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SAR Studies on 1,2,4-Triazolo[3,4-b][1,3,4]thiadiazoles as Inhibitors of *Mtb* Shikimate Dehydrogenase for the Development of Novel Antitubercular Agents

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ABSTRACT:

Shikimate dehydrogenase, an essential protein for the biosynthesis of the chorismate end product, is a highly promising therapeutic target, especially for the discovery and development of new-generation anti-TB agents. Following up the identification of one lead 3,6-disubstituted 1,2,4-triazolo[3,4-b][1,3,4]thiadiazole (**1**), targeting *Mt* SD in our previous study, an extensive SAR study for optimization of the lead compound was performed through systematic modification of the 3 and 6 positions. This study has successfully led to the discovery of two highly potent advanced leads **6d-4**, **6c-4** and several other compounds with comparable potencies (**6d-4**, MIC-H37Rv = 0.5 μg/mL; MIC-MDRTB = 4.0 μg/mL; MIC-RDRTB = 0.5 μg/mL; *Mt* SD-IC50 = 14.20 μg/mL; and **6c-4**, MIC-H37Rv = 0.5 μg/mL; MIC-MDRTB = 4.0 μg/mL; MIC-RDRTB = 1.0 μg/mL; *Mt* SD-IC50 = 6.82 μg/mL). These advanced lead compounds possess a para-halogen phenyl at the 3 position. *In vitro Mt* SD inhibitory assay indicates that *Mt* SD is the target for their antitubercular activity. Moreover, the BacT/ALERT 3D liquid culture technology and *in vitro Mt* SD inhibitory assay were initially applied.

Keywords:

Shikimate dehydrogenase

Antitubercular agent

3,6-Disubstituted triazolothiadiazole

SAR

BacT/ALERT 3D

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1. Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*) remains a leading cause of morbidity and mortality. Worldwide, there were approximately 9 million cases in 2013, of which 500,000 were multidrug-resistant (MDR).[1] The only FDA-approved TB drug, Bedaquiline, has been recently announced for the treatment of MDR-TB since the 1960s.[2] It specifically inhibits mycobacterial ATP (adenosine 5'-triphosphate) synthase which is essential for the final step in ATP production by oxidative phosphorylation.[3] Its associated risk of potentially lethal heart problems has emphasized the unmet and urgent need for the development of safer antitubercular drugs with novel targets and mechanisms of action to treat resistant forms of the disease.

The shikimate pathway is used in a variety of bacteria, including *Mycobacterium tuberculosis*, for the production of chorismate, a precursor for aromatic amino acids and other aromatic compounds. Mammals do not need the shikimate pathway enzymes necessary for de novo synthesis of these amino acids but rather obtain them from the diet, making these potential targets considerably less toxic in humans.[4] Shikimate pathway enzymes may offer attractive targets for new TB drug and vaccine development.[5] One favorable mechanism for antitubercular agents is the inhibition of shikimate dehydrogenase (SD) in the shikimate pathway. Consequently, inhibitors of SD are anticipated to be selective antitubercular drugs. SD is the fourth of seven enzymes involved in the shikimate pathway. SD is responsible for catalyzing the NADPH-dependent reduction of 3-dehydroshikimate to form shikimate and $NADP^+$.[4] *M. tuberculosis* shikimate dehydrogenase (*Mt* SD) is encoded by *aroE* and is essential for the survival of *M. tuberculosis*.[6] Recently, we have established a high-throughput screening (HTS) model targeting *Mycobacterium tuberculosis* shikimate dehydrogenase (*Mt* SD) for the discovery of novel antituberculosis drugs. 80, 000 compounds have been screened by using this model and

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evaluated for their activity against *Mtb* H37Rv, MDRTB (Isoniazid and Rifampin resistant strains) and RDRTB (Rifampin resistant strains) initially using the BacT/ALERT 3D (MB/BacT) liquid culture technology [7]. One 3,6-disubstituted triazolothiadiazole (**1**, MIC-H37Rv = 8.0 μg/mL; MIC-MDRTB = 8.0 μg/mL; MIC-RDRTB = 8.0 μg/mL; *Mt* SD-IC_{50} = 23.00 μg/mL) has been identified as a promising lead for antitubercular drug development.

Triazolothiadiazoles have been attracting increasing interest over the past decade because of their utility in various applications such as antibacterial [8-16], anti-inflammatory [8,11,112], analgesic [8,11,12,13], antifungal [11-15], urease inhibitory [16], antitubercular [13,14,17], antitumor [13,18-21], antioxidant [16,19], acetylcholinesterase inhibitory [21], PDE4 inhibitory [22] and anticonvul activity [23]. Among these, interesting observations on the antitubercular activity of triazolothiadiazole derivatives were made. At present, several triazole bearing compounds, like Flutrox, Nefazodone, Trazodone, Triazoledione, etc., are used in modern medicine. Fluorine incorporated heterocycles, triazoles and thiadiazoles displayed varied pharmacological properties. Since there have been few reports on dichlorofluorophenyl containing triazolothiadiazoles, it was contemplated to synthesize them and to pursue antitubercular screening so as to obtain a promising lead **2**[13] (Fig. 1). Isopropylthiazole moiety has already been identified for its antimicrobial activity and its coupling with other heterocyclic rings furnishes novel biologically active compounds [24]. It was contemplated to synthesize a series of clubbed isopropylthiazole derivatives triazolothiadiazoles, study their antitubercular activity against H37Rv strain to get two potent and low-toxicity lead **3**[14] and **4**[14] (Fig. 1). Literature survey revealed that the pyrazine ring is important for antimycobacterial activity [25]. Some novel fused heterocycles incorporation of the pyrazine in triazolothiadiazoles have been synthesized and evaluated for their antimycobacterial activity to obtain two promising leads **5**[17] and **6**[17] (Fig. 1). However, against *Mt* SD, no compound has

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emerged as a promising lead in the past years. Since the early lead compounds **1** (MIC-H37Rv = 8.0 μg/mL), **5** (MIC-H37Rv = 0.4 μg/mL) and **6** (MIC-H37Rv = 1.0 μg/mL) possess a methyleneoxo group at the 6 or 3 position, we consider the effect of unsubstituted and substituted aryloxymethylene groups at the 6 position as well as unsubstitution and different substitution of aromatic and hetero-aromatic groups at the 3 position on the potency against *Mtb*. Considering above facts and the principles of group replacement, scaffold hopping, bioisosterism, we set out for the structure–activity relationships (SAR) study and optimization of the lead through systematic structural modifications at the 3 and 6 positions, keeping the methyleneoxo group at the 6 position intact as shown in Fig. 2. We describe here our SAR study and successful optimization of the early lead compound, which has led to the identification of two highly potent lead compounds **6d-4**, **6c-4** (MIC-H37Rv = 0.5 μg/mL) and others with comparable potencies.

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2. Chemical synthesis

Synthesis of compounds for the optimization library of 3,6-disubstituted triazolothiadiazoles (72 compounds in total) is outlined in Scheme 1. The commercially available aromatic and hetero-aromatic acid was firstly activated by 1,1'-carbonyldiimidazole and then hydrazinolysized to give corresponding aroyl hydrazides **3a–h** in 79–95% yields in one-pot, which reacted with carbon disulfide and potassium hydroxide in ethanol to yield potassium dithiocarbazinate **4a–h**, which later cyclized to 4-amino-3-mercapto-1,2,4-triazole **5a–h** by reacting with hydrazine hydrate (80%) in 60–70% yields. The resulting triazoles **5a–h** were further converted to triazolo thiadiazoles **6** in one pot-reaction by condensation with unsubstituted and substituted aryloxyacetic acids in the presence of phosphorous oxychloride under microwave irradiation (MWI) in 91–98% yields.

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3. Results and discussions

3.1. Evaluation of antitubercular activity and SAR study

The 3,6-disubstituted triazolothiadiazoles **6a-h** (72 compounds) were evaluated for their activity against *Mtb* H37Rv, MDRTB and RDRTB using the BacT/ALERT 3D liquid culture technology[7]. The antitubercular activities of the compounds are indicated by MIC values. Results are summarized in Table 1. Compounds were tested in 8.0 μg/mL (MIC) for the preliminary assessment of the activity against *Mtb* H37Rv. 54 compounds result inactive at 8.0 μg/mL concentration, whereas 17 compounds are found to inhibit the growth of *Mtb* H37Rv, MDRTB and RDRTB at variable concentrations (Table 1). Some of the most representative compounds are also tested toward Vero and HepG2 cells to ascertain the cytotoxicity profile. To further analyze the biological profile of these compounds, *in vitro Mt* SD inhibitory assay has been carried out as well. These modifications lead to a variable range of activities and allow us to construct a plausible SAR as will be described below.

Selected 17 derivatives were tested for their ability to inhibit the growth of the acquired clinical MDRTB and RDRTB strains from Jiangsu province hospital, China (Table 1). To our delight, although these triazolothiadiazoles are less potent than positive control medicine INH and RFP against H37Rv, maintain similar excellent activities against the susceptible *M. tuberculosis* strain H37Rv in two tested drug-resistant strains.

As Table 1 shows, the substituents at both 3 and 6 positions have substantial effects on antibacterial activity, but it is very clear that electron-donating group (the 4-methoxy group) attached at para position and unsubstituted of the phenyl ring at the 3 position are detrimental to the potency of compounds (**6a-1–9**, **6e-1–9**). On the contrary, different electron-withdrawing groups, i.e., fluoro, chloro and bromo groups, attached at para position of the phenyl ring at the 3

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position exert a remarkable enhancement in potency (**6b-5**, **6b-7**, **6c-2**, **6c-4**, **6c-7**, **6c-8**, **d-2–6**, **6d-8**, **6d-9**), leading to the discovery of two highly active lead compounds **6d-4** and **6c-4** (MIC-H37Rv = 0.5 μg/mL), which are 16-fold more potent than **1**. The nature of phenoxymethyl group at the 6 position exhibits substantial effects on the potency. Bromo, chloro, fluoro, nitro and methoxy groups in the *para* position of the benzene ring at the 6 position are well tolerated, and **6c-4** and **6d-4** bearing a bromo moiety possess high potency.

In the 3-(4-fluorophenyl) series of compounds (**6b-1–9**), the potency is very sensitive to the substitution pattern of the 6-phenoxymethyl moiety. Thus, the compounds with 4-fluoro-, 4-chloro-, 4-bromo-, 2,4-dichloro-, 3,4-butenyl-, 4-nitro- and 2-methyl-4-chlorophenoxymethyl moieties at the 6 position do not show appreciable antitubercular activity (MIC > 8.0 μg/mL). 3,4-butenylphenoxymethyl moiety shows only modest activities. Compound **6b-5** shows better potency than the parent compound **1** (MIC-H37Rv = 0.5 μg/mL; MIC- RDRTB = 0.5 μg/mL), which is approximately 2-fold more active than INH (MIC-RDRTB = 1.0 μg/mL), the standard first-line drug used against RDRTB.

In the 3-(4-chlorophenyl) series of compounds (**6c-1–9**), the potency of the compounds is also sensitive to the substitution pattern of the 6-phenoxymethyl moiety. Nevertheless, 4-chloro-, 4-methoxy-, 2,4-dichloro-, 2-methyl-4-chloro- and phenoxymethyl moieties decrease the potency. Two compounds bearing 4-fluorophenoxymethyl and 3,4-butenylphenoxymethyl moieties possess better activity than compound **1**. Meanwhile, Two compounds bearing 4-bromophenoxymethyl and 4-nitrophenoxymethyl moieties, **6c-4** (MIC-MDRTB = 4.0 μg/mL) and **6c-8** (MIC-MDRTB= 2.0 μg/mL) exhibit good activity, which are approximately 4-fold and 8-fold more potent than RFP (MIC-MDRTB ≥ 16.0 μg/mL), the standard first-line drug used against MDRTB, respectively.

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In the 3-(4-bromophenyl) series of compounds (**6d-1–9**), almost all compounds (except **6d-1** and **6d-7**) examined possess good to excellent antitubercular activities, wherein five compounds have MIC less than 4.0 μg/mL. The results clearly indicate that the introduction of the 4-bromophenyl group to the 3 position is a breakthrough in this SAR study. Also, 4-bromo, 4-chloro, 4-fluoro, 4-nitro, 4-methoxy, 2,4-dichloro and 2-methyl-4-chloro groups of the benzene ring at the 6 position are found to be beneficial to increase the potency. The most potent compounds in this series, at present, are **6d-3** and **6d-4** (MIC-MDRTB = 4.0 μg/mL; MIC-RDRTB = 0.5 μg/mL), which are approximately 2-fold and 32-fold more potent than INH (MIC-RDRTB = 1.0 μg/mL) and RFP (MIC-RDRTB = 16.0 μg/mL) against RDRTB, and 4-fold in activity to RFP (MIC-MDRTB ≥ 16.0 μg/mL) against MDRTB.

On the basis of these data, the next investigation is to introduce a methylene spacer between carbon atom at the 3 position and the β-naphthalene ring. Quite surprisingly, small electron-donating groups such as methoxy, or electron-withdrawing groups such as the chloro, bromo and 2,4-dichloro of the benzene ring at the 6 position, fail to show any activity (MIC-H37Rv > 8.0 μg/mL). However, compound **6f-8**, bearing 4-nitrophenoxymethyl moiety at the 6 position, is found to be the most active compound of the series (MIC-H37Rv = 0.5 μg/mL; MIC-MDRTB = 4.0 μg/mL; MIC-RDRTB = 1.0 μg/mL), which is comparable in activity to INH. Besides, 4-fluorophenoxymethyl moiety is also tolerated.

Aiming to improve the water solubility by introduction of ionizable nitrogen groups, **6g-1–9** have been synthesized whereby the benzene ring at the 3 position is replaced with a pyridine ring. Unfortunately, these modifications result in a dramatic attenuation of activity, compared to the MIC value of compound **1**. Further attempts to introduce fluoro attached at meta position of the pyridine ring (**6h-1–9**). Fortunately, the modifications lead to the identification of two active

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compounds in the series, **6h-1** (MIC-MDRTB = 2.0 μg/mL; MIC-RDRTB = 2.0 μg/mL) and **6h-6** (MIC-MDRTB = 2.0 μg/mL; MIC-RDRTB = 2.0 μg/mL), which are approximately 2- to 16-fold more than INH and RFP against MDRTB and RDRTB.

Assessment of the collocation of these data within the SAR is difficult, but we might speculate that antitubercular activity of the newly synthesized heterocyclic compounds, containing 1,2,4-triazole moiety fused with 1,3,4-thiadiazole ring depends on the substituents rather the basic skeleton of the molecule.

3.2. *In vitro Mt* SD inhibitory activity

To confirm that this series of compounds actually target *Mt* SD, the 17 potent compounds were selected for further evaluation of their *in vitro Mt* SD inhibitory activity (Table 1). As Table 1 shows, the potent derivatives, **6c-4**, **6c-8** and **6d-4** display potential inhibitory activity on *Mt* SD (**6c-4**, *Mt* SD-IC_{50} = 6.82 μg/mL; **6c-8**, *Mt* SD-IC_{50} = 9.37 μg/mL; **6d-4**, *Mt* SD-IC_{50} = 14.42 μg/mL), which demonstrates the extent of inhibitory effect on *Mt* SD correlates to the extent of antitubercular activity.

3.3. Cytotoxicity

Some of the potent derivatives synthesized were tested to assess their apparent cytotoxicity toward Vero and HepG2 cells (Table 2). In general, the selectivity index (SI), that in this case is the ratio between IC_{50} toward Vero or HepG2 cells and the MIC toward *Mtb* H37Rv, for a compound to be considered a valuable lead has usually to be > 10. As Table 2 displays, we are pleased to notice that most compounds are apparently not toxic (SI>10), while maintaining good activity compared with the hit compound **1**. Unfortunately, modifications of **6c-8** and **6d-9** leading to an enhancement of activity, also result in a counterproductive improvement of cytotoxicity. Summarizing, the extended investigation around the SAR for these antitubercular

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triazolothiadiazoles, leads to the synthesis of more active compounds, moreover devoid of apparent cytotoxicity. Thus, **6c-4** and **6d-4** have emerged as advanced lead compounds for further preclinical drug development.

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4. Conclusions

The extensive SAR study on triazolothiadiazoles for their antitubercular activities against *Mtb* H37Rv strain was performed, building upon the identification of promising early lead compounds **1** obtained from an high-throughput screening campaign. The purpose of this SAR study was to optimize the aromatic or hetero-aromatic substituents at the 3 and 6 positions of the lead compound **1** through systematic modifications. It has been found that the nature of the substitute group on the *para* site of 3-phenyl exerts remarkable effects on the antitubercular activity. For example, electron-donating (4-methoxy) and unsubstituted groups are detrimental to the activity. On the contrary, halogen group at this position dramatically increases the potency. This breakthrough finding in this SAR study has led to the discovery of **6c-4** and **6d-4** with exceptional potency (MIC-H37Rv = 0.5 μg/mL), which bear a *p*-bromophenoxymethyl group at the 6 position and a *p*-chlorophenyl or a *p*-bromophenyl at the 3 position. Also, 15 other compounds were found to possess comparable potencies (MIC-H37Rv ≤ 4.0 μg/mL), including **6d-3** (MIC-H37Rv = 0.25 μg/mL), bearing a *p*-chlorophenoxymethyl group at the 6 position and a *p*-bromophenyl group at the 3 position. These advanced lead compounds do not show appreciable cytotoxicity against Vero and HepG2 cells (SI > 10). The advanced leads **6c-4** and **6d-4** exhibit the similar potencies against drug-resistant *Mtb* clinical isolates, as anticipated. Other important findings in this SAR study include the fact that unsubstituted pyridyl group at the 3 position is detrimental to the antitubercular activity of this series of triazolothiadiazoles, while 3-fluoropyridyl group is tolerated, although only unsubstituted and 2,4-dichlorophenoxymethyl moieties at the 6 position are favorable. *In vitro Mt* SD inhibitory activity by the most potent compounds, shows effective inhibition, which validates that *Mt* SD is the target of these compounds. The observation strongly suggests that the advanced lead

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triazolothiadiazoles have a novel mechanism of action on the inhibition of *Mt* SD. Extensive preclinical evaluations for the pharmacological properties of these advanced lead compounds as well as in vivo efficacy evaluations are actively underway, and the results will be reported in due course.

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5. Experimental section

5.1. Methods and materials

Methods. ^{1}H and ^{13}C NMR spectra were measured on a Bruker or Varian 400 or 500 MHz NMR spectrometer. Melting points were measured on a Mettler Toledo capillary melting point apparatus and are uncorrected. TLC experiments were carried out on pre-coated silica gel plates (F 254 Merck). APCI high-resolution mass spectra (HRMS) was recorded on an Autospec Ultima-TOF spectrometer. MB/BacT ALERT 3D system, which includes a computerized database management system. Carbon dioxide released into the medium by actively growing mycobacteria is detected through a gas-permeable sensor containing a colorimetric indicator embedded at the bottom of culture vials. Ensprire^{TM} enzyme-labelling measuring instrument (Perkin Elmer) was used in vitro *Mt SD* inhibitory activity and cell cytotoxicity experiments.

Materials. Modified Middlebrook 7H9 broth, MB reconstituting fluid, and the buffers were purchased from Biomerieux. RFP and INH were purchased from National Institute for Food and Drug Control.

5.2. Synthetic procedure and analytical data of aryl or hetero-aryl acid hydrazides(3a-h)

Isonicotinohydrazide (3g). In 250 mL reaction flask, 1,1'-carbonyldiimidazole (10.27 g, 63.36 mmol) was added to a solution of isonicotinic acid (6.0 g, 48.74 mmol) in tetrahydrofuran (THF) (60 mL). After stirring at room temperature for 3 h, upon completion of the reaction, the reaction mixture was added dropwise to the hydrazine hydrate 80% (v/v) (9.15 g, 146.22 mmol) in 40 mL THF over 3 h. The reaction mixture was stirred at room temperature overnight. After completion of the reaction, solvent was evaporated in vacuum to give a pale yellow precipitate. The crude product was recrystallized in ethanol to give isonicotinohydrazide (**3g**, 5.25 g, 79% yield) as a

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colorless solid: mp 173-174 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.71 (dd, J = 4.4, 1.6 Hz, 2H), 7.74 (dd, J = 4.4, 1.6 Hz, 2H), 4.64 (s, 2H); MS (ESI) m/z 137 $(M+1)^+$.

The same procedure was followed for the synthesis of **3a–h**.

Benzohydrazide (3a). White solid (95% yield); mp 128 – 129 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.50 (dd, J = 7.2, 2.0 Hz, 1H), 7.45 (m, 2H), 4.44 (s, 2H); MS (ESI) m/z 137.2 $(M+1)^+$.

4-Fluorobenzohydrazide (3b). White solid (93% yield); mp 157 – 158 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.82 (s, 1H), 7.91 – 7.88 (m, 2H), 7.27 (m, 2H), 4.46 (s, 2H); MS (ESI) m/z 155.2 $(M+1)^+$.

4-Chlorobenzohydrazide (3c). White solid (86% yield); mp 167 – 168 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 7.84 (dd, J = 8.4, 1.2 Hz, 2H), 7.53 – 7.51 (m, 2H), 4.51 (s, 2H); MS (ESI) m/z 171.1 $(M+1)^+$.

4-Bromobenzohydrazide (3d). White solid (85% yield); mp 166 – 167 °C;^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 7.77 (d, J = 8.8 Hz, 2H), 7.67 – 7.65 (m, 2H), 4.52 (s, 2H); MS (ESI) m/z 215.1 $(M+1)^+$.

4-Methoxybenzohydrazide (3e). White solid (92% yield); mp 135 – 136 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.61 (s, 1H), 7.80 (d, J = 8.0 Hz, 2H), 6.97 (d, J = 7.2 Hz, 2H), 4.38 (s, 2H), 3.80 (s, 3H); MS (ESI) m/z 167.2 $(M+1)^+$.

2-(Naphthalen-2-yl)acetohydrazide (3f). White solid (89% yield); mp 167 – 168 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (s, 1H), 8.12 (d, J = 7.6 Hz, 1H), 7.96 – 7.86 (m, 1H), 7.81

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(dd, *J* = 5.6, 2.8 Hz, 1H), 7.52 (m, 2H), 7.44 (dd, *J* = 6.4, 3.2 Hz, 2H), 4.23 (s, 2H), 3.84 (s, 2H); MS (ESI) m/z 201.2 $(M+1)^+$.

3-Fluoroisonicotinohydrazide (3h). White solid (91% yield); mp 152 – 153 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.68 (s, 1H), 8.52 (d, *J* = 4.8 Hz, 1H), 7.55 (t, *J* = 5.2 Hz, 1H), 4.64 (s, 2H); MS (ESI) m/z 156.2 $(M+1)^+$.

5.3. General method for the synthesis of potassium dithiocarbazinate 4a-g [11]

Potassium hydroxide (0.15 mol) was dissolved in absolute ethanol (200 mL). To the above solution, aryl or hetero-aryl acid hydrazide (0.1 mol) was added. To this, carbon disulfide (0.15 mol) was added in small portions with constant stirring. The reaction mixture was agitated continuously for a period of 9-10 h. Then, it was diluted with anhydrous ether. The precipitated potassium dithiocarbazinate was collected by filtration. The precipitate was further washed with anhydrous ether (100 mL) and dried under vacuum. The potassiumsalt thus obtained was in quantitative yield and was used in the next step without further purification.

5.4. Synthetic procedure and analytical data of 4-amino-5-substituted -3-mercapto-1,2,4-triazoles (5a-h)

4-Amino-5-phenyl-3-mercapto-1,2,4-triazole (5a) [11]. A suspension of potassium dithiocarbazinate of the aromatic esters **4a**, (11.10 g, 44.33 mmol) in water (50 mL) and hydrazine hydrate 80% (v/v) (5.55 g, 88.66 mmol) was refluxed for 6-7 h with constant stirring. The color of the reaction mixture changed to green with the production of hydrogen sulfide gas (lead acetate paper and odor). A homogenous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with water. On acidification with concentrated hydrochloric acid, the required triazole was precipitated. It was

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filtered, washed thoroughly with cold water, and recrystallized from ethanol to give **5a** (5.25 g, 61% yield) as a colorless solid: mp 219 – 220 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.93 (s, 1H), 8.03 (dd, J = 5.2, 1.6 Hz, 2H), 7.54 (dd, J = 5.2, 1.6 Hz, 3H), 5.80 (s, 2H). MS (ESI) m/z 193.14 $(M+1)^+$.

The same procedure was followed for the synthesis of **5b–h**.

4-Amino-5-(4-fluorophenyl)-3-mercapto-1,2,4-triazole (5b). White solid (68% yield); mp 200 – 201 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (s, 1H), 7.89 (m, 2H), 7.28 (m, 2H), 4.57 (s, 2H); MS (ESI) m/z 212.1 $(M+1)^+$.

4-Amino-5-(4-chlorophenyl)-3-mercapto-1,2,4-triazole (5c). White solid (65% yield); mp 201 – 202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.00 (s, 1H), 8.08 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 5.81 (s, 2H); MS (ESI) m/z 227.1 $(M+1)^+$.

4-Amino-5-(4-bromophenyl)-3-mercapto-1,2,4-triazole (5d). White solid (60% yield); mp 204 – 205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.99 (s, 1H), 8.00 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 8.0 Hz, 2H), 5.79 (s, 2H); MS (ESI) m/z 271.1 $(M+1)^+$.

4-Amino-5-(4-methoxyphenyl)-3-mercapto-1,2,4-triazole (5e). White solid (70% yield); mp 185 – 186 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.82 (s, 1H), 7.99 (d, J = 8.8 Hz, 2H), 7.08 (d, J = 8.8 Hz, 2H), 5.77 (s, 2H), 3.83 (s, 3H); MS (ESI) m/z 223.13 $(M+1)^+$.

4-Amino-5-(naphthalen-2-ylmethyl)-3-mercapto-1,2,4-triazole (5f). White solid (68% yield); mp 206 – 207 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.48 (s, 1H), 8.09 – 8.04 (m, 1H), 7.97 – 7.93 (m, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.57 – 7.51 (m, 2H), 7.46 (t, J = 7.2 Hz, 1H), 7.40 (d, J = 6.4 Hz, 1H), 5.55 (s, 2H), 4.47 (s, 2H); MS (ESI) m/z 257.2 $(M+1)^+$.

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4-Amino-5-(pyridin-4-yl)-3-mercapto-1,2,4-triazole (5g). White solid (67% yield); mp 217 – 218 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.17 (s, 1H), 8.76 (dd, J = 4.0, 1.5 Hz, 2H), 8.03 (dd, J = 3.6, 1.2 Hz, 2H), 5.86 (s, 2H); MS (ESI) m/z 194.11 $(M+1)^+$.

4-Amino-5-(3-fluoropyridin-4-yl)-3-mercapto-1,2,4-triazole (5h). White solid (61% yield); mp 211 – 212 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.23 (s, 1H), 8.82 (d, J = 2.0 Hz, 1H), 8.64 (d, J = 5.0 Hz, 1H), 7.89 (t, J = 5.5 Hz, 1H), 5.70 (s, 2H); MS (ESI) m/z 212.1 $(M+1)^+$.

5.5. Synthetic procedure and analytical data of 3,6-disubstituted triazolothiadiazoles (6a-h)

6-(Phenoxymethyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6a-1). A mixture of **5a** (0.10 g, 0.52 mmol), phenoxyacetic acid (0.10 g, 0.62 mmol), 4-dimethylaminopyridine (0.006 g, 0.052 mmol), tetrabutyl ammonium bromide (0.05 g, 0.156 mmol) in phosphorus oxychloride (2.5 mL) was heated at 95℃ in the microwave for 25 min. After cooling, the mixture was gradually poured onto crushed ice with stirring. The mixture was allowed to stand overnight, separated solid was filtered, washed thoroughly with cold water, and dried. The crude product was stirred in 5 mL ether for 2 h , filtered, washed thoroughly with ether, and dried to obtain **6a-1** (0.15 g, 94% yield) as a white solid: mp 157 – 158 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 – 8.20 (m, 2H), 7.62 (t, J = 7.0 Hz, 2H), 7.57 (t, J = 7.0 Hz, 1H), 7.37 (dd, J = 8.5, 7.5 Hz, 2H), 7.14 (d, J = 8.0 Hz, 2H), 7.05 (t, J = 7.5 Hz, 1H), 5.64 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.26, 157.06, 154.40, 145.29, 130.43, 129.81, 129.21, 125.84, 125.39, 122.16, 115.08, 64.85. HRMS (APCI) m/z calcd. for $C_{16}H_{12}N_4OSH^+$: 309.0805. Found: 309.0811.

The same procedure was followed for the synthesis of **6a-2–9**, **6b-1–9**, **6c-1–9**, **6d-1–9**, **6e-1–9**, **6f-1–9**, **6g-1–9**, **6h-1–9**.

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6-((4-Fluorophenoxy)methyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6a-2). White solid (96% yield); mp 176 – 177 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 (d, J = 7.0 Hz, 2H), 7.62 (t, J = 7.0 Hz, 2H), 7.57 (m, J = 7.0 Hz, 1H), 7.23 – 7.15 (m, 4H), 5.62 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.00, 156.19, 154.41, 153.38, 145.29, 130.43, 129.21, 125.83, 125.38, 116.76, 116.67, 116.31, 116.08, 65.51. HRMS (APCI) m/z calcd. for $C_{16}H_{11}FN_4OSH^+$: 327.0710. Found: 327.0722.

6-((4-Chlorophenoxy)methyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6a-3). White solid (98% yield); mp 182 – 183 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.24 – 8.19 (m, 2H), 7.64 – 7.60 (m, 2H), 7.59 – 7.55 (m, 1H), 7.41 (dd, J = 7.0, 2.5 Hz, 2H), 7.18 (dd, J = 7.0, 2.5 Hz, 2H), 5.65 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.71, 155.93, 154.42, 145.29, 130.43, 129.55, 129.20, 125.91, 125.83, 125.37, 116.97, 65.18. HRMS (APCI) m/z calcd. for $C_{16}H_{11}Cl\,N_4OSH^+$: 343.0415. Found: 343.0413.

6-((4-Bromophenoxy)methyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6a-4). White solid (95% yield); mp 190 – 191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (dd, J = 8.4, 1.6 Hz, 2H), 7.65 – 7.56 (m, 3H), 7.54 (dd, J = 6.8, 2.4 Hz, 2H), 7.13 (dd, J = 6.8, 2.4 Hz, 2H), 5.65 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.67, 156.38, 154.41, 145.29, 132.44, 130.43, 129.20, 125.83, 125.37, 117.46, 113.69, 65.11. HRMS (APCI) m/z calcd. for $C_{16}H_{11}BrN_4OSH^+$: 388.9890. Found: 388.9886.

6-((4-Methoxyphenoxy)methyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6a-5). White solid (92% yield); mp 170 – 171 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 (dd, J = 8.5, 1.5 Hz, 2H), 7.64 – 7.60 (m, 2H), 7.59 – 7.55 (m, 1H), 7.08 (dd, J = 6.5, 2.0 Hz, 2H), 6.91 (dd, J = 6.5, 2.0 Hz, 2H), 5.56 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.03, 154.93,

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154.84, 151.44, 145.73, 130.89, 129.67, 126.29, 125.84 , 116.78, 115.24, 66.02, 55.85. HRMS (APCI) m/z calcd. for $C_{17}H_{14}N_4O_2SH^+$: 339.0910. Found: 339.0907.

6-((2,4-Dichlorophenoxy)methyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6a-6). White solid (91% yield); mp 202 – 203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, J = 6.8 Hz, 2H), 7.70 (d, J = 2.4 Hz, 1H), 7.64-7.55 (m, 3H), 7.458-7.40 (m, 2H), 5.76 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.21, 154.42, 151.62, 145.29, 130.44, 129.63, 129.19, 128.35, 126.23, 125.82, 125.36, 122.83, 116.23, 66.02. HRMS (APCI) m/z calcd. for $C_{16}H_{10}Cl_2N_4OSH^+$: 377.0025. Found: 377.0025.

6-((2-Naphthyloxy)methyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6a-7). White solid (95% yield); mp 215 – 216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (dd, J = 8.4, 1.6 Hz, 2H), 7.95 – 7.83 (m, 3H), 7.66 – 7.57 (m, 4H), 7.53 – 7.48 (m, 1H), 7.44 – 7.39 (m, 1H), 7.35 (dd, J = 8.8, 2.4 Hz, 2H), 5.77 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.01, 154.91, 145.30, 133.93, 130.44, 129.82, 129.22, 129.12, 127.65, 126.96, 126.75, 125.85, 125.41, 118.24, 108.32, 64.95. HRMS (APCI) m/z calcd. for $C_{20}H_{14}N_4OSH^+$: 359.0961. Found: 359.0956.

6-((4-Nitrophenoxy)methyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6a-8). White solid (95% yield); mp 216 – 217 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.28 (dd, J = 7.0, 2.5 Hz, 2H), 8.24 – 8.19 (m, 2H), 7.64 – 7.60 (m, 2H), 7.59– 7.56 (m, 1H), 7.37 (dd, J = 7.0, 2.0 Hz, 2H), 5.81 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.69, 162.12, 154.53, 145.34, 141.95, 130.47, 129.21, 126.01, 125.85, 125.35, 115.75, 65.46. HRMS (APCI) m/z calcd. for $C_{16}H_{11}N_5O_3SH^+$: 354.0655. Found: 354.0654.

6-((2-Methyl-4-chlorophenoxy)methyl)-3-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6a-9). White solid (93% yield); mp 197 – 198 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 – 8.20

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(m, 2H), 7.64 – 7.60 (m, 2H), 7.59 – 7.55 (m, 1H), 7.32 (d, *J* = 2.5 Hz, 1H), 7.26 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.17 (d, *J* = 9.0 Hz, 1H), 5.65 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.20, 154.35, 154.07, 145.29, 130.45, 130.34, 129.22, 128.73, 126.68, 125.84, 125.44, 125.39, 113.96, 65.28, 15.73. HRMS (APCI) m/z calcd. for $C_{17}H_{13}ClN_4OSH^+$: 357.0571. Found: 357.0561.

3-(4-Fluorophenyl)-6-(phenoxymethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b-1). White solid (97% yield); mp 183 – 184 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (dd, *J* = 8.8, 5.6 Hz, 2H), 7.48 (t, *J* = 8.8 Hz, 2H), 7.36 (dd, *J* = 8.8, 7.6 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 2H), 7.05 (t, *J* = 7.2 Hz, 1H), 5.63 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.34, 164.35, 161.88, 157.03, 154.34, 144.53, 129.78, 128.32, 128.23, 122.15, 116.49, 116.27, 115.07, 64.83. HRMS (APCI) m/z calcd. for $C_{16}H_{11}FN_4OSH^+$: 327.0710. Found: 327.0696.

6-((4-Fluorophenoxy)methyl)-3-(4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b-2). White solid (95% yield); mp 188 – 189 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (dd, *J* = 8.8, 5.2 Hz, 2H), 7.48 (t, *J* = 9.2 Hz, 2H), 7.20-7.17(m, 4H), 5.62 (s, 2H). HRMS (APCI) m/z calcd. for $C_{16}H_{10}F_2N_4OSH^+$: 345.0616. Found: 345.0630.

6-((4-Chlorophenoxy)methyl)-3-(4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b-3). White solid (97% yield); mp 183 – 184 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (dd, *J* = 9.0, 5.5 Hz, 2H), 7.50-7.44 (m, 2H), 7.41 (dd, *J* = 6.5, 2.0 Hz, 2H), 7.17 (dd, *J* = 7.0, 2.5 Hz, 2H), 5.64 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.83, 155.90, 154.37, 144.53, 129.54, 128.26, 125.92, 122.03, 116.95, 116.47, 116.29, 65.17. HRMS (APCI) m/z calcd. for $C_{16}H_{10}ClFN_4OSH^+$: 361.0321. Found: 361.0322.

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6-((4-Bromophenoxy)methyl)-3-(4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b-4). White solid (96% yield); mp 179 – 180 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.48 (t, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 9.0 Hz, 2H), 5.64 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.80, 161.88, 156.37, 140.81, 132.45, 128.30, 122.05, 117.47, 116.51, 116.29, 113.71, 65.10. HRMS (APCI) m/z calcd. for $C_{16}H_{10}BrFN_4OSH^+$: 406.9796. Found: 406.9791.

3-(4-Fluorophenyl)-6-((4-methoxyphenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b-5). White solid (95% yield); mp 201 – 202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (dd, *J* = 8.8,5.6 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 9.2 Hz, 2H), 5.56 (s, 2H), 3.71 (s, 3H). HRMS (APCI) m/z calcd. for $C_{17}H_{13}FN_4O_2S$ H^+: 357.0816. Found: 357.0823.

6-((2,4-Dichlorophenoxy)methyl)-3-(4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b-6). White solid (93% yield); mp 186 – 187 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (dd, *J* = 9.0, 5.5 Hz, 2H), 7.69 (d, *J* = 2.5 Hz, 1H), 7.50 – 7.44 (m, 3H), 7.41 (d, *J* = 8.5 Hz, 1H), 5.75 (s, 2H). HRMS (APCI) m/z calcd. for $C_{16}H_9Cl_2FN_4OSH^+$: 394.9931. Found: 394.9933.

3-(4-Fluorophenyl)-6-((2-naphthyloxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b-7). White solid (96% yield); mp 226 – 227 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 – 8.25 (m, 2H), 7.94 – 7.82 (m, 3H), 7.59 (d, *J* = 2.4 Hz, 1H), 7.53 – 7.46 (m, 3H), 7.43 – 7.38 (m, 1H), 7.35 (dd, *J* = 9.2, 2.8 Hz, 1H), 5.76 (s, 2H). HRMS (APCI) m/z calcd. for $C_{20}H_{13}FN_4OSH^+$: 377.0867. Found: 377.0863.

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3-(4-Fluorophenyl)-6-((4-nitrophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b-8). White solid (94% yield); mp 212 – 213 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 – 8.24 (m, 4H), 7.51 – 7.45 (m, 2H), 7.36 (dd, J = 7.2, 2.4 Hz, 2H), 5.81 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.80, 162.09, 154.49, 144.60, 141.96, 128.31, 126.00, 122.04, 116.52, 116.30, 115.75, 65.44. HRMS (APCI) m/z calcd. for $C_{16}H_{10}FN_5O_3SH^+$: 372.0561. Found: 372.0548.

3-(4-Fluorophenyl)-6-((2-methyl-4-chlorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b-9). White solid (95% yield); mp 178 – 179 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (dd, J = 8.8, 5.2 Hz, 2H), 7.49 (t, J = 8.8 Hz, 2H), 7.32 (d, J = 2.0 Hz, 1H), 7.26 (dd, J = 8.8, 2.4 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H), 5.64 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.30, 154.05, 144.55, 130.33, 128.72, 128.32, 128.24, 126.65, 125.45, 122.06, 116.51, 116.28, 113.96, 65.26, 15.70 . HRMS (APCI) m/z calcd. for $C_{17}H_{12}ClFN_4OSH^+$: 375.0477. Found: 375.0465.

3-(4-Chlorophenyl)-6-(phenoxymethyl)-1,2,4-triazolo[3,4-b][1,3,4]thiadiazole (6c-1). White solid (96% yield); mp 191 – 192 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (dd, J = 7.0, 2.0 Hz, 2H), 7.71 (dd, J = 6.5, 2.0 Hz, 2H), 7.39 – 7.34 (m, 2H), 7.14 (dd, J = 8.5, 0.5 Hz, 2H), 7.05 (t, J = 2.0 Hz, 1H), 5.63 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.55, 157.04, 154.62, 144.41, 135.03, 129.80, 129.38, 127.47, 124.27, 122.17, 115.07, 64.82. HRMS (APCI) m/z calcd. for $C_{16}H_{11}Cl\,N_4OSH^+$: 343.0415. Found: 343.0422.

3-(4-Chlorophenyl)-6-((4-fluorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c-2). White solid (96% yield); mp 227 – 228 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 8.8 Hz, 2H), 7.23 – 7.13 (m, 4H), 5.62 (s, 2H); ^{13}C NMR (101 MHz,

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DMSO-d_6) δ 168.28, 154.63, 153.38, 144.42, 135.03, 129.38, 127.47, 124.26, 116.72, 116.30, 116.07, 65.50. HRMS (APCI) m/z calcd. for $C_{16}H_{10}ClFN_4OSH^+$: 361.0321. Found: 361.0326.

6-((4-Chlorophenoxy)methyl)-3-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c-3). White solid (97% yield); mp 181 – 182 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 (dd, *J* = 6.5, 2.0 Hz, 2H), 7.71 (dd, *J* = 6.5, 2.0 Hz, 2H), 7.41 (dd, *J* = 7.0, 2.5 Hz, 2H), 7.17 (dd, *J* = 6.5, 2.0 Hz, 2H), 5.64 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.02, 155.92, 154.66, 144.44, 135.05, 129.56, 129.39, 127.49, 125.93, 124.26, 116.98, 65.18. HRMS (APCI) m/z calcd. for $C_{16}H_{10}Cl_2N_4OSH^+$: 377.0025. Found: 377.0018.

6-((4-Bromophenoxy)methyl)-3-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c-4). White solid (98% yield); mp 181 – 182 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, *J* = 8.4 Hz, 2H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.57 – 7.50 (dd, *J* = 7.2, 2.0 Hz, 2H), 7.15 – 7.10 (dd, *J* = 6.8, 2.0 Hz, 2H), 5.64 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.98, 156.36, 154.64, 144.40, 135.04, 132.45, 129.37, 127.44, 124.23, 117.44, 113.73, 65.08. HRMS (APCI) m/z calcd. for $C_{16}H_{10}BrClN_4OSH^+$: 422.9498. Found: 422.9480.

3-(4-Chlorophenyl)-6-((4-methoxyphenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c-5). White solid (96% yield); mp 146 – 147 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (dd, *J* = 6.4, 1.6 Hz, 2H), 7.70 (dd, *J* = 8.4, 1.6 Hz, 2H), 7.08 (dd, *J* = 6.8, 2.4 Hz, 2H), 6.91 (dd, *J* = 7.2, 2.4 Hz, 2H), 5.56 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.84, 154.59, 154.48, 150.98, 144.39, 135.01, 129.36, 127.46, 124.25, 116.32, 114.78, 65.56, 55.39. HRMS (APCI) m/z calcd. for $C_{17}H_{13}ClN_4O_2SH^+$: 373.0521. Found: 373.0527.

3-(4-Chlorophenyl)-6-((2,4-dichlorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c-6). White solid (95% yield); mp 208 – 209 °C; ^{1}H NMR (400 MHz,

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DMSO-d_6) δ 8.22 (dd, J = 6.4, 1.6 Hz, 2H), 7.72 – 7.69 (m, 3H), 7.46 (dd, J = 8.8, 2.4 Hz, 1H), 7.41 (d, J = 8.8 Hz, 1H), 5.75 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.49, 154.63, 151.60, 144.42, 135.04, 129.61, 129.36, 128.33, 127.45, 126.24, 124.23, 122.82, 116.23, 66.00. HRMS (APCI) m/z calcd. for $C_{16}H_9Cl_3N_4OSH^+$: 412.9607. Found: 412.9589.

3-(4-Chlorophenyl)-6-((2-naphthyloxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c-7). White solid (96% yield); mp 190 – 191 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (dd, J = 6.8, 2.0 Hz, 2H), 7.94 – 7.82 (m, 3H), 7.71 (dd, J = 6.8, 2.0 Hz, 2H), 7.59 (d, J = 2.8 Hz, 1H), 7.53 – 7.48 (m, 1H), 7.44 – 7.39 (m, 1H), 7.35 (dd, J = 9.2, 2.8 Hz, 1H), 5.77 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.26, 154.88, 154.63, 144.41, 135.02, 133.91, 129.79, 129.35, 129.11, 127.62, 127.46, 126.94, 126.71, 124.34, 124.26, 118.19, 108.32, 64.93. HRMS (APCI) m/z calcd. for $C_{20}H_{13}ClN_4OSH^+$: 393.0571. Found: 393.0556.

3-(4-Chlorophenyl)-6-((4-nitrophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c-8). White solid (95% yield); mp 201 – 202 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (dd, J = 7.2, 2.4 Hz, 2H), 8.23 (dd, J = 6.8, 2.0 Hz, 2H), 7.71 (dd, J = 6.8, 2.0 Hz, 2H), 7.36 (dd, J = 6.8, 2.0 Hz, 2H), 5.81 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.97, 162.09, 154.75, 144.48, 141.96, 135.07, 129.40, 127.50, 126.00, 124.24, 115.75, 65.44. HRMS (APCI) m/z calcd. for $C_{16}H_{10}ClN_5O_3SH^+$: 388.0265. Found: 388.0266.

3-(4-Chlorophenyl)-6-((2-methyl-4-chlorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c-9). White solid (93% yield); mp 202 – 203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 2.0 Hz, 1H), 7.26 (dd, J = 8.8, 2.8 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H), 5.65 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.47, 154.55, 154.04, 144.41, 135.03, 130.32, 129.37, 128.71, 127.46,

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126.65, 125.45, 124.25, 113.96, 65.25, 15.69. HRMS (APCI) m/z calcd. for $C_{17}H_{12}Cl_2N_4OSH^+$: 391.0182. Found: 391.0173.

3-(4-Bromophenyl)-6-(phenoxymethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d-1). White solid (96% yield); mp 191 – 192 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (dd, J = 6.8, 2.0 Hz, 2H), 7.85 (dd, J = 6.8, 2.0 Hz, 2H), 7.37 (dd, J = 8.8, 7.6 Hz, 2H), 7.15 – 7.13 (m, 2H), 7.08 – 7.03 (m, 1H), 5.64 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.58, 157.05, 154.67, 144.51, 132.30, 129.81, 127.65, 124.62, 123.82, 122.17, 115.08, 64.83. HRMS (APCI) m/z calcd. for $C_{16}H_{11}BrN_4OSH^+$: 388.9890. Found: 388.9899.

3-(4-Bromophenyl)-6-((4-fluorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d-2). White solid (95% yield); mp 222 – 223 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (d, J = 8.8 Hz, 2H), 7.84 (d, J = 8.4 Hz, 2H), 7.22 – 7.15 (m, 4H), 5.62 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.77, 155.13, 153.83, 144.96, 132.75, 128.09, 125.05, 124.27, 117.16, 116.76, 116.53, 65.95. HRMS (APCI) m/z calcd. for $C_{16}H_{10}BrFN_4OSH^+$: 406.9796. Found: 406.9788.

3-(4-Bromophenyl)-6-((4-chlorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d-3). White solid (97% yield); mp 204 – 205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (dd, J = 6.8, 1.6 Hz, 2H), 7.84 (dd, J = 6.8, 1.6 Hz, 2H), 7.41 (dd, J = 6.8, 2.0 Hz, 2H), 7.17 (dd, J = 6.8, 2.4 Hz, 2H), 5.65 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.03, 155.92, 154.68, 144.52, 132.30, 129.55, 127.65, 125.93, 124.60, 123.83, 116.98, 65.17. HRMS (APCI) m/z calcd. for $C_{16}H_{10}BrClN_4OSH^+$: 422.9498. Found: 422.9494.

6-((4-Bromophenoxy)methyl)-3-(4-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d-4). White solid (98% yield); mp 176 – 177 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (dd, J = 6.4, 1.6 Hz, 2H), 7.84 (dd, J = 6.8, 2.0 Hz, 2H), 7.53 (dd, J = 6.8, 2.4 Hz, 2H), 7.13 (dd, J =

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6.8, 2.0 Hz, 2H), 5.64 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6)) δ 168.00, 156.35, 154.68, 144.48, 132.45, 132.27, 127.59, 124.56, 123.83, 117.44, 113.73, 65.08. HRMS (APCI) m/z calcd. for $C_{16}H_{10}Br_2N_4OSH^+$: 466.8995. Found: 466.8998.

3-(4-Bromophenyl)-6-((4-methoxyphenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d-5). White solid (96% yield); mp 162 – 163 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (dd, *J* = 6.8, 2.0 Hz, 2H), 7.84 (dd, *J* = 6.8, 2.0 Hz, 2H), 7.08 (dd, *J* = 6.8, 2.0 Hz, 2H), 6.91 (dd, *J* = 6.8, 2.4 Hz, 2H), 5.56 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.90, 154.66, 154.48, 150.98, 144.49, 132.30, 127.64, 124.61, 123.82, 116.33, 114.79, 65.56, 55.41. HRMS (APCI) m/z calcd. for $C_{17}H_{13}BrN_4O_2SH^+$: 418.9996. Found: 418.9980.

3-(4-Bromophenyl)-6-((2,4-dichlorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d-6). White solid (95% yield); mp 208 – 209 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, *J* = 8.8 Hz, 2H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.69 (d, *J* = 2.4 Hz, 1H), 7.46 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 5.75 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.51, 154.67, 151.60, 144.51, 132.27, 129.62, 128.33, 127.61, 126.24, 124.57, 123.82, 122.82, 116.23, 66.00. HRMS (APCI) m/z calcd. for $C_{16}H_9BrCl_2N_4OSH^+$: 456.9101. Found: 456.9108.

3-(4-Bromophenyl)-6-((2-naphthyloxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d-7). White solid (97% yield); mp 185 – 186 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (dd, *J* = 6.8, 2.0 Hz, 2H), 7.91 (dd, *J* = 15.6, 8.8 Hz, 2H), 7.87 – 7.82 (m, 3H), 7.59 (d, *J* = 2.8 Hz, 1H), 7.53 – 7.48 (m, 1H), 7.43 – 7.39 (m, 1H), 7.35 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.77 (s, 2H). HRMS (APCI) m/z calcd. for $C_{20}H_{13}BrN_4OSH^+$: 439.0047. Found: 439.0044.

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3-(4-Bromophenyl)-6-((4-nitrophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d-8). White solid (93% yield); mp 210 – 211 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (d, J = 9.2 Hz, 2H), 8.15 (d, J = 8.4 Hz, 2H), 7.84 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 9.2 Hz, 2H), 5.81 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.98, 162.08, 154.78, 144.55, 141.95, 132.30, 127.65, 125.99, 124.57, 123.85, 115.74, 65.43. HRMS (APCI) m/z calcd. for $C_{16}H_{10}BrN_5O_3SH^+$: 433.9741. Found: 433.9736.

3-(4-Bromophenyl)-6-((2-methyl-4-chlorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d-9). White solid (95% yield); mp 202 – 203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (dd, J = 6.8, 2.0 Hz, 2H), 7.85 (dd, J = 6.8, 2.0 Hz, 2H), 7.32 (d, J = 2.8 Hz, 1H), 7.26 (dd, J = 8.8, 2.4 Hz, 1H), 7.17 (d, J = 8.8 Hz, 1H), 5.65 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.52, 154.61, 154.05, 132.30, 130.34, 128.72, 127.64, 126.66, 125.45, 124.61, 123.83, 113.96, 65.26, 15.72. HRMS (APCI) m/z calcd. for $C_{17}H_{12}BrClN_4OSH^+$: 436.9654. Found: 436.9647.

3-(4-Methoxyphenyl)-6-(phenoxymethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6e-1). White solid (95% yield); mp 127 – 128 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, J = 8.8 Hz, 2H), 7.37 (t, J = 8.4 Hz, 2H), 7.16 (dd, J = 14, 8.8 Hz, 4H), 7.05 (t, J = 7.6 Hz, 1H), 5.62 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.96, 160.83, 157.05, 153.78, 145.22, 129.81, 127.50, 122.15, 117.74, 115.06, 114.60, 64.84, 55.43. HRMS (APCI) m/z calcd. for $C_{17}H_{14}N_4O_2SH^+$: 339.0910. Found: 339.0894.

6-((4-Fluorophenoxy)methyl)-3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6e-2). White solid (96% yield); mp 151 – 152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (dd, J = 6.8, 2.0 Hz, 2H), 7.23 – 7.14 (m, 6H), 5.61 (s, 2H), 3.85 (s, 3H); ^{13}C

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NMR (126 MHz, DMSO-d_6) δ 167.65, 160.80, 158.32, 156.43, 153.77, 153.39, 145.22, 127.47, 117.77, 116.66, 116.28, 116.10, 114.58, 65.50, 55.40. HRMS (APCI) m/z calcd. for $C_{17}H_{13}FN_4O_2SH^+$: 357.0816. Found: 357.0811.

6-((4-Chlorophenoxy)methyl)-3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6e-3). White solid (94% yield); mp 149 – 150 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.14 (dd, *J* = 7.0, 2.5 Hz, 2H), 7.41 (dd, *J* = 6.5, 2.0 Hz, 2H), 7.17 (dd, *J* = 7.0, 5.0 Hz, 4H), 5.63 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.40, 160.84, 155.93, 153.80, 145.25, 129.55, 127.52, 125.90, 117.78, 116.97, 114.64, 65.20, 55.43. HRMS (APCI) m/z calcd. for $C_{17}H_{13}Cl\,N_4O_2SH^+$: 373.0521. Found: 373.0520.

6-((4-Bromophenoxy)methyl)-3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6e-4). White solid (96% yield); mp 150 – 151 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (dd, *J* = 7.2, 2.4 Hz, 2H), 7.54 (dd, *J* = 6.8, 2.4 Hz, 2H), 7.18 (dd, *J* = 7.2, 2.4 Hz, 2H), 7.12 (dd, *J* = 6.8, 2.0 Hz, 2H), 5.64 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.38, 160.85, 156.40, 147.70, 145.25, 132.45, 127.53, 117.78, 117.47, 114.65, 113.70, 65.12 , 55.44. HRMS (APCI) m/z calcd. for $C_{17}H_{13}BrN_4O_2SH^+$: 418.9996. Found: 418.9994.

6-((4-Methoxyphenoxy)methyl)-3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6e-5). White solid (93% yield); mp 141 – 142 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15 (dd, *J* = 7.0, 2.0 Hz, 2H), 7.17 (dd, *J* = 7.0, 2.0 Hz, 2H), 7.08 (dd, *J* = 6.5, 2.0 Hz, 2H), 6.91 (dd, *J* = 6.5, 2.5 Hz, 2H), 5.55 (s, 2H), 3.85 (s, 3H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.24, 160.83, 154.47, 153.77, 150.99, 145.23, 127.51, 117.83, 116.33, 114.79, 114.65, 65.58, 55.43, 55.40. HRMS (APCI) m/z calcd. for $C_{18}H_{16}N_4O_3SH^+$: 369.1016. Found: 369.1003.

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6-((2,4-Dichlorophenoxy)methyl)-3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6e-6). White solid (95% yield); mp 194 – 195 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (dd, *J* = 6.8, 2.0 Hz, 2H), 7.70 (d, J = 2.4 Hz, 1H), 7.46 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.17 (dd, *J* = 6.8, 2.0 Hz, 2H), 5.74 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.88, 160.84, 151.63, 129.63, 128.35, 127.50, 126.22, 122.83, 117.80, 116.23, 114.64, 66.03, 55.43. HRMS (APCI) m/z calcd. for $C_{17}H_{12}Cl_2N_4O_2SH^+$: 407.0131. Found: 407.0143.

3-(4-Methoxyphenyl)-6-((2-naphthyloxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6e-7). White solid (95% yield); mp 159 – 160 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (dd, *J* = 6.8, 2.0 Hz, 2H), 7.94 – 7.82 (m, 3H), 7.59 (d, *J* = 2.4 Hz, 1H), 7.52-7.48 (m, 1H), 7.43-7.39 (m, 1H), 7.35 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.17 (d, *J* = 8.8 Hz, 2H), 5.75 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.06, 161.20, 155.29, 154.19, 145.61, 134.32, 130.20, 129.50, 128.04, 127.90, 127.35, 127.13, 124.74, 118.62, 118.19, 115.00, 108.65, 65.32, 55.81. HRMS (APCI) m/z calcd. for $C_{21}H_{16}N_4O_2SH^+$: 389.1067. Found: 389.1090.

3-(4-Methoxyphenyl)-6-((4-nitrophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6e-8). White solid (92% yield); mp 211 – 212 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.28 (dd, *J* = 7.0, 2.0 Hz, 2H), 8.15 (dd, *J* = 7.0, 2.5 Hz, 2H), 7.36 (dd, *J* = 7.0, 2.0 Hz, 2H), 7.17 (dd, *J* = 7.0, 2.0 Hz, 2H), 5.80 (s, 2H), 3.85 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.37, 162.12, 160.86, 153.90, 145.30, 141.94, 127.53, 126.01, 117.79, 115.75, 114.65, 65.47, 55.44. HRMS (APCI) m/z calcd. for $C_{17}H_{13}N_5O_4SH^+$: 384.0761. Found: 384.0752.

3-(4-Methoxyphenyl)-6-((2-methyl-4-chlorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6e-9). White solid (93% yield); mp 187 – 188 °C; ^{1}H NMR (500 MHz,

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DMSO-d_6) δ 8.15 (dd, J = 6.5, 1.5 Hz, 2H), 7.32 (d, J = 2.5 Hz, 1H), 7.26 (dd, J = 8.5, 2.5 Hz, 1H), 7.17 (dd, J = 8.5, 2.0 Hz, 3H), 5.63 (s, 2H), 3.85 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.87, 160.84, 154.07, 153.72, 145.24, 130.34, 128.72, 127.50, 126.67, 125.43, 117.82, 114.65, 113.95, 65.28, 55.44, 15.72. HRMS (APCI) m/z calcd. for $C_{18}H_{15}ClN_4O_2SH^+$: 387.0678. Found: 387.0680.

3-(β-Naphthylmethyl)-6-(phenoxymethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6f-1). White solid (96% yield); mp 149 – 150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (d, J = 7.6 Hz, 1H), 7.96 (dd, J = 6.8, 2.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.60 – 7.52 (m, 2H), 7.49-7.42 (m, 2H), 7.36 – 7.31 (m, 2H), 7.10 – 7.01 (m, 3H), 5.52 (s, 2H), 4.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.44, 157.01, 153.10, 146.05, 133.44, 131.36, 131.35, 129.75, 128.55, 127.80, 127.23, 126.39, 125.95, 125.60, 123.94, 122.08, 115.02, 64.70, 28.10. HRMS (APCI) m/z calcd. for $C_{21}H_{16}N_4OSH^+$: 373.1118. Found: 373.1107.

6-((4-Fluorophenoxy)methyl)-3-(β-naphthylmethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6f-2). White solid (93% yield); mp 120 – 121 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 7.5 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.59 – 7.53 (m, 2H), 7.48 – 7.41 (m, 2H), 7.18 – 7.15 (m, 2H), 7.10 (dd, J = 9.0, 4.5 Hz, 2H), 5.50 (s, 2H), 4.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.16, 153.33, 146.06, 133.44, 131.33, 128.55, 127.79, 127.22, 126.39, 125.95, 125.58, 123.93, 116.70, 116.62, 116.24, 116.01, 65.38, 28.10. HRMS (APCI) m/z calcd. for $C_{21}H_{15}FN_4OSH^+$: 391.1023. Found: 391.1020.

6-((4-Chlorophenoxy)methyl)-3-(β-naphthylmethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6f-3). White solid (95% yield); mp 135 – 136 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 – 8.24 (m, 1H), 7.97-7.94 (m, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.60 – 7.52 (m,

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2H), 7.48-7.41 (m, 2H), 7.38 (dd, *J* = 6.8, 2.4 Hz, 2H), 7.11 (dd, *J* = 6.8, 2.0 Hz, 2H), 5.53 (s, 2H), 4.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.90, 155.87, 153.14, 146.06, 133.44, 131.36, 131.31, 129.49, 128.55, 127.79, 127.24, 126.39, 125.95, 125.84, 125.58, 123.93, 116.92, 65.03, 28.10. HRMS (APCI) m/z calcd. for $C_{21}H_{15}Cl\ N_4OSH^+$: 407.0728. Found: 407.0732.

6-((4-Bromophenoxy)methyl)-3-(β-naphthylmethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6f-4). White solid (95% yield); mp 129 – 130 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.58 – 7.52 (m, 2H), 7.51 – 7.40 (m, 4H), 7.05 (d, *J* = 8.5 Hz, 2H), 5.53 (s, 2H), 4.86 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.83, 156.31, 146.06, 133.44, 132.38, 131.3, 131.32, 128.55, 127.79, 127.23, 126.39, 125.95, 125.58, 123.93, 117.41, 113.62, 100.04, 64.95, 28.11. HRMS (APCI) m/z calcd. for $C_{21}H_{15}BrN_4OSH^+$: 453.0204.. Found: 453.0212.

6-((4-Methoxyphenoxy)methyl)-3-(β-naphthylmethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6f-5). White solid (94% yield); mp 128 – 129 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (d, *J* = 8.0 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.59 – 7.53 (m, 2H), 7.48 – 7.41 (m, 2H), 7.01 (d, *J* = 9.0 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 5.44 (s, 2H), 4.86 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.72, 154.40, 153.09, 150.92, 146.03, 133.44, 131.35, 128.55, 127.79, 127.22, 126.39, 125.95, 125.59, 123.93, 116.28, 114.72, 65.44, 55.39, 28.10. HRMS (APCI) m/z calcd. for $C_{22}H_{18}N_4O_2SH^+$: 403.1223. Found: 403.1216.

6-((2,4-Dichlorophenoxy)methyl)-3-(β-naphthylmethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6f-6). White solid (93% yield); mp 171 – 172 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 1H), 7.66 (s,

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1H), 7.57-7.54 (m, 2H), 7.48 – 7.38 (m, 3H), 7.33 (d, *J* = 9.0 Hz, 1H), 5.63 (s, 2H), 4.86 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.38, 151.56, 146.08, 133.44, 131.35, 131.29, 129.60, 128.55, 128.27, 127.79, 127.25, 126.39, 126.14, 125.95, 125.57, 123.92, 122.76, 116.13, 99.53, 65.85, 28.13. HRMS (APCI) m/z calcd. for $C_{21}H_{14}Cl_2N_4OSH^+$: 441.0338. Found: 441.0335.

3-(β-Naphthylmethyl)-6-((2-naphthyloxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6f-7). White solid (95% yield); mp 172 – 173 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 – 8.25 (m, 1H), 7.96 – 7.94 (m, 1H), 7.90 – 7.84 (m, 3H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.57 – 7.53 (m, 2H), 7.51 – 7.48 (m, 2H), 7.44 – 7.39 (m, 3H), 7.29 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.65 (s, 2H), 4.88 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.24, 154.85, 146.06, 133.88, 133.44, 131.36, 131.32, 129.76, 129.07, 128.54, 127.79, 127.62, 127.26, 126.92, 126.71, 126.39, 125.94, 125.92, 125.58, 124.33, 123.94, 118.22, 108.18, 64.82, 28.13. HRMS (APCI) m/z calcd. for $C_{25}H_{18}N_4OSH^+$: 423.1274. Found: 423.1265.

3-(β-Naphthylmethyl)-6-((4-nitrophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6f-8). White solid (93% yield); mp 193 – 194 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 – 8.23 (m, 3H), 7.96 – 7.94 (m, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.58 – 7.52 (m, 2H), 7.45 – 7.40 (m, 2H), 7.29 (dd, *J* = 7.0, 2.5 Hz, 2H), 5.70 (s, 2H), 4.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.85, 162.05, 153.24, 146.09, 141.85, 133.42, 131.34, 131.27, 128.54, 127.77, 127.23, 126.38, 125.92, 125.54, 123.91, 115.67, 65.28, 28.12. HRMS (APCI) m/z calcd. for $C_{21}H_{15}N_5O_3SH^+$: 418.0968. Found: 418.0953.

6-((2-Methyl-4-chlorophenoxy)methyl)-3-(β-naphthylmethyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6f-9). White solid (95% yield); mp 172 – 173 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 7.5 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.59 –

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7.53 (m, 2H), 7.48 – 7.42 (m, 2H), 7.29 (d, *J* = 2.5 Hz, 1H), 7.21 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.09 (d, *J* = 8.5 Hz, 1H), 5.53 (s, 2H), 4.87 (s, 2H), 2.21 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.35, 154.00, 146.06, 133.45, 131.36, 131.32, 130.29, 128.629, 128.56, 127.81, 127.26, 126.58, 126.40, 125.96, 125.58, 125.36, 125.29, 123.93, 113.89, 65.13, 28.13, 15.70. HRMS (APCI) m/z calcd. for $C_{22}H_{17}ClN_4OSH^+$: 421.0884. Found: 421.0883.

6-(Phenoxymethyl)-3-(pyridin-4-yl)-1,2,4-triazolo[3,4-b][1,3,4]thiadiazole (6g-1). Light yellow powder (92% yield); mp 180 – 181 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (dd, *J* = 4.4, 1.6 Hz, 2H), 8.16 (dd, *J* = 4.8, 1.6 Hz, 2H), 7.40 – 7.35 (m, 2H), 7.16 – 7.14 (m, 2H), 7.08 – 7.04 (m, 1H), 5.66 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.17, 157.04, 155.62, 150.73, 143.42, 132.38, 129.82, 122.19, 119.40, 115.08, 64.82. HRMS (APCI) m/z calcd. for $C_{15}H_{11}N_5OSH^+$: 310.0757. Found: 310.0746.

6-((4-Fluorophenoxy)methyl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6g-2). White solid (94% yield); mp 301 – 302 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.83 (dd, *J* = 4.5, 2.0 Hz, 2H), 8.14 (dd, *J* = 4.5, 1.5 Hz, 2H), 7.23 – 7.16 (m, 4H), 5.64 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.92, 153.38, 150.79, 143.45, 132.33, 119.40, 116.77, 116.69, 116.33, 116.10, 65. 49. HRMS (APCI) m/z calcd. for $C_{15}H_{10}FN_5OSH^+$: 328.0663. Found: 328.0658.

6-((4-Chlorophenoxy)methyl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6g-3). Light yellow powder (96% yield); mp 196 – 197 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.85 (dd, *J* = 5.0, 1.5 Hz, 2H), 8.18 (dd, *J* = 4.5, 1.5 Hz, 2H), 7.42 (dd, *J* = 6.5, 2.0 Hz, 2H), 7.18 (dd, *J* = 7.0, 2.0 Hz, 2H), 5.67 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.71 (s), 155.91, 155.79, 150.18, 143.32, 132.93, 129.56, 125.96, 119.59, 116.99, 65.16. HRMS (APCI) m/z calcd. for $C_{15}H_{10}ClN_5OSH^+$: 344.0367. Found: 344.0370.

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6-((4-Bromophenoxy)methyl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6g-4). White solid (93% yield); mp 246 – 247 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.93 (d, J = 4.5 Hz, 2H), 8.34 (dd, J = 4.5, 1.5 Hz, 2H), 7.54 (dd, J = 7.0, 2.5 Hz, 2H), 7.13 (dd, J = 7.0, 2.5 Hz, 2H), 5.68 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.07, 156.37, 147.76, 142.82, 132.47, 120.43, 117.49, 113.77, 65.07. HRMS (APCI) m/z calcd. for $C_{15}H_{10}BrN_5OSH^+$: 389.9842. Found: 389.9842.

6-((4-Methoxyphenoxy)methyl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6g-5). White solid (95% yield); mp 200 – 201 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (d, J = 6.0 Hz, 2H), 8.15 (dd, J = 6.0, 1.6 Hz, 2H), 7.09 (dd, J = 6.8, 2.4 Hz, 2H), 6.92 (dd, J = 6.8, 2.4 Hz, 2H), 5.59 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.53, 155.66, 154.49, 150.98, 150.50, 143.35, 132.60, 119.48, 116.32, 114.79, 65.54, 55.40. HRMS (APCI) m/z calcd. for $C_{16}H_{13}N_5O_2SH^+$: 340.0863. Found: 340.0867.

6-((2,4-Dichlorophenoxy)methyl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6g-6). White solid (91% yield); mp 241 – 242 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (dd, J = 5.0, 1.5 Hz, 2H), 8.36 (dd, J = 5.0, 1.5 Hz, 2H), 7.70 (d, J = 2.5 Hz, 1H), 7.47 (dd, J = 9.0, 2.5 Hz, 1H), 7.43 (d, J = 9.0 Hz, 1H), 5.79 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.68, 156.44, 151.61, 147.49, 142.78, 135.65, 129.66, 128.37, 126.30, 122.81, 120.52, 116.26, 65.98. HRMS (APCI) m/z calcd. for $C_{15}H_9Cl_2N_5OSH^+$: 377.9978. Found: 377.9984.

6-((2-Naphthyloxy)methyl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6g-7). Light yellow powder (96% yield); mp 254 – 255 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.84 (dd, J = 4.5, 1.5 Hz, 2H), 8.17 (dd, J = 4.5, 1.5 Hz, 2H), 7.93 (d, J = 9.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.60 (d, J = 2.0 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 7.41 (t, J =

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8.0 Hz, 1H), 7.36 (dd, *J* = 9.0, 3.0 Hz, 1H), 5.79 (s, 2H). HRMS (APCI) m/z calcd. for $C_{19}H_{13}N_5OSH^+$: 360.0914. Found: 360.0940.

6-((4-Nitrophenoxy)methyl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6g-8). Light yellow powder (94% yield); mp 244 – 245 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.84 (dd, *J* = 4.5, 1.5 Hz, 2H), 8.28 (dd, *J* = 7.0, 2.5 Hz, 2H), 8.15 (dd, *J* = 4.5, 1.5 Hz, 2H), 7.37 (dd, *J* = 7.0, 2.5 Hz, 2H), 5.84 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.60, 162.09, 155.79, 150.63, 143.46, 141.97, 132.48, 126.02, 119.46, 117.57, 65.42. HRMS (APCI) m/z calcd. for $C_{16}H_{12}ClN_5OSH^+$: 355.0607. Found: 355.0617.

6-((2-Methyl-4-chlorophenoxy)methyl)-3-(pyridin-4-yl)--[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6g-9). White solid (93% yield); mp 258 – 259 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.96 (d, *J* = 5.0 Hz, 2H), 8.39 (t, *J* = 2.5 Hz, 2H), 7.33 (d, *J* = 2.0 Hz, 1H), 7.27 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.19 (d, *J* = 9.0 Hz, 1H), 5.69 (s, 2H), 2.27 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.68, 156.37, 154.04, 147.37, 142.74, 130.37, 128.72, 127.65, 126.68, 125.51, 120.56, 114.00, 65.24, 15.74. HRMS (APCI) m/z calcd. for $C_{16}H_{12}ClN_5OSH^+$: 358.0524. Found: 358.0518.

3-(3-Fluoropyridin-4-yl)-6-(phenoxymethyl)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole (6h-1). Yellow powder (94% yield); mp 293 – 294 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.87 (s, 1H), 8.69 (d, *J* = 4.5 Hz, 1H), 8.11 (bs, 1H), 7.35 (t, *J* = 7.0 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 7.04 (t, *J* = 7.0 Hz, 1H), 5.62 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.99, 157.01, 155.47, 146.55, 146.50, 139.73, 139.50, 129.78, 122.54, 122.17, 120.49, 115.07, 64.78. HRMS (APCI) m/z calcd. for $C_{15}H_{10}FN_5OSH^+$: 328.0663. Found: 328.0664.

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6-((4-Fluorophenoxy)methyl)-3-(3-fluoropyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6h-2). Yellow powder (93% yield); mp 161 – 162 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (d, J = 2.0 Hz, 1H), 8.70 (d, J = 4.5 Hz, 1H), 8.11 (t, J = 6.0 Hz, 1H), 7.22 – 7.14 (m, 4H), 5.61 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.73, 156.19, 153.35, 146.55, 146.49, 139.74, 139.51, 122.54, 116.76, 116.68, 116.29, 116.05, 65.45. HRMS (APCI) m/z calcd. for $C_{15}H_9F_2N_5OSH^+$: 346.0569. Found: 346.0574.

6-((4-Chlorophenoxy)methyl)-3-(3-fluoropyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6h-3). Yellow powder (94% yield); mp 208 – 209 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.70 (d, J = 5.0 Hz, 1H), 8.11 (t, J = 5.5 Hz, 1H), 7.41 (d, J = 9.0 Hz, 2H), 7.16 (d, J = 9.0 Hz, 2H), 5.64 (s, 2H). HRMS (APCI) m/z calcd. for $C_{15}H_9ClFN_5OSH^+$: 362.0273. Found: 362.0266.

6-((4-Bromophenoxy)methyl)-3-(3-fluoropyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6h-4). Yellow powder (96% yield); mp 135 – 136 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (s, 1H), 8.70 (d, J = 4.5 Hz, 1H), 8.11 (d, J = 5.0 Hz, 1H), 7.53 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 5.64 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.40, 156.33, 153.53, 146.54, 139.74, 139.50, 132.42, 132.08, 122.54, 117.46, 116.79, 113.72, 65.05. HRMS (APCI) m/z calcd. for $C_{15}H_9BrFN_5OSH^+$: 407.9748. Found: 407.9741.

3-(3-Fluoropyridin-4-yl)-6-((4-methoxyphenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6h-5). Yellow powder (94% yield); mp 175 – 176 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (d, J = 2.0 Hz, 1H), 8.70 (d, J = 4.5 Hz, 1H), 8.11 (t, J = 6.0 Hz, 1H), 7.07 (dd, J = 7.0, 2.5 Hz, 1H), 6.91 (dd, J = 6.5, 2.0 Hz, 1H), 5.55 (s, 2H), 3.71 (s, 3H). HRMS (APCI) m/z calcd. for $C_{16}H_{12}FN_5O_2SH^+$: 358.0769. Found: 358.0759.

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6-((2,4-Dichlorophenoxy)methyl)-3-(3-fluoropyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6h-6). Yellow powder (93% yield); mp 181 – 182 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 (d, J = 2.5 Hz, 1H), 8.70 (d, J = 5.0 Hz, 1H), 8.10 (t, J = 6.0 Hz, 1H), 7.69 (d, J = 2.5 Hz, 1H), 7.46 (dd, J = 9.0, 2.5 Hz, 1H), 7.40 (d, J = 9.0 Hz, 1H), 5.74 (s, 2H). HRMS (APCI) m/z calcd. for $C_{15}H_8Cl_2FN_5OSH^+$: 395.9883. Found: 395.9872.

3-(3-Fluoropyridin-4-yl)-6-((2-naphthyloxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6h-7). Yellow powder (93% yield); mp 169 – 170 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.89 (d, J = 2.0 Hz, 1H), 8.70 (d, J = 4.5 Hz, 1H), 8.13 (t, J = 6.0 Hz, 1H), 7.92 (d, J = 9.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 2.5 Hz, 1H), 7.50 (t, J = 7.0 Hz, 1H), 7.41 (t, J = 7.0 Hz, 1H), 7.34 (dd, J = 9.0, 2.5 Hz, 1H), 5.76 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.77, 155.52, 154.85, 146.52, 139.97, 133.90, 129.80, 129.12, 127.63, 126.95, 126.73, 124.36, 122.49, 120.58, 120.50, 118.20, 108.30, 64.88. HRMS (APCI) m/z calcd. for $C_{19}H_{12}FN_5OSH^+$: 378.0819. Found: 378.0812.

3-(3-Fluoropyridin-4-yl)-6-((4-nitrophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6h-8). Yellow powder (95% yield); mp 145 – 146 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (d, J = 2.0 Hz, 1H), 8.70 (d, J = 4.8 Hz, 1H), 8.27 (dd, J = 7.2, 2.4 Hz, 2H), 8.11 (t, J = 5.2 Hz, 1H), 7.35 (dd, J = 7.2, 2.4 Hz, 2H), 5.81 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.40, 162.04, 153.54, 146.56, 146.50, 141.96, 139.75, 139.52, 125.97, 122.55, 120.56, 115.74, 65.38. HRMS (APCI) m/z calcd. for $C_{15}H_9FN_6O_3SH^+$: 373.0514. Found: 373.0513.

3-(3-Fluoropyridin-4-yl)-6-((2-methyl-4-chlorophenoxy)methyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6h-9). Yellow powder (95% yield); mp 183 – 184 °C; ^{1}H NMR (500 MHz,

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DMSO-d_6) δ 8.88 (d, J = 2.0 Hz, 1H), 8.70 (d, J = 5.0 Hz, 1H), 8.11 (t, J = 5.5 Hz, 1H), 7.32 (d, J = 2.5 Hz, 1H), 7.25 (dd, J = 9.0, 2.5 Hz, 1H), 7.16 (d, J = 9.0 Hz, 1H), 5.64 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.92, 155.42, 154.02, 146.51, 139.74, 139.51, 130.32, 128.71, 126.64, 125.47, 122.53, 120.58, 120.48, 113.98, 65.21, 15.69. HRMS (APCI) m/z calcd. for $C_{16}H_{11}ClFN_5OSH^+$: 376.0430. Found: 376.0446.

5.6. Biological assays

5.6.1. Bacterial strains and growth

H37Rv and clinical *Mtb* strains MDRTB and RDRTB exhibiting resistant profiles to Isoniazid and Rifampin resistant strains were used. For evaluation of drug sensitivity all strains were grown in Difco 7H9 Middlebrook liquid medium (BD Biosciences, 271310) supplemented with casein, bovine albumen and catalase at 37 °C.

5.6.2. Antibacterial Activity [7]

M. tuberculosis H37Rv strain and clinical isolates were used in the antibacterial studies, which were grown at 37 °C in Difco 7H9 Middlebrook liquid medium supplemented with casein, bovine albumen and catalase. The BacT/Alert MP bottle containing the growth of *Mycobacterium tuberculosis* (≤36 hours, subculture, then the growth was diluted 1:1 in sterile distilled water). This formed the direct growth control of approximately Mc Farland no.2 (DGC). MIC of the compounds was determined by MB/BacT in triplicate. All the synthesized compounds, isonizaid and rifampcin were dissolved in DMSO to make the working solution (2,560 µg/mL). Working solution (2,560 µg/mL) was added aseptically to 0.5 mL of reconstitution fluid (Tween 80, glycerol and amaranth), 0.5 mL DGC and MB Bact medium to achieve the required concentration (16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06 µg/mL) in a final volume

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of 10 mL each BacT/Alert MP bottle. To 9 mL of MB Bact medium, 0.5 mL of reconstitution fluid and 0.5mL DGC were added to a bottle and this was the positive control. To 9.5 mL of MB Bact medium, 0.5 mL of reconstitution fluid was inoculated into another bottle formed the negative control. These bottles were incubated in the system at 35°C for 8-9 days and monitored to detect growth.

The compound was considered as ineffective if the bottles containing it flagged positive at the same time or before the positive control. The compound was considered effective if the bottle containing it remained negative during the test period or flagged positive 2 days after the positive control. If the positive control did not flag positive in 12 days the test was invalidated and had to be repeated.

5.6.3. *In vitro Mt* SD inhibitory assay [26,27]

Mtb SD Protein Preparation was carried out as described [26]. SD catalyzes the NADPH-dependent reduction of 3-dehydroshikimate to form shikimate and $NADP^+$. Enzyme activity was assayed by continuously monitoring the increase in NADPH absorbance at 340 nm (εNADPH = $6.22 \times 103\ M^{-1}\ cm^{-1}$) (Chaudhuri, *et al*., 1987). All spectrophotometric assays were performed at 25°C, and the increase in NADPH was monitored at 340 nm. Briefly, the assays were conducted in a final volume of 100 µL, containing the following components: 100 mM Tris HCl, pH 9.0, 1 mM shikimic acid, 0.5 mM $NADP^+$ and 25 U/100 µL *Mt*SD. All of the components except for the SD enzyme were premixed in a reservoir and dispensed. The reaction mixture was incubated at 25°C for 5 min to reach a stable background. The SD enzyme was added in the end to trigger the reaction as described [27]. Stock solutions of all compounds were prepared in DMSO such that the final concentration of this co-solvent was constant at 1% v/v in a final volume 100 µL for all

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kinetic reactions. Negative control reactions were carried out with the same conditions as described above but without inhibitor. The inhibitory activity of each derivative was expressed as the percentage inhibition of *Mt* SD activity with respect to the negative control reaction without inhibitor. All activity assays were performed in triplicate.

5.6.4. Cytotoxicity assay

The cytotoxicity of the compounds was tested against Vero and HepG2 cells. Vero and HepG2 cells were grown without CO_2 in L15 medium supplemented with antibiotics and heat inactivated calf serum. Serial 2-fold dilutions of the drugs were prepared in the 96-well microplates. The Vero and HepG2 cells, in medium containing 2× Alamar Blue, were added to the wells to a final concentration of 1.3×10^4 cells per well. The plates were incubated for 3 days at 37 °C. The IC_{50} was calculated according to manufacturer directions.

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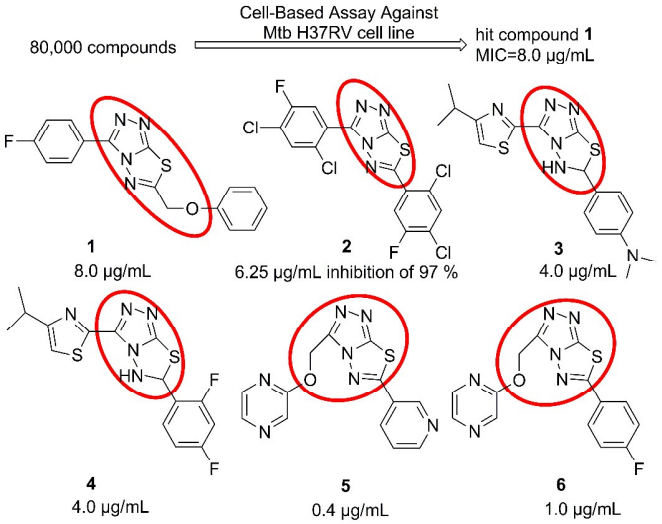
Figures

Fig. 1. Lead compounds from triazolothiadiazoles.

Fig. 2. Optimization of 3,6-disubstituted triazolothiadiazoles.



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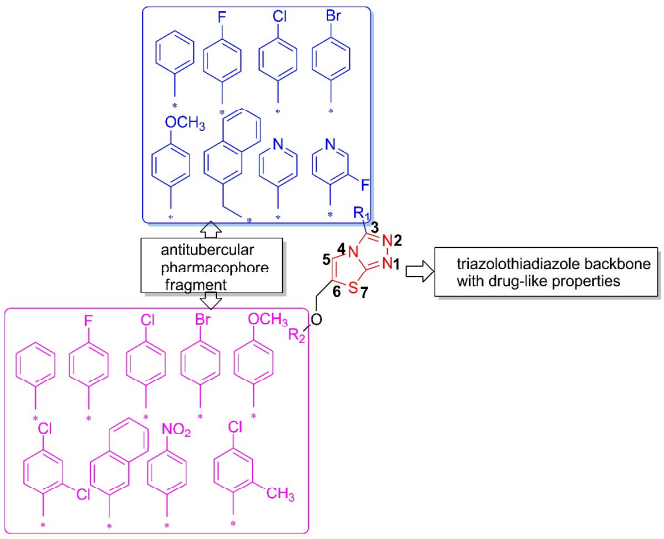

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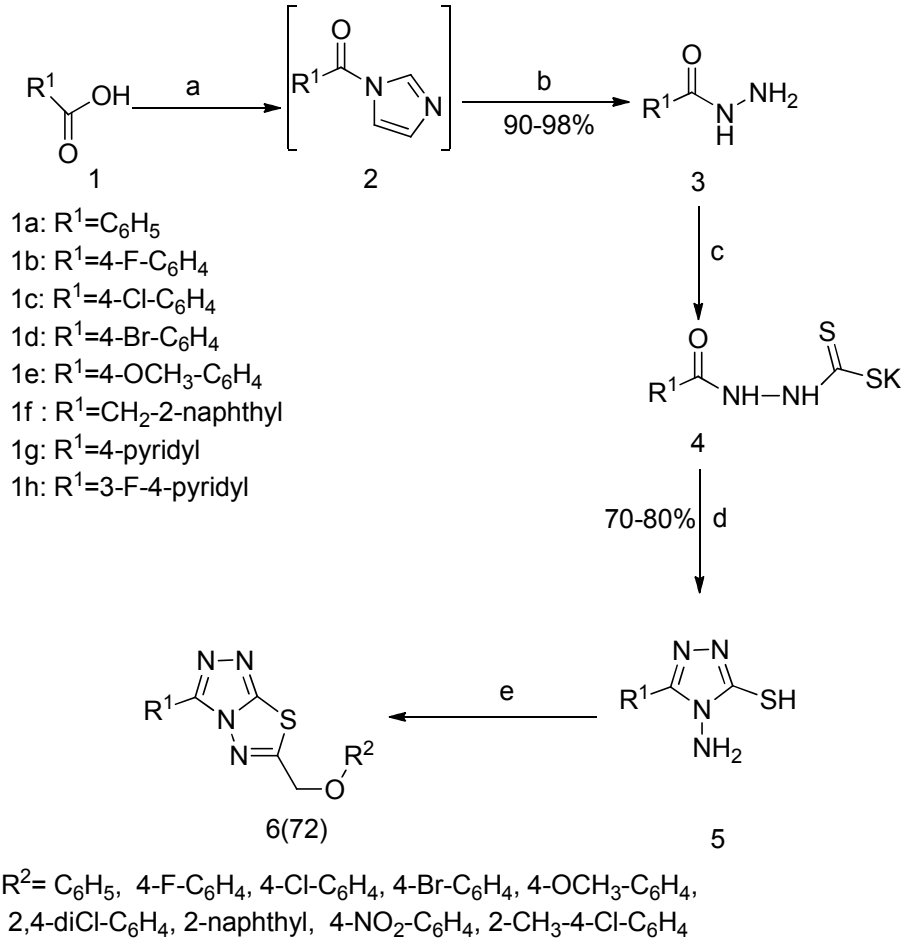

Scheme 1. Synthesis of 3,6-disubstituted triazolothiadiazoles. Reagents and conditions: (a)1,1'-Carbonyldiimidazole, THF, 2 h, room temperature (rt); (b) $N_2H_4H_2O$, rt, overnight; (c) CS_2, KOH, EtOH, rt, 8 h; (d) $N_2H_4H_2O$, rt, H_2O, 2 h, rt/5 h, reflux; (e) $ArOCH_2CO_2H$, $POCl_3$, MWI, 30 min, 155 ℃.

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Table 1 Antibacterial (MIC) activity against H37Rv, MDRTB and RDRTB and inhibition of *Mt* SD activity of 17 promising triazolothiadiazoles

compound	*Mtb* H37Rv MIC(μg/mL)	*Mtb* MDRTB MIC(μg/mL)	*Mtb* RDRTB MIC(μg/mL)	Inhibition of *Mt* SD IC_{50}(μg/mL)
1	8	8	8	23.00±1.18
6b-5	0.5	4.0	0.5	66.26±1.24
6b-7	4.0	4.0	4.0	ND[a]
6c-2	4.0	8.0	8.0	5052.00±4.72
6c-4	0.5	4.0	1.0	6.82±1.32
6c-7	2.0	8.0	2.0	168.30±2.43
6c-8	2.0	2.0	2.0	9.37±1.12
6d-2	2.0	8.0	4.0	79.53±1.29
6d-3	0.25	4.0	0.5	36.32±1.24
6d-4	0.5	4.0	0.5	14.42±1.12
6d-5	2.0	8.0	4.0	11.99±1.07
6d-6	4.0	16.0	8.0	39.57±9.10

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6d-8	1.0	4.0	4.0	58.56±1.45
6d-9	4.0	8.0	8.0	21.56±1.14
6f-2	2.0	8.0	4.0	27.71±1.10
6f-8	0.5	4.0	1.0	40.66±1.39
6h-1	2.0	2.0	2.0	ND
6h-6	1.0	2.0	1.0	ND
RFP[b]	0.25	≥16.0	16.0	
INH[c]	0.12	4.0	1.0	

IC_{50} values are indicated as means ± SD of three independent experiments; ND[a], not determined; RFP[b], Rifampin; INH[c], Isoniazid.

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Table 2 Cytotoxicity (IC_{50} in μM) and SI of 10 promising triazolothiadiazoles against Vero, and HepG2 cell lines

compound	*Mtb* H37Rv MIC(μM)	Vero Cytotoxicity IC_{50}(μM)	Vero SI*	HepG2 Cytotoxicity IC_{50}(μM)	HepG2 SI*
1	24.54	23.17±2.53	0.94	15.19±2.28	0.62
6b-5	1.40	84.95±2.92	60.68	59.17±3.06	42.26
6c-4	1.18	92.31±2.70	78.23	29.08±2.49	25.25
6c-8	5.15	27.27±2.63	5.30	10.95±2.94	2.13
6d-3	0.59	57.16±2.46	96.88	44.77±2.58	75.88
6d-4	1.07	123.52±2.79	115.44	31.53±2.23	29.47
6d-5	4.77	64.22±2.58	13.46	50.17±2.48	10.52
6d-8	2.31	243.90±4.95	105.58	38.39±2.45	16.62
6d-9	9.15	32.52±2.44	3.55	19.57±2.36	2.14
6f-8	1.20	79.26±2.69	66.05	168.50±4.68	140.42
6h-6	2.53	1562.82±8.71	617.72	71.50±3.01	28.26

SI* is the ratio of cytotoxicity (IC_{50} in μM) to in vitro activity against *M. tuberculosis* H37Rv (ATCC 25618 strain) expressed as MIC in μM.

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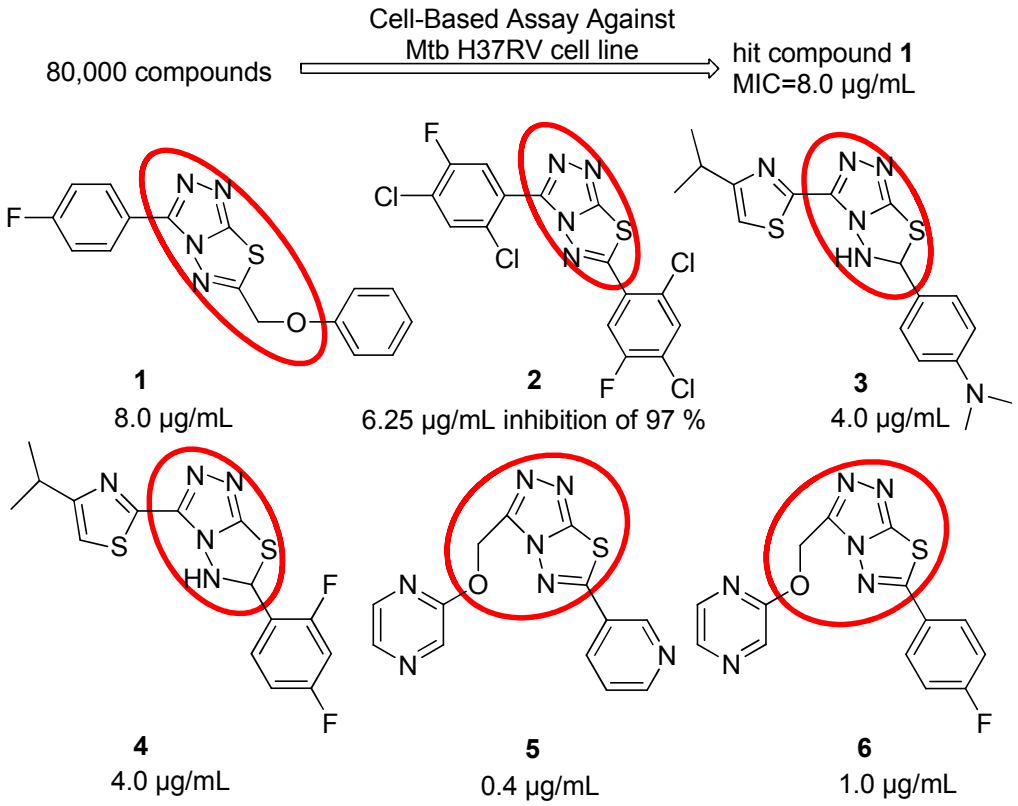

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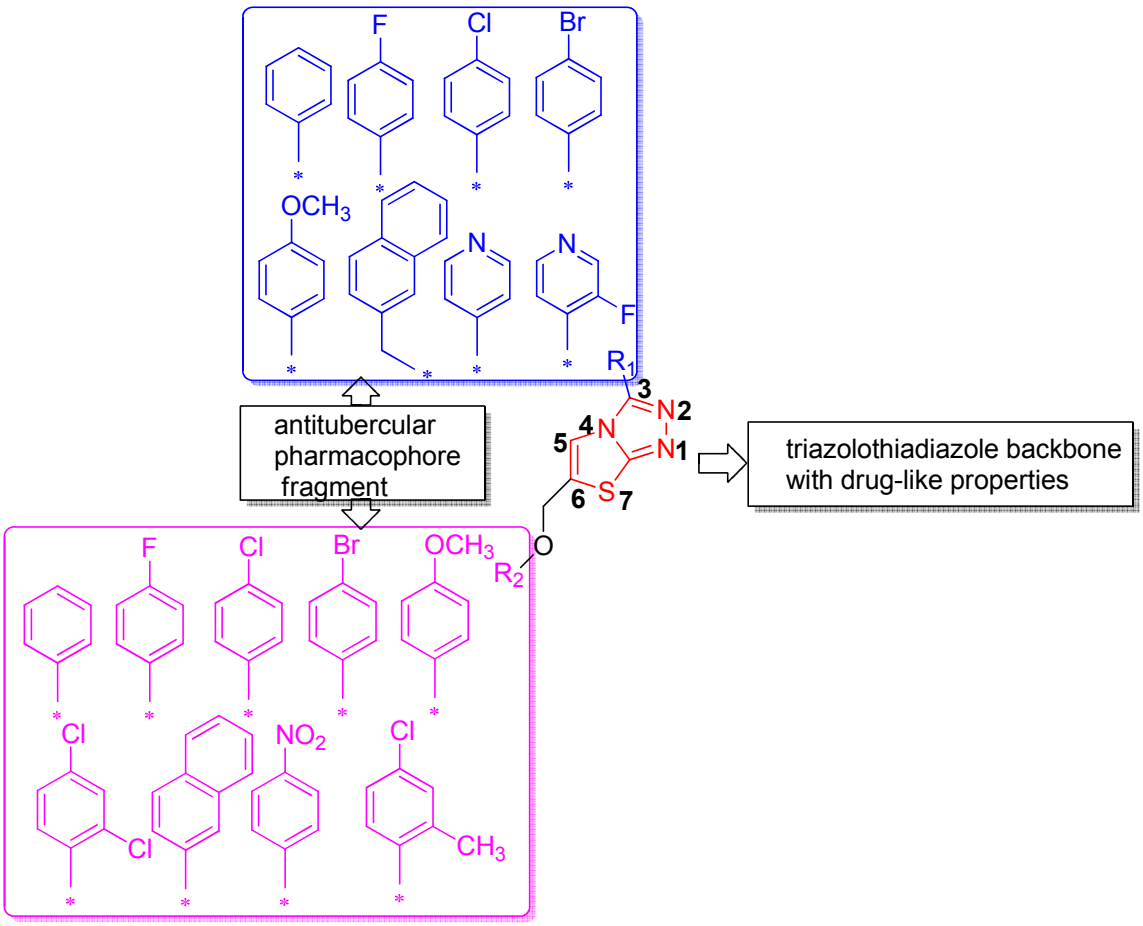

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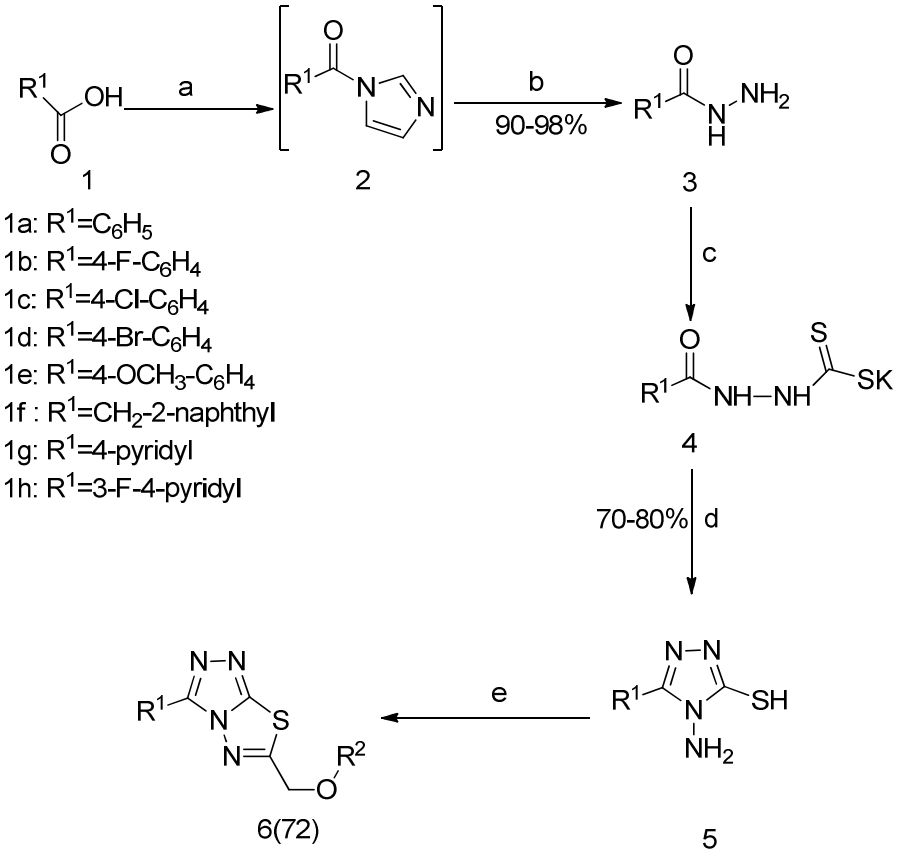

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