169.16; IR (KBr, ν_{max}, cm^{-1}): 3307, 3154, 3027, 2960, 2825, 1678, 1611, 1513, 1372, 1223, 1165, 1093, 968; Anal. Calcd. for $C_{14}H_{15}N_3O_3S$: C, 55.07; H, 4.95; N, 13.76; S, 10.50; Found: C, 55.11; H, 4.96; N, 13.80; S, 10.56; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{15}N_3O_3S$: 305.08341; Found *m/z* $[M + H]^+$: 305.08346.

*4.3.12. Ethyl 2-[(E)-2-[(4-methoxyphenyl)methylidene]hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2l**)*

Antique white solid from EtOH (81% yield). m.p. 180–182 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.33 (t, *J* = 7.08 Hz, 3H), 3.84 (s, 3H), 2.57 (s, 3H), 4.23 (q, *J* = 7.08 Hz, 2H), 5.57 (s, 1H), 6.91 (d, *J* = 8.76 Hz, 2H), 7.58 (d, *J* = 8.76 Hz, 2H), 7.82 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 14.15, 16.82, 55.16, 60.24, 110.73, 114.00, 126.39, 128.28, 143.58, 156.94, 160.99, 162.31, 170.51; IR (KBr, ν_{max}, cm^{-1}): 3168, 3056, 298, 2845, 1700, 1606, 1581, 1512, 1432, 1370, 1314, 1253, 1166; Anal. Calcd. for $C_{15}H_{17}N_3O_3S$: C, 56.41; H, 5.37; N, 13.16; S, 10.04; Found: C, 56.37; H, 5.39; N, 13.19; S, 10.08; HR-MS (ESI+): Calcd. *m/z* for $C_{15}H_{17}N_3O_3S$: 319.37878; Found *m/z* $[M + H]^+$: 319.37890.

*4.3.13. Ethyl 2-[(E)-2-[(3-hydroxy-4-methoxyphenyl)methylidene] hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2m**)*

Light yellow from EtOH (88% yield). m.p. 226–229 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.25 (t, *J* = 7.2, 3H), 2.46 (s, 3H), 3.82 (s, 3H), 4.18 (q, *J* = 7.2, 2H), 6.82 (d, *J* = 8.0, 1H), 7.09 (d, *J* = 8.0, 1H), 7.23 (s, 1H), 9.55 (s, 1H), 12.25 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.35, 17.17, 55.59, 60.03, 109.62, 115.68, 121.01, 125.40, 145.09, 147.95, 148.82, 161.98, 169.01; IR (KBr, ν_{max}, cm^{-1}): 3235, 3069, 2933, 2834, 1666, 1584, 1516, 1422, 1372, 1289, 1164, 1091; Anal. Calcd. for $C_{15}H_{17}N_3O_4S$: C, 53.72; H, 5.11; N, 12.53; S, 9.56 Found: C, 53.76; H, 5.15; N, 12.56; S, 9.60; HR-MS (ESI+): Calcd. *m/z* for $C_{15}H_{17}N_3O_4S$: 335.09398; Found *m/z* $[M + H]^+$: 335.09311.

*4.3.14. Ethyl 2-[(E)-2-[(3-ethoxy-4-hydroxyphenyl)methylidene] hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2n**)*

Antique white solid from EtOH (76% yield). m.p. 204–206 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.25 (t, *J* = 7.09 Hz, 3H), 1.36 (t, *J* = 6.98 Hz, 3H), 2.46 (s, 3H), 4.06 (q, *J* = 6.93 Hz, 2H), 4.19 (q, *J* = 7.07 Hz, 2H), 6.84 (d, *J* = 8.13 Hz, 1H), 7.09 (d, *J* = 8.14 Hz, 1H), 7.21 (d, *J* = 1.92 Hz, 1H), 7.96 (s, 1H), 9.45 (s, 1H), 12.26 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.36, 14.74, 17.19, 38.90, 39.10, 39.31, 39.52, 39.73, 39.94, 40.15, 60.04, 63.92, 110.96, 115.76, 120.97, 125.39, 145.08, 147.10, 149.07, 161.98, 169.02; IR (KBr, ν_{max}, cm^{-1}): 3321, 3171, 3063, 2921, 1674, 1580, 1518, 1423, 1371, 1276, 1175, 1088; Anal. Calcd. for $C_{16}H_{19}N_3O_4S$: C, 55.00; H, 5.48; N, 12.03; S, 9.18; Found: C, 55.03; H, 5.46; N, 11.96; S, 9.15; HR-MS (ESI+): Calcd. *m/z* for $C_{16}H_{19}N_3O_4S$: 349.10962; Found *m/z* $[M + H]^+$: 349.10975.

*4.3.15. Ethyl 2-[(E)-2-[(3-hydroxy-4-methoxyphenyl)methylidene] hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2o**)*

Sandy brown solid from EtOH (80% yield). m.p. 215–217 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.26 (t, *J* = 7.12 Hz, 3H), 2.46 (s, 3H), 3.79 (s, 3H), 4.19 (q, *J* = 7.12 Hz, 2H), 6.94 (d, *J* = 8.36 Hz, 1H), 7.00 (dd, *J* = 1.94, 8.36 Hz, 1H), 7.23 (d, *J* = 1.90 Hz, 1H), 7.94 (s, 1H),

9.30 (s, 1H), 12.18 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.35, 17.16, 55.58, 60.06, 99.56, 111.67, 111.86, 120.12, 126.83, 144.20, 146.90, 149.72, 161.95, 169.15; IR (KBr, ν_{max}, cm^{-1}): 3235, 3069, 2933, 2834, 1666, 1584, 1516, 1422, 1372, 1289, 1164, 1091; Anal. Calcd. for $C_{15}H_{17}N_3O_4S$: C, 53.72; H, 5.11; N, 12.53; S, 9.56; Found: C, 53.79; H, 5.15; N, 12.55; S, 9.58; HR-MS (ESI+): Calcd. *m/z* for $C_{15}H_{17}N_3O_4S$: 335.09398; Found *m/z* [M + H]$^+$: 335.09409.

4.3.16. Ethyl 2-[(E)-2-[(3,4-dimethoxyphenyl)methylidene] hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2p**)

Antique white solid from EtOH (76% yield). m.p. 211–213 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.33 (t, *J* = 7.13 Hz, 3H), 3.91 (s, 3H), 2.58 (s, 3H), 3.96 (s, 3H), 4.25 (q, *J* = 7.19 Hz, 2H), 6.85 (d, *J* = 8.30 Hz, 1H), 7.06 (d, *J* = 8.23 Hz, 1H), 7.82 (s, 1H), 7.31 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): δ 14.50, 17.19, 56.09, 60.67, 108.12, 110.86, 111.18, 121.75, 126.94, 144.26, 149.51, 151.12, 157.09, 162.66, 170.59; IR (KBr, ν_{max}, cm^{-1}): 3211, 3106, 2986, 2935, 2831, 1664, 1510, 1424, 1268, 1088, 1024; Anal. Calcd. for $C_{16}H_{19}N_3O_4S$: C, 55.00; H, 5.48; N, 12.03; S, 9.18; Found: C, 54.03; H, 5.49; N, 12.07; S, 9.19; HR-MS (ESI+): Calcd. *m/z* for $C_{16}H_{19}N_3O_4S$: 349.10962; Found *m/z* [M + H]$^+$: 349.10973.

4.3.17. Ethyl 2-[(E)-2-(2H-1,3-benzodioxol-4-ylmethylidene) hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2q**)

Light green solid from EtOH (80% yield). m.p. 242–245 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.26 (t, *J* = 7.15 Hz, 3H), 2.46 (s, 3H), 4.19 (q, *J* = 7.15 Hz, 2H), 6.08 (s, 2H), 6.96 (d, *J* = 8.16 Hz, 1H), 7.13 (d, *J* = 8.16 Hz, 1H), 7.23 (s, 1H), 7.99 (s, 1H), 12.31 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.30, 17.12, 60.05, 101.54, 104.92, 108.50, 121.10, 122.74, 128.42, 143.92, 147.97, 148.90, 155.86, 161.90, 169.09; IR (KBr, ν_{max}, cm^{-1}): 3156, 3039, 2986, 2907, 1707, 1583, 1497, 1430, 1374, 1256, 1095, 972, 931; Anal. Calcd. for $C_{15}H_{15}N_3O_4S$: C, 54.04; H, 4.54; N, 12.60; S, 9.62; Found: C, 54.09; H, 4.55; N, 12.55; S, 9.68; HR-MS (ESI+): Calcd. *m/z* for $C_{15}H_{15}N_3O_4S$: 333.07833; Found *m/z* [M + H]$^+$: 333.07838.

4.3.18. Ethyl 4-methyl-2-[(E)-2-[(3,4,5-trimethoxyphenyl) methylidene]hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**2r**)

Light green solid from EtOH (79% yield). m.p. 189–191 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.34 (t, *J* = 7.10 Hz, 3H), 2.59 (s, 3H), 3.89 (d, *J* = 8.79 Hz, 9H), 4.27 (q, *J* = 7.10 Hz, 2H), 6.87 (s, 2H), 7.78 (s, 1H), 10.91 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 14.50, 17.10, 56.36, 60.77, 61.05, 104.21, 111.43, 129.28, 140.14, 144.18, 153.65, 156.75, 162.57, 170.50; IR (KBr, ν_{max}, cm^{-1}): 3200, 3099, 2975, 2976, 2834, 1675, 1578, 1504, 1371, 1129, 1089, 1008; Anal. Calcd. for $C_{17}H_{21}N_3O_5S$: C, 53.81; H, 5.58; N, 11.07; S, 8.45; Found: C, 53.89; H, 5.50; N, 11.11; S, 8.43; HR-MS (ESI+): Calcd. *m/z* for $C_{17}H_{21}N_3O_5S$: 379.12019; Found *m/z* [M + H]$^+$: 379.12021.

4.3.19. Ethyl 4-methyl-2-[(E)-2-[(4-methylphenyl)methylidene] hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**2s**)

Antique white solid from EtOH (85% yield). m.p. 189–191 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.34 (t, *J* = 7.11 Hz, 3H), 2.59 (s, 3H), 2.38 (s, 3H), 4.25 (q, *J* = 7.11 Hz, 2H), 7.20 (d, *J* = 8.08 Hz, 2H), 7.55 (d, *J* = 8.08 Hz, 2H), 7.85 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): δ 14.50, 17.23, 21.62, 60.63, 111.36, 127.09, 129.59, 131.20, 140.45, 144.27, 157.34, 162.67, 170.81; IR (KBr, ν_{max}, cm^{-1}): 3459, 3194, 3101, 2979, 1665, 1570, 1428, 1371, 1274, 1123, 1091; Anal. Calcd. for $C_{15}H_{17}N_3O_2S$: C, 59.38; H, 5.65; N, 13.85; S, 10.57; Found: C, 59.36; H, 5.62; N, 13.87; S, 16.00; HR-MS (ESI+): Calcd. *m/z* for $C_{15}H_{17}N_3O_2S$: 303.10414; Found *m/z* [M + H]$^+$: 303.10416.

4.3.20. Ethyl 4-methyl-2-[(E)-2-{[3-(propan-2-yl)phenyl] methylidene}hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**2t**)

Pale green solid from EtOH (89% yield). m.p. 210–212 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.25 (t, *J* = 6.8, 6H), 1.34 (t, *J* = 7.2, 3H), 2.59 (s, 3H), 2.93 (m, 1H), 4.25 (q, *J* = 7.2, 2H), 7.24 (d, *J* = 8.0, 2H), 7.57 (d, *J* = 8.0, 2H), 7.86 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 14.53, 17.26, 23.92, 34.25, 60.63, 111.36, 126.99, 127.21, 131.55, 144.27, 151.41, 157.36, 162.69, 170.86; IR (KBr, ν_{max}, cm^{-1}): 3193, 3094, 2963, 1666, 1569, 1429, 1370, 1275, 1124, 1092; Anal. Calcd. for $C_{17}H_{21}N_3O_2S$: C, 61.61; H, 6.39; N, 12.68; S, 9.67; Found: C, 61.64; H, 6.45; N, 12.63; S, 9.60; HR-MS (ESI+): Calcd. *m/z* for $C_{17}H_{21}N_3O_2S$: 331.13545; Found *m/z* [M + H]$^+$: 331.13549.

4.3.21. Ethyl 2-[(E)-2-{[4-(dimethylamino)phenyl]methylidene} hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**2u**)

Light brown solid from EtOH (82% yield). m.p. 231–232 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.36 (t, *J* = 7.17 Hz, 3H), 2.57 (s, 3H), 3.02 (s, 6H), 4.29 (q, *J* = 7.17 Hz, 2H), 6.69 (d, *J* = 8.85 Hz, 2H), 7.54 (d, *J* = 8.85 Hz, 2H), 7.80 (s, 1H); ^{13}C NMR (400 MHz, δ, ppm, $CDCl_3$): 13.72, 16.58, 39.45, 59.69, 109.76, 111.11, 120.87, 127.73, 144.52, 150.93, 156.84, 162.10, 169.80; IR (KBr, ν_{max}, cm^{-1}): 3367, 3187, 3073, 2979, 2925, 1696, 1608, 1527, 1430, 1369, 1315, 1262, 1181, 1079; Anal. Calcd. for $C_{16}H_{20}N_4O_2S$: C, 57.81; H, 6.06; N, 16.85; S, 9.65; Found: C, 57.86; H, 6.10; N, 16.81; S, 9.71; HR-MS (ESI+): Calcd. *m/z* for $C_{16}H_{20}N_4O_2S$: 332.13070; Found *m/z* [M + H]$^+$: 332.13073.

4.3.22. Ethyl 4-methyl-2-[(E)-2-[(3-nitrophenyl)methylidene] hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**2v**)

Yellow solid from EtOH (80% yield). m.p. 239–243 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.28 (t, *J* = 7.2 Hz, 3H), 2.50 (s, 3H), 4.23 (q, *J* = 8.0 Hz, 2H), 7.89 (d, *J* = 8 Hz, 2H), 8.14 (s, 1H), 8.24 (d, *J* = 8 Hz, 2H), 9.88 (s, 1H), 12.67 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.28, 16.87, 60.25, 124.05, 127.42, 140.33, 147.41, 161.72, 169.08; IR (KBr, ν_{max}, cm^{-1}): 3140, 1704, 1566, 1513, 1432, 1342, 1321, 1274, 1088 cm^{-1}; Anal. Calcd. for $C_{14}H_{14}N_4O_4S$: C, 50.29; H, 4.22; N, 16.76; S, 9.59; Found: C, 50.27; H, 4.25; N, 16.75; S, 9.58; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{14}N_4O_4S$: 334.07358; Found *m/z* [M + H]$^+$: 334.07371.

4.3.23. Ethyl 4-methyl-2-[(E)-2-[(2-nitrophenyl)methylidene] hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**2w**)

Yellow solid from EtOH (80% yield). m.p. 261–262 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.26 (t, *J* = 7.2 Hz, 3H), 2.48 (s, 3H), 4.20 (q, *J* = 7.2 Hz, 2H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 8.04 (d, *J* = 7.6 Hz, 2H), 8.47 (s, 1H), 12.71 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.24, 16.71, 60.20, 117.52, 124.68, 127.80, 130.23, 131.93, 133.60, 143.17, 147.74, 157.92, 161.75, 172.31; IR (KBr, ν_{max}, cm^{-1}): 3147, 3106, 3033, 2984, 1705, 1575, 1510, 1426, 1372, 1339, 1262, 1119, 1090, 970; Anal. Calcd. for $C_{14}H_{14}N_4O_4S$: C, 50.29; H, 4.22; N, 16.76; S, 9.59; Found: C, 50.32; H, 4.24; N, 16.70; S, 9.61; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{14}N_4O_4S$: 334.07358; Found *m/z* [M + H]$^+$: 334.07362.

4.3.24. 1-{2-[(E)-2-[(2,4-Dichlorophenyl)methylidene]hydrazin-1-yl]-4-methyl-1,3-thiazol-5-yl}ethan-1-one (**2x**)

Antique white solid from EtOH (88% yield). m.p. 240–242 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 2.31 (s, 1H), 2.61 (s, 1H), 4.27 (q, *J* = 7.1, 1H), 4.78 (s, 1H), 7.28 (dd, *J* = 8.6, 1.7, 1H), 7.39 (d, *J* = 1.9, 1H), 7.99 (d, *J* = 8.6, 1H), 8.24 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.36. 17.19, 115.76, 120.97, 125.39, 145.08, 147.10, 149.07, 161.98, 169.02; IR (KBr, ν_{max}, cm^{-1}): 3459, 3221, 3092, 2976, 2933, 1756, 1591, 1565, 1422, 1472, 1334, 1100, 876; Anal. Calcd. for $C_{14}H_{11}Cl_2N_3OS$: C, 47.57; H, 3.38; N, 12.08; S, 9.77; Found: C, 47.59; H, 3.37; N, 12.07; S, 9.75; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{11}Cl_2N_3OS$: 326.9999; Found *m/z* [M + H]$^+$: 327.0004.

4.3.25. 1-{4-Methyl-2-[(E)-2-[(3,4,5-trimethoxyphenyl) methylidene]hydrazin-1-yl]-1,3-thiazol-5-yl}ethan-1-one (**2y**)

Light yellow solid from EtOH (88% yield). m.p. 177–180 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.40 (s, 3H), 2.50 (s, 3H), 3.68 (s,

3H), 3.81 (s, 6H), 7.00 (s, 1H), 8.11 (s, 1H), 8.60 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 16.06, 55.85, 62.07, 104.23, 128.46, 133.72, 140.68, 144.62, 154.47, 157.27, 170.96, 195.54; IR (KBr, ν_{max}, cm^{-1}): 3100, 2975, 2966, 2844, 1715, 1571, 1504, 1381, 1159, 1099, 1008; Anal. Calcd. for $C_{16}H_{19}N_3O_4S$: C, 55.00; H, 5.48; N, 12.03; S, 9.18; Found: C, 55.05; H, 5.51; N, 12.07; S, 9.21; HR-MS (ESI+): Calcd. *m/z* for $C_{16}H_{19}N_3O_4S$: 349.10963; Found *m/z* $[M + H]^+$: 349.10966.

4.3.26. 1-{2-[(E)-2-(2H-1,3-Benzodioxol-4-ylmethylidene) hydrazin-1-yl]-4-methyl-1,3-thiazol-5-yl}ethan-1-one (**2z**)

Light yellow solid from EtOH (80% yield). m.p. 172–180 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.41 (s, 3H), 2.50 (s, 3H), 5.81 (s, 1H), 6.09 (s, 2H), 6.98 (d, J = 8.0, 1H), 7.15 (d, J = 8.0, 1H), 7.26 (s, 1H), 8.07 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 16.39, 26.23, 102.12, 115.80, 117.56, 119.66, 124.25, 133.36, 144.60, 148.48, 153.40, 156.54, 173.05, 194.13; IR (KBr, ν_{max}, cm^{-1}): 3146, 3030, 2976, 2915, 1697, 1590, 1491, 1422, 1370, 1245, 1088, 975; Anal. Calcd. for $C_{14}H_{13}N_3O_3S$: C, 55.43; H, 4.32; N, 13.85; S, 10.57; Found: C, 55.45; H, 4.37; N, 13.88; S, 10.60; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{13}N_3O_3S$: 303.06776; Found *m/z* $[M + H]^+$: 303.06780.

4.3.27. Ethyl 2-[(E)-2-(furan-2-ylmethylidene)hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**4a**)

Brown solid from EtOH (71% yield). m.p. 194–196 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.34 (t, J = 7.12 Hz, 3H), 2.58 (s, 3H), 4.27 (q, J = 7.12 Hz, 2H), 6.48 (dd, J = 1.72, 3.42 Hz, 1H), 6.69 (d, J = 3.42 Hz, 1H), 7.53 (d, J = 1.72 Hz, 1H), 7.77 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 14.41, 17.08, 60.27, 109.08, 112.36, 113.49, 134.77, 145.19, 149.27, 157.85, 162.02, 168.99; IR (KBr, ν_{max}, cm^{-1}): 3210, 3093, 2979, 1669, 1617, 1567, 1528, 1411, 1318, 1274, 1092; Anal. Calcd. for $C_{12}H_{13}N_3O_3S$: C, 51.60; H, 4.69; N, 15.04; S, 11.48; Found: C, 51.64; H, 4.68; N, 15.07, S, 11.42; HR-MS (ESI+): Calcd. for *m/z* $C_{12}H_{13}N_3O_3S$: 279.06776; Found *m/z* $[M + H]^+$ 279.06781.

4.3.28. Ethyl 4-methyl-2-[(E)-2-[1-(thiophen-2-yl)ethylidene] hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**4b**)

Gray solid from EtOH (76% yield). m.p. 209–210 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.35 (t, J = 7.2, 3H), 2.24 (s, 3H), 2.57 (s, 3H), 4.29 (2H, q, J = 7.2), 7.01 (m, 1H), 7.25 (d, J = 3.6, 1H), 7.32 (m, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 13.98, 14.42, 17.11, 60.58, 111.47, 126.34, 127.26, 128.02, 143.01, 145.56, 156.76, 162.71, 170.28; IR (KBr, ν_{max}, cm^{-1}): 3212, 3098, 2980, 1615, 1565, 1368, 1317, 1280, 1119; Anal. Calcd. for $C_{13}H_{15}N_3O_2S_2$: C, 50.46; H, 4.89; N, 13.58; S, 20.73; Found: C, 50.61; H, 4.85; N, 13.50; S, 22.00; HR-MS (ESI+): Calcd. *m/z* for $C_{13}H_{15}N_3O_2S_2$: 309.06057; Found *m/z* $[M + H]^+$: 309.06066.

4.3.29. Ethyl 2-[(E)-2-(1H-indol-3-ylmethylidene)hydrazin-1-yl]-4-methyl-1,3-thiazole-5-carboxylate (**4c**)

Antique white solid from EtOH (86% yield). m.p. 282–285 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.26 (t, J = 7.2 Hz, 3H), 2.50 (s, 3H), 4.23 (q, J = 7.2 Hz, 2H), 7.23 (dd, J = 4 Hz, 2.8 Hz, 2H), 7.47 (dd, J = 4 Hz, 2.8, 1H), 7.83 (d, J = 2.4 Hz, 1H), 8.21 (dd, J = 4 Hz, 2.8, 1H), 8.34 (s, 1H), 11.6 (s, 1H), 12.17 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.43, 17.37, 59.99, 108.15, 111.45, 112.02, 120.76, 121.58, 122.75, 124.10, 130.53, 137.18, 142.29, 158.61, 162.19, 168.99; IR (KBr, ν_{max}, cm^{-1}): 3306, 3052, 2898, 1686, 1613, 1550, 1419, 1119, 1093 cm^{-1}; Anal. Calcd. for $C_{16}H_{16}N_4O_2S$: C, 58.52; H, 4.91; N, 17.06; S, 9.76; Found: C, 58.55; H, 4.99; N, 17.02; S, 9.72; HR-MS (ESI+): Calcd. *m/z* for $C_{16}H_{16}N_4O_2S$: 328.09940; Found *m/z* $[M + H]^+$: 328.09944.

4.3.30. Ethyl 4-methyl-2-[(E)-2-[1-(pyridin-2-yl)ethylidene] hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**4d**)

Yellow solid from EtOH (81% yield). m.p. 206–208 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.28 (t, J = 7.0, 3H), 2.40 (s, 3H), 4.22 (q, J = 7.0, 2H), 2.51 (s, 3H), 7.42–7.37 (t, J = 7.3, 1H), 7.86 (t, J = 7.3, 1H), 8.05 (d, J = 7.8, 1H), 8.60 (d, J = 7.8, 1H), 11.91 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 12.86, 14.25, 18.43, 60.11, 111.54, 119.83, 123.72, 136.56, 143.86, 148.55, 154.74, 161.06, 170.55, 178.61; IR (KBr, ν_{max}, cm^{-1}): 3200, 3048, 2988, 2947, 1687, 1566, 1469, 1372, 1271, 1099; Anal. Calcd. for $C_{14}H_{16}N_4O_2S$: C, 55.25; H, 5.30; N, 18.41; S, 10.53; Found: C, 55.24; H, 5.28; N, 18.44; S, 10.63; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{16}N_4O_2S$: 304.09519; Found *m/z* $[M + H]^+$: 304.09523.

4.3.31. Ethyl 4-methyl-2-[(E)-2-[1-(pyridin-3-yl)ethylidene] hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**4e**)

Brown color solid from EtOH (yield 78%). m.p. 244–245 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.23 (t, J = 7.1, 3H), 2.44 (s, 3H), 2.32 (s, 3H), 4.17 (q, J = 7.1, 2H), 7.42 (dd, J = 8.0, 4.8, 1H), 8.10 (d, J = 8.0, 1H), 8.54 (d, J = 3.3, 1H), 8.92 (d, J = 3.3, 1H), 11.87 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.25, 16.35, 18.11, 60.12, 119.84, 123.73, 127.35, 136.56, 148.56, 151.94, 157.92, 162.13, 167.04, 169.86; IR (KBr, ν_{max}, cm^{-1}): 3123, 3045, 2978, 2923, 2796, 1671, 1618, 1556, 1474, 1367, 1321, 1097, 1037; Anal. Calcd. for $C_{14}H_{16}N_4O_2S$: C, 55.25; H, 5.30; N, 18.41; S, 10.53; Found: C, 55.30; H, 5.32; N, 18.47; S, 10.51; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{16}N_4O_2S$: 304.09940; Found *m/z* $[M + H]^+$: 304.09943.

4.3.32. Ethyl 4-methyl-2-[(E)-2-[1-(pyridin-4-yl)ethylidene] hydrazin-1-yl]-1,3-thiazole-5-carboxylate (**4f**)

Yellow solid from EtOH (80% yield). m.p. 217 °C –218 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 1.27 (t, J = 7.1, 1H), 2.32 (s, 1H), 2.48 (s, 1H), 4.21 (q, J = 7.1, 1H), 7.71 (dd, J = 4.6, 1.6, 1H), 8.62 (dd, J = 4.6, 1.6, 1H), 11.94 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 15.00, 18.14, 22.02, 62.06, 120.03, 126.34, 139.00, 149.86, 154.80, 163.93, 168.48, 171.29; IR (KBr, ν_{max}, cm^{-1}): 3123, 2993, 2899, 1706, 1617, 1591, 1559, 1368, 1316, 1271, 1216, 1131, 1092; Anal. Calcd. for $C_{14}H_{16}N_4O_2S$: C, 55.25; H, 5.30; N, 18.41; S, 10.53; Found: C, 55.28; H, 5.37; N, 18.46; S, 10.58; HR-MS (ESI+): Calcd. *m/z* for $C_{14}H_{16}N_4O_2S$: 304.09940; Found *m/z* $[M + H]^+$: 304.09946.

4.3.33. 1-{2-[(E)-2-(Furan-2-ylmethylidene)hydrazin-1-yl]-4-methyl-1,3-thiazol-5-yl}ethan-1-one (**5a**)

Light green solid from EtOH (80% yield). m.p. 202–203 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.37 (s, 3H), 2.46 (s, 3H), 6.61–6.58 (m, 1H), 6.86 (d, J = 3.46 Hz, 1H), 7.81 (d, J = 3.46 Hz, 1H), 7.96 (s, 1H), 12.39 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 16.39, 26.23, 112.64, 119.32, 133.36, 142.86, 146.71, 150.57, 155.85, 171.29, 195.89; IR (KBr, ν_{max}, cm^{-1}): 3212, 3073, 2999, 1690, 1617, 1577, 1528, 1411, 1328; Anal. Calcd. for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86; S, 12.86; Found: C, 53.04; H, 4.46; N, 16.88; S, 12.89; HR-MS (ESI+): Calcd. *m/z* for $C_{11}H_{11}N_3O_2S$: 249.05720; Found *m/z* $[M + H]^+$: 249.05723.

4.3.34. 1-{4-Methyl-2-[(E)-2-[1-(thiophen-2-yl)ethylidene] hydrazin-1-yl]-1,3-thiazol-5-yl}ethan-1-one (**5b**)

Yellow solid from EtOH (80% yield). m.p. 192–193 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.33 (s, 3H), 2.39 (s, 3H), 2.48 (s, 3H), 7.09–7.06 (m, 1H), 7.42 (d, J = 3.60 Hz, 1H), 7.54 (d, J = 4.99 Hz, 1H), 11.86 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 14.69, 18.44, 26.24, 125.41, 127.90, 129.47, 133.20, 155.66, 158.78, 170.62, 197.76; IR (KBr, ν_{max}, cm^{-1}): 3210, 3072, 2975, 1692, 1545, 1364, 1327, 1285; Anal. Calcd. for $C_{12}H_{13}N_3OS_2$: C, 51.59; H, 4.69; N, 15.04; S, 22.95; Found: C, 51.63; H, 4.71; N, 15.08; S, 22.99; HR-MS (ESI+): Calcd. *m/z* for $C_{12}H_{13}N_3OS_2$: 279.05000; Found *m/z* $[M + H]^+$: 279.05002.

4.3.35. 1-{2-[(E)-2-(1H-Indol-3-ylmethylidene)hydrazin-1-yl]-4-methyl-1,3-thiazol-5-yl}ethan-1-one (**5c**)

Light green solid from EtOH (81% yield). m.p. 182–184 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.44 (s, 3H), 2.53 (s, 3H), 6.03 (s, 1H), 7.26–7.20 (m, 2H), 7.49–7.44 (m, 1H), 7.86 (d, J = 2.6, 1H), 8.17

(dd, $J = 5.8, 3.1$, 1H), 8.42 (s, 1H), 11.73 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 16.39, 26.58, 52.23, 63.47, 121.07, 124.25, 128.08, 133.36, 135.83, 138.28, 140.39, 158.29, 170.61, 177.63, 195.89; IR (KBr, ν_{max}, cm^{-1}): 3316, 3042, 2890, 1696, 1620, 1570, 1439, 1109, 1087. Anal. Calcd. for $C_{15}H_{16}N_4OS$: C, 59.98; H, 5.37; N, 18.65; S, 10.67; Found: C, 59.95; H, 5.39; N, 18.69; S, 10.65; HR-MS (ESI+): Calcd. m/z for $C_{15}H_{16}N_4OS$: 300.10448; Found m/z [M + H]$^+$: 300.10452.

4.3.36. 1-{4-Methyl-2-[(E)-2-[1-(pyridin-2-yl)ethylidene]hydrazin-1-yl]-1,3-thiazol-5-yl}ethan-1-one (**5d**)

Light red solid from EtOH (86% yield). m.p. 237–238 °C; ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.45 (s, 3H), 2.62 (s, 3H), 2.80 (s, 3H), 7.44–7.35 (t, $J = 7.3$, 1H), 7.78 (t, $J = 7.3$, 1H), 8.11 (d, $J = 7.8$, 1H), 8.65 (d, $J = 7.8$, 1H), 11.88 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, DMSO-d_6): 12.74, 14.34, 18.63, 61.01, 110.89, 120.33, 124.12,137.50, 142.96, 149.55, 155.77, 162.16, 171.25, 177.61; IR (KBr, ν_{max}, cm^{-1}): 3200, 3048, 2988, 2947, 1687, 1636, 1459, 1376, 1267, 1105; Anal. Calcd. for $C_{13}H_{14}N_4OS$: C, 56.91; H, 5.14; N, 20.42; S, 11.69; Found: C, 56.95; H, 5.18; N, 20.44; S, 11.67; HR-MS (ESI+): Calcd. m/z for $C_{13}H_{14}N_4OS$: 274.08883; Found m/z [M + H]$^+$: 274.08888.

4.3.37. Ethyl 2-{2-[(E)-2-[1-(pyridin-3-yl)ethylidene]hydrazin-1-yl]-1,3-thiazol-4-yl}acetate (**6a**)

Yellow green solid from EtOH (80% yield). m.p. 215–218 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.28 (t, $J = 7.1$, 3H), 2.26 (s, 3H), 3.64 (s, 2H), 4.20 (q, $J = 7.1$, 2H), 6.55 (s, 1H), 7.33 (dd, $J = 8.0, 4.8$, 1H), 8.08 (dd, $J = 8.0, 1.6$, 1H), 8.59 (d, $J = 4.7$, 1H), 8.75 (s, 1H), 8.97 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 14.2, 17.4, 35.4, 61.4, 104.3, 124.2, 126.5, 137.5, 150.9, 152.1, 153.5, 168.2, 169.0, 171.1; IR (KBr, ν_{max}, cm^{-1}): 3133, 3003, 2886, 1708, 1611, 1587, 1559, 1358, 1312, 1268, 1226, 1121, 1088; Anal. Calcd. for $C_{14}H_{16}N_4O_2S$: C, 55.25; H, 5.30; N, 18.41; S, 10.53; Found: C, 55.29; H, 5.34; N, 18.47; S, 10.59; HR-MS (ESI+): Calcd. m/z for $C_{14}H_{16}N_4O_2S$: 304.09940; Found m/z [M + H]$^+$: 304.09943.

4.3.38. Ethyl 2-{2-[(E)-2-[1-(pyridin-2-yl)ethylidene]hydrazin-1-yl]-1,3-thiazol-4-yl}acetate (**6b**)

Brown solid from EtOH (80% yield). m.p. 197–198 °C; ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.28 (t, $J = 7.1$, 3H), 2.37 (s, 3H), 3.64 (s, 2H), 4.19 (q, $J = 7.1$, 2H), 6.55 (s, 1H), 7.25–7.19 (m, 1H), 7.69 (t, $J = 7.8$, 1H), 8.10 (d, $J = 8.1$, 1H), 8.56 (d, $J = 4.8$, 1H), 8.78 (s, 1H); ^{13}C NMR (101 MHz, δ, ppm, $CDCl_3$): 11.2, 14.1, 15.2, 61.3, 104.3, 122.2, 126.2, 136.1, 145.6, 149.1, 150.8, 154.8, 169.2, 170.4; IR (KBr, ν_{max}, cm^{-1}): 3208, 3038, 2983, 2956, 1690, 1558, 1479, 1362, 1277, 1120; Anal. Calcd. for $C_{14}H_{16}N_4O_2S$: C, 55.25; H, 5.30; N, 18.41; S, 10.53; Found: C, 55.28; H, 5.32; N, 18.46; S, 10.57; HR-MS (ESI+): Calcd. m/z for $C_{14}H_{16}N_4O_2S$: 304.09940; Found m/z [M + H]$^+$: 304.09947.

4.4. In vitro assay for evaluation of antimycobacterial assay

All test compound stocks as well as dilutions were prepared in DMSO. *M. tuberculosis* MICs of test compounds were determined in 7H9 broth by a standard microdilution method [48] with some modifications. Briefly, 1 μl of serial twofold dilutions of test compound made in DMSO were added to wells of a 384 well plate, with the final concentrations ranging from 100 mM to 0.19 mM. Two sets of control wells were prepared: a media control with only medium (Middlebrook 7H9 medium) supplemented with 0.2% glycerol, 0.05% Tween 80 (Sigma), and 10% albumin dextrose catalase (Difco Laboratories, Detroit, Mich.) and a culture control with a bacterial suspension but no compound. Then, 40 μl (3–7 × 10^5 CFU/ml) of the bacterial culture was added to all the wells except the media control wells. The plates were packed in gas permeable polyethylene bags and incubated at 37 °C for 5 days. Following this incubation period, 8 μL of a freshly prepared 1:1 mixture of Resazurin (0.02% in water) and 10% Tween 80 was added to all the wells. The plates were re-incubated for an additional 24 h at 37 °C and the color conversion of all wells were recorded. A blue color in the well indicated no growth and a pink color indicated the growth. Absorbance at 575 nm and 610 nm was monitored and the ratio (A_{575}/A_{610}) calculated. The least concentration which yielded 80% inhibition was considered as MIC; the media control is considered 100% inhibition and the culture control as 0% inhibition. Isoniazid was used as a reference drug for the assay.

4.5. In vitro assay for evaluation of cytotoxicity assay

4.5.1. Cell culture

The human lung carcinoma type II epithelial cells (A549) cells were grown in RPMI-1640 medium containing 5% (v/v) fetal bovine serum (FBS). Cultures of A549 cells were maintained in medium containing 100 U/ml penicillin, 100 mg/ml streptomycin and 2 mM L-glutamine in 75 cm^2 culture flasks at 37 °C in a humidified atmosphere with a supply of 5% CO_2. The cells were sub cultured twice per week by seeding at a density of about 3 × 10^5 cells/ml.

4.5.2. MTT assay

The cell viability assay was carried out using MTT as per the protocol described earlier with slight modifications [47]. In brief, A549 cells (3 × 10^3 cells/well) in RPMI-1640 medium with a final volume of 200 μL were seeded into 96 wells culture plate and incubated overnight at 37 °C with the supply of 5% CO_2. The cells were treated with various synthesized drugs and incubated for 48 h. Further, following a wash with Phosphate Buffered Saline (PBS), the cells were treated with 20 μL of MTT (5 mg/ml) alone and incubated for 4 h at 37 °C in CO_2 incubator. The blue Formosan products formed in cells were dissolved in DMSO (200 μL) and spectrophotometrically measured at 540 nm. The concentration of the compound that inhibited cell growth by 50% (IC_{50}) was determined from cell survival plots and the percentage of inhibition results were expressed as mean ± SE ($n = 5$) and percentage of growth in the treated samples was calculated with respect to control. The cell toxicity effect of various drugs on A549 cells were calculated and graphically represented.

4.6. Molecular docking

The Auto Dock 4.2 [49] was used to perform docking simulations. From the RCSB Protein Data Bank, the recently solved X-ray crystal structure of Kas A of *Mtb* (PDB code: 2WGD) in complex with TLM has been retrieved and all heteroatoms were removed. The 2WGD was setup for a standard protocol for docking. Lamarckian Genetic Algorithm was used to carry out the simulation. For evaluating binding energy in the docking step, Coulombic electrostatic potential, Vander Waals interaction represented as a Lennard-Jones 12-6 dispersion/repulsion term and hydrogen bonding represented as a directional 12-10 term were the three parameters taken into account. The most favorable free energy of binding were attained by considering the docking orientations lying within the range of 2.0 Å in the root-mean square deviation (rmsd) tolerance and clustering the each other to get the result. The top-posed docking conformations obtained were subjected to post-docking energy minimization on Discovery Studio 2.5.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2013.08.054. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] H. Herzog, History of tuberculosis, Respiration 65 (1998) 5–15.
[2] A. Schatz, E. Bugie, S.A. Waksman, Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria, Proc. Soc. Exp. Biol. Med. 55 (1944) 66–69.
[3] Tuberculosis: WHO Global Tuberculosis Report 2012, in, World Health Organization, World Health Organization, Geneva, 2012.
[4] T.S. Balganesh, P.M. Alzari, S.T. Cole, Rising standards for tuberculosis drug development, Trends Pharmacol. Sci. 29 (2008) 576–581.
[5] C. Dye, Global epidemiology of tuberculosis, Lancet 367 (2006) 938–940.
[6] N.R. Gandhi, A. Moll, A.W. Sturm, R. Pawinski, T. Govender, U. Lalloo, K. Zeller, J. Andrews, G. Friedland, Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa, Lancet 368 (2006) 1575–1580.
[7] C.K. Kwan, J.D. Ernst, HIV and tuberculosis: a deadly human syndemic, Clin. Microbiol. Rev. 24 (2011) 351–376.
[8] M.R. Ward, Notes on the 2004 to 2009 United States Food and Drug Administration approval of new molecular entities (NMEs), in: KEI Research Note, 2010, pp. 1–14.
[9] P. Trouiller, P. Olliaro, E. Torreele, J. Orbinski, R. Laing, N. Ford, Drug development for neglected diseases: a deficient market and a public-health policy failure, Lancet 359 (2002) 2188–2194.
[10] C. Lienhardt, S.V. Cook, M. Burgos, V.Y. Edwards, L. Rigouts, G. Anyo, K.S. Jae, A. Jindani, D.A. Enarson, A.J. Nunn, Efficacy and safety of a 4-drug fixed-dose combination regimen compared with separate drugs for treatment of pulmonary tuberculosis: the study c randomized controlled trial, J. Am. Med. Assoc. 305 (2011) 1415–1423.
[11] Z.F. Udwadia, R.A. Amale, K.K. Ajbani, C. Rodrigues, Totally drug-resistant tuberculosis in India, Clin. Infect. Dis. 54 (2012) 579–581.
[12] S.E. Dorman, R.E. Chaisson, From magic bullets back to the magic mountain: the rise of extensively drug-resistant tuberculosis, Nat. Med. 13 (2007) 295–298.
[13] A. Faustini, A.J. Hall, C.A. Perucci, Risk factors for multidrug resistant tuberculosis in Europe: a systematic review, Thorax 61 (2006) 158–163.
[14] A. Fraser, M. Paul, A. Attamna, L. Leibovici, Treatment of latent tuberculosis in persons at risk for multidrug-resistant tuberculosis: systematic review, Int. J. Tuberc. Lung Dis. 10 (2005) 19–23.
[15] M. Zignol, M.S. Hosseini, A. Wright, C.L.V. Weezenbeek, P. Nunn, C.J. Watt, B.G. Williams, C. Dye, Global incidence of multidrug-resistant tuberculosis, J. Infect. Dis. 194 (2006) 479–485.
[16] A. Zumla, P. Nahid, S.T. Cole, Advances in the development of new tuberculosis drugs and treatment regimens, Nat. Rev. Drug Discovery 12 (2013) 388–404.
[17] K. Andries, P. Verhasselt, J. Guillemont, H.W.H. Göhlmann, J.-M. Neefs, H. Winkler, J.V. Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D.d. Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis, V. Jarlier, A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*, Science 307 (2005) 223–227.
[18] S. Ananthan, E.R. Faaleolea, R.C. Goldman, J.V. Hobrath, C.D. Kwong, B.E. Laughon, J.A. Maddry, A. Mehta, L. Rasmussen, R.C. Reynolds, J.A. Secrist 3rd, N. Shindo, D.N. Showe, M.I. Sosa, W.J. Suling, E.L. White, High-throughput screening for inhibitors of *Mycobacterium tuberculosis* H37Rv, Tuberculosis 89 (2009) 334–353.
[19] E.L. Luzina, A.V. Popov, Synthesis and anticancer activity of *N*-bis(trifluoromethyl)alkyl-*N*′-thiazolyl and *N*-bis(trifluoromethyl)alkyl-*N*′-benzothiazolyl ureas, Eur. J. Med. Chem. 44 (2009) 4944–4953.
[20] S.M. El-Messery, G.S. Hassan, F.A.M. Al-Omary, H.I. El-Subbagh, Substituted thiazoles VI. Synthesis and antitumor activity of new 2-acetamido- and 2 or 3-propanamido-thiazole analogs, Eur. J. Med. Chem. 54 (2012) 615–625.
[21] J. Cai, M. Sun, X. Wu, J. Chen, P. Wang, X. Zong, M. Ji, Design and synthesis of novel 4-benzothiazole amino quinazolines Dasatinib derivatives as potential anti-tumor agents, Eur. J. Med. Chem. 63 (2013) 702–712.
[22] A. Cohen, P. Verhaeghe, M.D. Crozet, S. Hutter, P. Rathelot, P. Vanelle, N. Azas, Tandem synthesis and *in vitro* antiplasmodial evaluation of new naphtho[2,1-*d*]thiazole derivatives, Eur. J. Med. Chem. 55 (2012) 315–324.
[23] R.P. Karuvalam, K.R. Haridas, S.K. Nayak, T.N. Guru Row, P. Rajeesh, R. Rishikesan, N.S. Kumari, Design, synthesis of some new (2-aminothiazol-4-yl)methylester derivatives as possible antimicrobial and antitubercular agents, Eur. J. Med. Chem. 49 (2012) 172–182.
[24] M.H.M. Helal, M.A. Salem, M.S.A. El-Gaby, M. Aljahdali, Synthesis and biological evaluation of some novel thiazole compounds as potential anti-inflammatory agents, Eur. J. Med. Chem. 65 (2013) 517–526.
[25] S.N. Mokale, P.T. Sanap, D.B. Shinde, Synthesis and hypolipidemic activity of novel 2-(4-(2-substituted aminothiazole-4-yl)phenoxy) acetic acid derivatives, Eur. J. Med. Chem. 45 (2010) 3096–3100.
[26] M.A. Metwally, E. Abdel-Latif, F.A. Amer, G. Kaupp, Versatile 2-amino-4-substituted-1,3-thiazoles: synthesis and reactions, J. Sulfur Chem. 25 (2004) 63–85.
[27] G. Pappenberger, T. Schulz-Gasch, E. Kusznir, F. Muller, M. Hennig, Structure-assisted discovery of an aminothiazole derivative as a lead molecule for inhibition of bacterial fatty-acid synthesis, Acta Crystallogr. Sect. D: Biol. Crystallogr. 63 (2007) 1208–1216.
[28] S.R. Luckner, C.A. Machutta, P.J. Tonge, C. Kisker, Crystal structures of *Mycobacterium tuberculosis* Kas A show mode of action within cell wall biosynthesis and its inhibition by thiolactomycin, Structure 17 (2009) 1004–1013.
[29] Q. Al-Balas, N.G. Anthony, B. Al-Jaidi, A. Alnimr, G. Abbott, A.K. Brown, R.C. Taylor, G.S. Besra, T.D. McHugh, S.H. Gillespie, B.F. Johnston, S.P. Mackay, G.D. Coxon, Identification of 2-aminothiazole-4-carboxylate derivatives active against *Mycobacterium tuberculosis* $H_{37}R_v$ and the β-ketoacyl-ACP synthase MtfabH, PLoS One 4 (2009) e5617.
[30] E.C. Dykhuizen, J.F. May, A. Tongpenyai, L.L. Kiessling, Inhibitors of UDP-galactopyranose mutase thwart mycobacterial growth, J. Am. Chem. Soc. 130 (2008) 6706–6707.
[31] L.M. Fox, L.D. Saravolatz, Nitazoxanide: a new thiazolide antiparasitic agent, Clin. Infect. Dis. 40 (2005) 1173–1180.
[32] L.G. Dover, G.D. Coxon, Current status and research strategies in tuberculosis drug development, J. Med. Chem. 54 (2011) 6157–6165.
[33] A. Arshad, H. Osman, M.C. Bagley, C.K. Lam, S. Mohamad, A.S. Zahariluddin, Synthesis and antimicrobial properties of some new thiazolyl coumarin derivatives, Eur. J. Med. Chem. 46 (2011) 3788–3794.
[34] G. Turan-Zitouni, Z.A. Kaplancikli, A. Ozdemir, Synthesis and antituberculosis activity of some *N*-pyridyl-*N*′-thiazolylhydrazine derivatives, Eur. J. Med. Chem. 45 (2010) 2085–2088.
[35] K.K. Roy, S. Singh, S.K. Sharma, R. Srivastava, V. Chaturvedi, A.K. Saxena, Synthesis and biological evaluation of substituted 4-arylthiazol-2-amino derivatives as potent growth inhibitors of replicating *Mycobacterium tuberculosis* H37RV, Bioorg. Med. Chem. Lett. 21 (2011) 5589–5593.
[36] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Delivery Rev. 46 (2001) 3–26.
[37] L.P.S. de Carvalho, G. Lin, X. Jiang, C. Nathan, Nitazoxanide kills replicating and nonreplicating *Mycobacterium tuberculosis* and evades resistance, J. Med. Chem. 52 (2009) 5789–5792.
[38] T.E. Ballard, X. Wang, I. Olekhnovich, T. Koerner, C. Seymour, P.S. Hoffman, T.L. Macdonald, Biological activity of modified and exchanged 2-amino-5-nitrothiazole amide analogues of nitazoxanide, Bioorg. Med. Chem. Lett. 20 (2010) 3537–3539.
[39] A. Alahari, X. Trivelli, Y.G. Rardel, L.G. Dover, G.S. Besra, J.C. Sacchettini, R.C. Reynolds, G.D. Coxon, L. Kremer, Thiacetazone, an antitubercular drug that inhibits cyclopropanation of cell wall mycolic acids in mycobacteria, PLoS One 2 (2007) e1343.
[40] G.D. Coxon, D. Craig, R.M. Corrales, E. Vialla, L. Gannoun-Zaki, L. Kremer, Synthesis, antitubercular activity and mechanism of resistance of highly effective thiacetazone analogues, PLoS One 8 (2013) e53162.
[41] M.H. Shih, Y.S. Su, C.L. Wu, Syntheses of aromatic substituted hydrazinothiazole derivatives to clarify structural characterization and antioxidant activity between 3-arylsydnonyl and aryl substituted hydrazino-thiazole, Chem. Pharm. Bull. 55 (2007) 1126–1135.
[42] W. Yi, C. Dubois, S. Yahiaoui, R. Haudecoeur, C. Belle, H. Song, R. Hardre, M. Reglier, A. Boumendjel, Refinement of arylthiosemicarbazone pharmacophore in inhibition of mushroom tyrosinase, Eur. J. Med. Chem. 46 (2011) 4330–4335.
[43] Y. Gu, T. Kar, S. Scheiner, Fundamental properties of the CH···O interaction: Is it a true hydrogen bond? J. Am. Chem. Soc. 121 (1999) 9411–9422.
[44] J.C. Palomino, A. Martin, M. Camacho, H. Guerra, J. Swings, F. Portaels, Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*, Antimicrob. Agents Chemother. 46 (2002) 2720–2722.
[45] A.R. Katritzky, C. Dennis Hall, B.E.D.M. El-Gendy, B. Draghici, Tautomerism in drug discovery, J. Comput. Aided Mol. Des. 24 (2010) 475–484.
[46] W.R. Porter, Warfarin: history, tautomerism and activity, J. Comput. Aided Mol. Des. 24 (2010) 553–573.
[47] A. Periyakaruppan, F. Kumar, S. Sarkar, C.S. Sharma, G.T. Ramesh, Uranium induces oxidative stress in lung epithelial cells, Arch. Toxicol. 81 (2006) 389–395.
[48] M. Balganesh, S. Kuruppath, N. Marcel, S. Sharma, A. Nair, U. Sharma, Rv1218c, an ABC transporter of *Mycobacterium tuberculosis* with implications in drug discovery, Antimicrob. Agents Chemother. 54 (2010) 5167–5172.
[49] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, AutoDock 4 and AutoDockTools 4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785–2791.