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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 3997–4008
Bioorganic & Medicinal Chemistry

# Synthesis of new *S*-derivatives of clubbed triazolyl thiazole as anti-*Mycobacterium tuberculosis* agents

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Received 6 March 2007; revised 2 April 2007; accepted 3 April 2007
Available online 6 April 2007

**Abstract**—In the present study, a series of *N*-{4-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-2-substituted-amide (**1a**–**d**) derivatives were synthesized in good yields and characterized by IR, $^1$H NMR, mass spectral and elemental analyses. The compounds were evaluated for their preliminary in vitro antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhosa* and then were screened for antitubercular activity against *Mycobacterium tuberculosis* H37 Rv strain by broth microdilution assay method. The antibacterial data of the tested compounds indicated that most of the synthesized compounds showed better activity against bacteria compared to reference drugs. The in vitro antitubercular activity reports of tested compounds against *M. tuberculosis* strain H37 Rv showed moderate to better activity.


## 1. Introduction

Tuberculosis is a contagious disease with comparatively high mortality worldwide. The statistics shows that around three million people throughout the world die annually from tuberculosis[1,2] and there are around eight million new cases each year, out of which developing countries shows major share.[3] In addition, about a third of the world's population harbours a dormant *Mycobacterium tuberculosis* infection, representing a significant reservoir of disease for the future.

The azole antitubercular may be regarded as a new class providing truly effective drugs, which are reported to inhibit bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanisms.[4,5] Triazole and thiazole derivatives[6–10] represent a novel emerging major chemical group as antimicrobial agent. Triazoles, in particular, substituted-1,2,4-triazoles and the open-chain thiosemicarbazide counterparts of 1,2,4-triazole, are among the various heterocycles that have received the most attention during the last two decades as potential antimicrobial agents.[11–14] Substitutions including thio,[15] alkylthio and alkenylthio[16] derivatives have been carried out primarily at the 3-position of the 1,2,4-triazole ring, as potential antimicrobial and antimycobacterial agents will overcome the above-mentioned resistance problems. Thiazole moiety has already been reported for its antimicrobial activity.[17,18] Thus in continuation of our in-house e to establish probable pharmacological activities of triazole[19–23] we herein report the synthesis of new 1,2,4-triazole derivatives clubbed with thiazole moiety. The synthesized compounds were also tested for their antimicrobial activity.

In recent years, environmentally benign synthetic methods have received considerable attention and solvent-free protocols are reported.[24–26] A fast, highly efficient and eco-friendly solvent-free chemical transformation, for the synthesis of title compounds, under microwave irradiation, using acidic alumina is designed. The main advantages of the synthetic approach presented here are considerable rate enhancement in comparison with

*Keywords*: Thiazole; Triazole; Antimicrobial; Antimycobacterial.
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doi:10.1016/j.bmc.2007.04.003

**Table 1.** First antituberculosis screening of compounds **1a–d**, **2a–h**, **3a–h**, **4a–h**, **5a–h**, **6a–h**, **7a–x**, **8a–h** and **9a–h**

| Compound | R | R′ | Ar | MIC[a] | GI (%)[b] |
|---|---|---|---|---|---|
| **1a** | $NHCOCH_2Cl$ | — | — | <6.25 | — |
| **1b** | $NHCOCH_3$ | — | — | <6.25 | 96 |
| **1c** | $NHCOC_6H_5$ | — | — | <6.25 | 96 |
| **1d** | $NHCH_2CH_2COOH$ | — | — | <6.25 | — |
| **2a** | $NHCOCH_2Cl$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **2b** | $NHCOCH_3$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **2c** | $NHCOC_6H_5$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **2d** | $NHCH_2CH_2COOH$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **2e** | $NHCOCH_2Cl$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 96 |
| **2f** | $NHCOCH_3$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 100 |
| **2g** | $NHCOC_6H_5$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 96 |
| **2h** | $NHCH_2CH_2COOH$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 96 |
| **3a** | $NHCOCH_2Cl$ | — | –4-Cl–$C_6H_4$ | <6.25 | — |
| **3b** | $NHCOCH_3$ | — | –4-Cl–$C_6H_4$ | <6.25 | — |
| **3c** | $NHCOC_6H_5$ | — | –4-Cl–$C_6H_4$ | <6.25 | — |
| **3d** | $NHCH_2CH_2COOH$ | — | –4-Cl–$C_6H_4$ | <6.25 | — |
| **3e** | $NHCOCH_2Cl$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **3f** | $NHCOCH_3$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **3g** | $NHCOC_6H_5$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **3h** | $NHCH_2CH_2COOH$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **4a** | $NHCOCH_2Cl$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **4b** | $NHCOCH_3$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **4c** | $NHCOC_6H_5$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **4d** | $NHCH_2CH_2COOH$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **4e** | $NHCOCH_2Cl$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 100 |
| **4f** | $NHCOCH_3$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 100 |
| **4g** | $NHCOC_6H_5$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 96 |
| **4h** | $NHCH_2CH_2COOH$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 96 |
| **5a** | $NHCOCH_2Cl$ | — | –4-Cl–$C_6H_4$ | <6.25 | — |
| **5b** | $NHCOCH_3$ | — | –4-Cl–$C_6H_4$ | <6.25 | — |
| **5c** | $NHCOC_6H_5$ | — | –4-Cl–$C_6H_4$ | <6.25 | — |
| **5d** | $NHCH_2CH_2COOH$ | — | –4-Cl–$C_6H_4$ | <6.25 | — |
| **5e** | $NHCOCH_2Cl$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **5f** | $NHCOCH_3$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **5g** | $NHCOC_6H_5$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **5h** | $NHCH_2CH_2COOH$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **6a** | $NHCOCH_2Cl$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **6b** | $NHCOCH_3$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **6c** | $NHCOC_6H_5$ | — | –4-Cl–$C_6H_4$ | <6.25 | 96 |
| **6d** | $NHCH_2CH_2COOH$ | — | –4-Cl–$C_6H_4$ | <6.25 | — |
| **6e** | $NHCOCH_2Cl$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 96 |
| **6f** | $NHCOCH_3$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 100 |
| **6g** | $NHCOC_6H_5$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | 100 |
| **6h** | $NHCH_2CH_2COOH$ | — | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **7a** | $NHCOCH_2Cl$ | $CH_3$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7b** | $NHCOCH_3$ | $CH_3$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7c** | $NHCOC_6H_5$ | $CH_3$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7d** | $NHCH_2CH_2COOH$ | $CH_3$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7e** | $NHCOCH_2Cl$ | $C_6H_5$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7f** | $NHCOCH_3$ | $C_6H_5$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7g** | $NHCOC_6H_5$ | $C_6H_5$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7h** | $NHCH_2CH_2COOH$ | $C_6H_5$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7i** | $NHCOCH_2Cl$ | $CH_2Cl$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7j** | $NHCOCH_3$ | $CH_2Cl$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7k** | $NHCOC_6H_5$ | $CH_2Cl$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7l** | $NHCH_2CH_2COOH$ | $CH_2Cl$ | –4-Cl–$C_6H_4$ | <6.25 | — |
| **7m** | $NHCOCH_2Cl$ | $CH_3$ | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **7n** | $NHCOCH_3$ | $CH_3$ | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **7o** | $NHCOC_6H_5$ | $CH_3$ | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **7p** | $NHCH_2CH_2COOH$ | $CH_3$ | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **7q** | $NHCOCH_2Cl$ | $C_6H_5$ | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **7r** | $NHCOCH_3$ | $C_6H_5$ | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **7s** | $NHCOC_6H_5$ | $C_6H_5$ | –3-$NO_2$–$C_6H_4$ | <6.25 | — |
| **7t** | $NHCH_2CH_2COOH$ | $C_6H_5$ | –3-$NO_2$–$C_6H_4$ | <6.25 | — |

[a] MIC in ($\mu g/mL^{-1}$). MIC of rifampin: 0.015–0.125 mg $mL^{-1}$ versus *M. tuberculosis* H37Rv (97% inhibition).
[b] Growth inhibition of virulent H37Rv strain of *M. tuberculosis*.

**Table 2.** First antituberculosis screening of synthesized compounds **1a–d**, **2a–h**, **3a–h**, **4a–h**, **5a–h**, **6a–h**, **7a–x**, **8a–h** and **9a–h**

| Compound | R | R′ | Ar | MIC[a] | GI (%)[b] |
|---|---|---|---|---|---|
| **7u** | $NHCOCH_2Cl$ | $CH_2Cl$ | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | — |
| **7v** | $NHCOCH_3$ | $CH_2Cl$ | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | — |
| **7w** | $NHCOC_6H_5$ | $CH_2Cl$ | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | — |
| **7x** | $NHCH_2CH_2COOH$ | $CH_2Cl$ | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | — |
| **8a** | $NHCOCH_2Cl$ | — | $-4\text{-}Cl\text{–}C_6H_4$ | <6.25 | 96 |
| **8b** | $NHCOCH_3$ | — | $-4\text{-}Cl\text{–}C_6H_4$ | <6.25 | 96 |
| **8c** | $NHCOC_6H_5$ | — | $-4\text{-}Cl\text{–}C_6H_4$ | <6.25 | 96 |
| **8d** | $NHCH_2CH_2COOH$ | — | $-4\text{-}Cl\text{–}C_6H_4$ | <6.25 | 96 |
| **8e** | $NHCOCH_2Cl$ | — | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | 100 |
| **8f** | $NHCOCH_3$ | — | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | 100 |
| **8g** | $NHCOC_6H_5$ | — | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | 96 |
| **8h** | $NHCH_2CH_2COOH$ | — | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | 96 |
| **9a** | $NHCOCH_2Cl$ | — | $-4\text{-}Cl\text{–}C_6H_4$ | <6.25 | — |
| **9b** | $NHCOCH_3$ | — | $-4\text{-}Cl\text{–}C_6H_4$ | <6.25 | — |
| **9c** | $NHCOC_6H_5$ | — | $-4\text{-}Cl\text{–}C_6H_4$ | <6.25 | — |
| **9d** | $NHCH_2CH_2COOH$ | — | $-4\text{-}Cl\text{–}C_6H_4$ | <6.25 | — |
| **9e** | $NHCOCH_2Cl$ | — | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | — |
| **9f** | $NHCOCH_3$ | — | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | — |
| **9g** | $NHCOC_6H_5$ | — | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | — |
| **9h** | $NHCH_2CH_2COOH$ | — | $-3\text{-}NO_2\text{–}C_6H_4$ | <6.25 | — |

[a] MIC in ($\mu g/mL^{-1}$). MIC of rifampin: 0.015–0.125 mg $mL^{-1}$ versus *M. tuberculosis* H37Rv (97% inhibition).
[b] Growth inhibition of virulent H37Rv strain of *M. tuberculosis*.

a thermal reaction, improved isolated yields of products and cleaner and environmentally safer reactions. Thus, this communication reports synthesis of the title compounds using MAOS.

**Table 3.** Second level antituberculosis screening

| SN | MIC[a] (μM) |
|---|---|
| **1b** | 3.13 |
| **1c** | 6.25 |
| **2a** | 6.25 |
| **2b** | 6.25 |
| **2c** | 6.25 |
| **2d** | 6.25 |
| **2e** | 6.25 |
| **2f** | 6.25 |
| **2g** | 6.25 |
| **2h** | 6.25 |
| **4a** | 3.13 |
| **4b** | 3.13 |
| **4c** | 3.13 |
| **4d** | 3.13 |
| **6a** | 6.25 |
| **6b** | 6.25 |
| **6c** | 6.25 |
| **6e** | 6.25 |
| **6f** | 6.25 |
| **6g** | 6.25 |
| **8a** | 3.13 |
| **8b** | 3.13 |
| **8c** | 3.13 |
| **8d** | 3.13 |
| **8e** | 1.56 |
| **8f** | 0.78 |
| **8g** | 0.39 |
| **8h** | 1.56 |

[a] Actual minimum inhibitory concentration (MABA assay).

## 2. Results and discussion

### 2.1. Synthesis

Compounds **1a–d** were synthesized as per the literature.[27] Compounds **1a–d**, adsorbed on acidic alumina (aluminium oxide, acidic, Brockmann I, ~150 mesh, 58 Å CAMAG 506-C-I, surface area 155 $m^2/g$, pH 6.0), when treated with substituted (4-chloro or 3-nitro)benzoyl chloride at 0 °C to give **2a–h**. The transformed compounds **2a–h** on treatment with diiodomethane in the presence of strong alkali, that is, sodium hydroxide gave **3a–h**. Title compounds **2a–h** were treated with chloroacetonitrile, which on neutralization with sodium carbonate gave precipitates of compounds **4a–h**. Compounds **2a–h**, when treated with methyl bromoacetate in basic condition, produced **5a–h**. Chemical transformation of **5a–h** to **6a–h** was achieved by treating it with hydrazine hydrate. While compounds **6a–h**, on treatment with appropriate acid chlorides, furnished **7a–x**. Schiff bases, the condensation products of **8a–h**, were synthesized by treating **6a–h** with benzaldehyde and confirmed by absence of triplet of NH of hydrazide. Compounds **6a–h** were converted to thiocarbazate salts by treatment with carbon disulfide and potassium hydroxide, which on treatment with hydrazine hydrate gave **9a–h**. The NMR spectra confirmed formation of triazole derivative from hydrazide, which shows presence of sulfhydryl proton at $\delta$ value 12.5. It was observed that there is remarkable loss of product (32% yield) in the second step for the conversion of **6a–h** to **9a–h** when performed in conventional method, while reaction involving MORE method gave good yield (70–82%).

### 2.2. Antitubercular activity

The results of the in vitro evaluation of antituberculosis activity are reported in Tables 1–3. During the prelimin-

ary screening four compounds (**1a–d**) were tested (Table 1) for their antimycobacterial activity, the compounds **1b** and **1c** have exhibited 96% inhibition at this concentration, while other compounds exhibited less than 90% inhibition at the same concentration.

Thus, we have considered **1** as a lead molecule and subsequent structural modifications were carried out as two of the substitutions were active. As a first step towards lead optimization, amino group was protected to the corresponding compounds (**2a–h**) wherein all the alterations made have shown promising activity proving that the modifications are towards synthesis of a pharmacophore. The next structural modification made was a dimeric product, **3a–h**, but these changes also resulted in a substantial loss of biological activity. This loss may indicate retardation in the intracellular transport of highly complex molecule.

Compounds **4a–h** have shown more than 90% of inhibition, which were obtained by S-alkylation with acetonitrile. As the cytotoxicity studies were performed for all the active compounds and none of them were found to be cytotoxic, it may be noted that cyano group may not have any role in increase in the activity. Thus looking at the activity, it was decided to modify the structure at SH group. In order to optimize the sulfhydryl component, compounds **5a–h** were synthesized and investigated, which revealed loss of activity. A further modification of compounds **5a–h** produced compounds **6a–h**. The results of the antimycobacterial activity are quite interesting because all of these compounds have shown inhibition above 96%. Compounds **6a–h** were selected for further studies as they have a free amino group, which opened an area for further modification at this point. Compounds **7a–x** were obtained by treatment with acid chlorides, which ultimately showed decreased antimycobacterial activity. Furthermore, compounds **6a–h** was converted to Schiff bases with benzaldehyde, and on investigation all **8a–h** have shown more than 95% inhibition. More interestingly, compounds **8f** and **8g** were the only ones, which have shown good activity in secondary screening. Compounds **9a–h** were found to be inactive.

All the compounds that were active in the first level screening were then tested to determine the actual minimum inhibitory concentration (MIC), wherein, compounds **8f** and **8g** have been proven to be the most active, with MIC values ranging from 0.39 to 0.78.

### 2.3. Antimicrobial activity

From the antibacterial screening it was observed that all the compounds exhibited activity against all the organisms employed. Looking at the structure–activity relationship, marked inhibition in bacteria was observed in the compounds **8e**, **8f** and **8g**, whereas **1b**, **1c**, **1d**, **3c**, **3h**, **4b**, **4c**, **4d**, **4e**, **4g**, **5d**, **5e**, **6c**, **6d**, **6e**, **7f**, **7m**, **7n**, **7r**, **7x** and **9h** have shown moderate activity and others showed least activity.

## 3. Conclusion

The antimycobacterial screening of the novel series has demonstrated emergence of potent derivatives that have highly electronegative part at sulfhydryl group. Specifically compounds (**8f** and **8g**), that is, Schiff bases, probably due to their ability to increase the penetration in the bacterial cell have shown the best of all. Due to the better activity against the mycobacteria, compounds (**8f** and **8g**) were the best choice for the preparations of new derivatives in order to improve its effectiveness on intracellular mycobacteria (macrophage) or in infected animal. Finally it can be concluded that an ideal antimycobacterial agent with minimal toxicity and potential activity can be designed using above-said compounds as lead molecules. The said inhibitor can be synthesized using MAOS so as to get the benefits of this novel technique.

## 4. Experimental

### 4.1. General

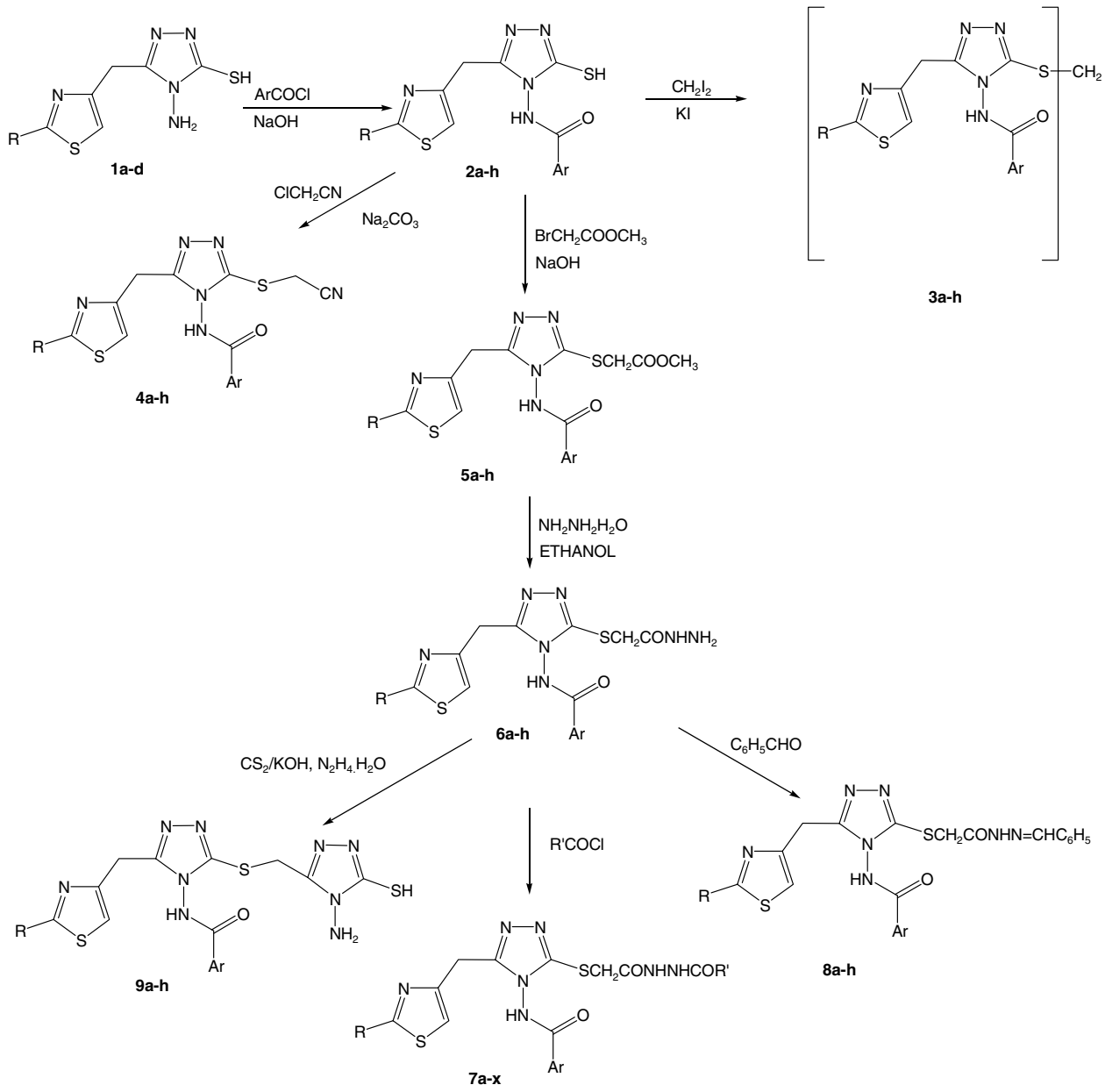
The melting points were recorded on electrothermal apparatus and are uncorrected. $^1$H NMR spectra were recorded on a Bruker Avance 300 MHz instrument using $CDCl_3$ as solvent using TMS as internal standard; the chemical shifts ($\delta$) are reported in ppm and coupling constants ($J$) are given in Hz. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet) and br s (broad singlet). Mass spectra were recorded on a Finning LCQ mass spectrometer. Microwave irradiation was carried out in Raga Scientific Microwave Systems, Model RG31L at 2450 MHz. Elemental analyses were performed on a Heracus CHN-Rapid Analyser. Analyses indicated by the symbols of the elements of functions were within ±/0.4% of the theoretical values. The purity of the compounds was checked on Merck precoated silica gel 60 F-254.

### 4.2. Preparation of *N*-{4-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-2-chloro acetamide (1a), *N*-{4-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-acetamide (1b), *N*-{4-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-benzamide (1c), 3-{*N*-(4-[(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-amino)}-propanoic acid (1d)

Above titled compounds were prepared as per the literature.[27] The method of synthesis for compounds **2a–d**, **3a–d**, **4a–d**, **5a–d**, **6a–d**, **7a–l**, **8a–d** and **9a–d** was as per the reported method.[28] Synthetic routes of the newly synthesized compounds are depicted in Scheme 1.

### 4.3. General preparation of *N*-[3-({2-[(substituted)amino]-1,3-thiazol-4-yl}methyl)-5-sulfanyl-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (2e–h)

The triazole (**1a–d**) (0.01 mol) in 20 mL of 10% NaOH was treated dropwise with an equimolar amount of the 3-nitrobenzoyl chloride at 0 °C, which was stirred for




**Scheme 1.**

30–45 min. At the end of stirring a precipitate was observed. The precipitate was then filtered, washed thoroughly with water and crystallized.

**4.3.1. *N*-[3-({2-[(2-Chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-5-sulfanyl-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (2e).** Yield 71%; yellow powder; mp 241–243 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.74 (s, 2H, $CH_2$), 4.13 (s, 2H, $CH_2$), 6.21 (s, 1H, thiazole CH), 7.12–7.36 (m, 4H, ArH), 8.06 (s, 2H, NH), 12.31 (s, 1H, SH); MS (%) 453 ($M^+$, 100), 323 (33.8), 309 (17.4), 248 (14.7), 247 (29.8), 233 (9.8), 220 (10.8), 180 (10.1), 095 (15.1), 86 (9.3); Anal. calcd for $C_{15}H_{12}ClN_7O_4S_2$: C, 39.69; H, 2.66; N, 21.60. Found: C, 39.84; H, 2.79; N, 21.85.

**4.3.2. *N*-[3-({2-[Acetyl-amino]-1,3-thiazol-4-yl}methyl)-5-sulfanyl-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (2f).** Yield 77%; buff white powder; mp 250–252 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.12 (s, 3H, $CH_3$), 3.48 (s, 2H, $CH_2$), 4.27 (s, 4H, $CH_2$), 6.11 (s, 1H, thiazole CH), 7.26–7.41 (m, 4H, ArH), 8.12 (s, 2H, NH), 12.53 (s, 1H, SH); MS (%) 419 ($M^+$, 100), 312 (29.6), 251 (9.8), 223 (15.1), 211 (10.8), 107 (14.7), 087 (10.1), 82 (7.6); Anal. calcd for $C_{15}H_{13}N_7O_4S_2$: C, 42.95; H, 3.12; N, 23.38. Found: C, 42.77; H, 3.23; N, 23.47.

**4.3.3. *N*-[3-({2-[Benzoyl-amino]-1,3-thiazol-4-yl}methyl)-5-sulfanyl-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (2g).** Yield 76%; yellow powder; mp 280–282 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.32 (s, 2H, $CH_2$), 4.18

(s, 2H, $CH_2$), 6.17 (s, 1H, thiazole CH), 7.21–7.86 (m, 9H, ArH), 8.13 (s, 2H, NH), 12.13 (s, 1H, SH); MS (%) 481 ($M^+$, 80), 306 (28), 292 (8.4), 251 (20), 214 (100), 195 (15), 154 (7), 106 (65); Anal. calcd for $C_{20}H_{15}N_7O_4S_2$: C, 49.89; H, 3.14; N, 20.36. Found: C, 49.74; H, 3.29; N, 20.51.

**4.3.4. 3-{*N*-[(4-[(4-(3-Nitrobenzoylamino)-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-aminopropanoic acid (2h).** Yield 69%; yellow powder; mp 271–273 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.46–2.51 (t, 2H, $CH_2$, $J = 4.3$ Hz), 3.33–3.39 (q, 2H, $CH_2$, $J = 7.6$ Hz), 4.01–4.11 (t, 1H, NH, $J = 8.1$), 4.32 (s, 2H, $CH_2$), 6.27 (s, 1H, thiazole CH), 7.43–7.62 (m, 4H, ArH), 8.16 (s, 1H, NH), 10.43 (br s, 1H, OH), 12.43 (s, 1H, SH); MS (%) 449 ($M^+$, 100), 323 (14), 309 (17.1), 280 (6), 134 (35.9); Anal. calcd for $C_{16}H_{15}N_7O_5S_2$: C, 42.76; H, 3.36; N, 21.81. Found: C, 42.58; H, 3.17; N, 21.56.

### 4.4. General preparation of *N*,*N*′-(methylenebis{sulfanedial-[5-({2-[(substituted)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazole-3,4-dial]})-di-3-nitrobenzamide (3e–h)

The triazole (**2e**–**h**) (0.01 mol), diiodomethane (0.01 mol) and 5.6 g (0.01 mol) potassium hydroxide were dissolved in 20 mL of dichloromethane. To the said mixture acidic alumina (20 g) was added. Dichloromethane was evaporated in vacuo, and mixture was kept inside the alumina bath and irradiated for 5–6 min at the power level of 300 W. The mixture was cooled. The solid thus separated was dissolved in hot ethanol and filtered. After cooling, the filtrate gave the product as crystals.

**4.4.1. *N*,*N*′-(Methylenebis{sulfanedial-[5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazole-3,4-dial]})-di-3-nitrobenzamide (3e).** Yield 84%; yellow powder; mp 278–280 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.34 (s, 4H, $CH_2$), 4.19 (s, 4H, $CH_2$), 4.58 (s, 2H, $CH_2$), 6.27 (s, 2H, thiazole CH), 7.13–7.62 (m, 9H, ArH), 7.84 (s, 4H, NH); MS (%) 874 ($M^+$, 7.1), 679 (27.5), 622 (5.5), 607 (100), 516 (3.4), 484 (4.7) 453 (8.2), 347(9.6), 234 (10.3), 185(13.8), 146(8.7), 123 (13.2), 104 (10.5), 87 (26.8), 78 (40); Anal. calcd for $C_{31}H_{24}Cl_2N_{14}O_8S_4$: C, 40.48; H, 2.63; N, 21.32. Found: C, 40.62; H, 2.47; N, 21.52.

**4.4.2. *N*,*N*′-(Methylenebis{sulfanedial-[5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazole-3,4-dial]})-di-3-nitrobenzamide (3f).** Yield 77%; yellow powder; mp 262–264 °C$^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.70 (s, 6H, $CH_3$), 4.34 (s, 4H, $CH_2$), 4.64 (s, 2H, $CH_2$), 5.93 (s, 2H, thiazole CH), 7.21–7.55 (m, 9H, ArH), 8.15 (s, 4H, NH); MS (%) 806 ($M^+$, 35.9), 709 (11.1), 659 (13.2), 667 (23.6), 619 (100), 541 (3.6), 454 (3.7) 419 (9.8), 307 (8.2), 277 (8.1), 254 (11.9), 241 (15.4), 223(35.8), 216 (24.9), 91 (23.8), 83 (54.2), 69 (25.7); Anal. calcd for $C_{31}H_{26}N_{14}O_8S_4$: C, 43.76; H, 3.08; N, 23.05. Found: C, 43.88; H, 3.24; N, 23.16.

**4.4.3. *N*,*N*′-(Methylenebis{sulfanedial-[5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazole-3,4-dial]})-di-3-nitrobenzamide (3g).** Yield 79%; yellow powder; mp 274–276 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.73 (s, 4H, $CH_2$), 4.43 (s, 2H, $CH_2$), 6.27 (s, 2H, thiazole CH), 7.13–7.80 (m, 19H, ArH), 8.16 (s, 4H, NH); MS (%) 930 ($M^+$, 14.1), 724 (15.7), 616 (14.3), 601 (100), 542 (23.5), 465 (3.9) 421 (13.2), 312 (5.8), 279 (7.2), 263 (11.0), 257 (11.7), 256(35.8), 216 (32.8), 91 (22), 83 (27.1), 69 (29.6); Anal. calcd for $C_{41}H_{30}N_{14}O_8S_4$: C, 50.51; H, 3.10; N, 20.11. Found: C, 50.68; H, 3.35; N, 20.38.

**4.4.4. 3-[4-(4-(3-Nitrobenzoylamino)-5-[([4-(3-nitrobenzoylamino)-5-(2-[(2-carboxy-ethyl)amino]-1,3-thiazol-4-ylmethyl)-4*H*-1,2,4-triazol-3-yl]sulfanylmethyl)sulfanyl]-4*H*-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino propanoic acid (3h).** Yield 89%; yellow powder; mp 255–257 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.21–2.30 (t, 4H, $CH_2$, $J = 4.5$ Hz), 3.28–3.35 (q, 4H, $CH_2$, $J = 7.4$ Hz), 3.76 (s, 4H, $CH_2$), 4.01–4.12 (t, 2H, NH, $J = 7.9$ Hz), 4.63 (s, 2H, $CH_2$), 6.2–6 (s, 2H, thiazole CH), 7.32–7.76 (m, 9H, ArH), 8.21 (s, 4H, NH), 10.65 (br s, 2H, OH); MS (%) 866 ($M^+$,13.6), 791 (100), 725 (40.9), 693 (6), 578 (7.3), 512 (4.1), 472 (13.6), 371 (5), 356 (3.4), 283 (13.7), 269 (6.4), 155 (14.4); Anal. calcd for $C_{35}H_{30}N_{14}O_{12}S_4$: C, 43.47; H, 3.13; N, 20.28. Found: C, 43.54; H, 3.27; N, 20.41.

### 4.5. General preparation of *N*-{3-({2-[(substituted)amino]-1,3-thiazol-4-yl}methyl)-5-[(cyanomethyl)sulfanyl]-4*H*-1,2,4-triazol-4-yl}-3-nitrobenzamide (4e–h)

The triazole (**2e**–**h**) (0.01 mol) was mixed with 1.2 mL (0.02 mol) of chloroacetonitrile and dissolved in 25 mL of water. Neutralization with sodium carbonate gave a precipitate. The precipitate was filtered, washed with cold water ($2 \times 20$ mL) and crystallized.

**4.5.1. N-{3-({2-[(Chloroacetyl)amino]-1,3-thiazol-4-yl}-methyl)-5-[(cyanomethyl)sulfanyl]-4*H*-1,2,4-triazol-4-yl}-3-nitrobenzamide (4e).** Yield 86%; yellow powder; mp 241–243 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.74 (s, 2H, $CH_2$), 4.17 (s, 2H, $CH_2$), 4.32 (s, 2H, $CH_2$), 6.26 (s, 2H, thiazole CH), 7.11–7.32 (m, 4H, ArH), 8.24 (s, 2H, NH); MS (%) 493 ($M^+$, 100), 384 (21.9), 369 (20.6), 272 (34.2), 270 (40.8), 256 (17.9), 83 (28.9), 69 (16.1), 55 (11.3); Anal. calcd for $C_{17}H_{13}ClN_8O_4S_2$: C, 41.42; H, 2.66; N, 22.73. Found: C, 41.63; H, 2.71; N, 22.64.

**4.5.2. *N*-{3-({2-[(Acetyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(cyanomethyl)sulfanyl]-4*H*-1,2,4-triazol-4-yl}-3-nitrobenzamide (4f).** Yield 82%; yellow powder; mp 264–266 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.31 (s, 3H, $COCH_3$), 3.62 (s, 2H, $CH_2$), 4.04 (s, 2H, $CH_2$), 6.18 (s, 1H, thiazole CH), 7.17–7.39 (m, 4H, ArH), 7.91 (s, 2H, NH); MS (%) 458 ($M^+$, 100), 384 (21.9), 369 (20.6), 272 (34.2), 270 (40.8), 256 (17.9), 83 (28.9), 69 (16.1), 55 (11.3); Anal. calcd for $C_{17}H_{14}N_8O_4S_2$: C, 44.54; H, 3.08; N, 24.44. Found: C, 44.73; H, 3.19; N, 24.62.

**4.5.3. *N*-{3-({2-[(Benzoyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(cyanomethyl)sulfanyl]-4*H*-1,2,4-triazol-4-yl}-3-nitrobenzamide (4g).** Yield 81%; yellow powder; mp 267–269 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.73 (s, 2H, $CH_2$), 4.14 (s, 2H, $CH_2$), 6.24 (s, 1H, Thiazole

CH), 7.41–7.83 (m, 9H, ArH), 8.24 (s, 2H, NH); MS (%) 520 ($M^+$, 93.3), 430 (10.9), 419 (4.1), 356 (31), 273 (46), 272 (100), 271 (14.3), 256 (9.3), 228 (4.4), 217 (3.2), 189 (3.6), 124 (8.9), 109 (5.8), 81 (4.5), 53 (3); Anal. calcd for $C_{22}H_{16}N_8O_4S_2$: C, 50.76; H, 3.10; N, 21.53. Found: C, 50.81; H, 3.26; N, 21.44.

**4.5.4. 3-{N-[4-(4-(3-nitrobenzoylamino)-5-[(cyanomethyl)sulfanyl]-4*H*-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino} propanoic acid (4h).** Yield 78%; yellow powder; mp 275–277 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.43–2.48 (t, 2H, $CH_2$, $J$ = 4.1 Hz), 3.31–3.37 (q, 2H, $CH_2$, $J$ = 7.3 Hz), 3.84 (s, 2H, $CH_2$), 4.11–4.18 (t, 1H, NH, $J$ = 7.8 Hz), 4.34 (s, 2H, $CH_2$), 6.26 (s, 1H, Thiazole CH), 7.22–7.43 (m, 4H, ArH), 8.25 (s, 1H, NH), 10.84 (br s, 1H, OH); MS (%) 488 ($M^+$, 56.8), 362 (100) 351 (6.9), 349 (16), 348 (12.8), 347 (26.8), 337 (9.7), 331 (12.4), 323 (9.7), 256 (8.8); Anal. calcd for $C_{18}H_{16}N_8O_5S_2$: C, 44.26; H, 3.30; N, 22.94. Found: C, 44.42; H, 3.49; N, 22.77.

### 4.6. General preparation of methyl{[4-(3-nitrobenzoylamino)-5-({2-[(chloroacetyl) amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-3-yl]sulfanyl} acetate (5e–h)

A solution of triazole (**2e**–**h**) (0.01 mol), 0.4 g (0.01 mol) of sodium hydroxide and methyl bromoacetate 1.53 g (0.01 mol) was prepared. To this, acidic alumina was added in 1:5 equivalent of triazole. The reaction mixture was mixed, and mixture was kept inside the alumina bath and irradiated for 4–5 min at the power level of 300 W. The mixture was cooled and poured on ice. The solid thus separated was extracted with hot ethanol, filtered. After cooling, filtrate gave almost pure product.

**4.6.1. Methyl{[4-(3-nitrobenzoylamino)-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-3-yl]sulfanyl} acetate (5e).** Yield 82%; yellow microcrystals; mp 259–251 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.11 (s, 3H, $OCH_3$), 3.81 (s, 2H, $CH_2$), 3.89 (s, 2H, $SCH_2$), 4.28 (s, 2H, $CH_2Cl$), 6.32 (s, 1H, CH of thiazole), 7.20-7.46 (m, 4H, ArH), 8.32 (br, 2H, NH); MS (%) 526 ($M^+$, 100), 417 (14), 386 (12.3), 385 (11.3), 373 (7.2), 316 (7.7), 279 (79), 278 (10), 363 (8.2), 262 (19.5), 248 (7.7), 234 (7.9), 222 (10.5), 220 (5.7), 250 (31.6); Anal. calcd for $C_{18}H_{16}ClN_7O_6S_2$: C, 41.11; H, 3.06; N, 18.64. Found: C, 41.28; H, 3.18; N, 18.79.

**4.6.2. Methyl{[4-(3-nitrobenzoylamino)-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-3-yl]sulfanyl} acetate (5f).** Yield 78%; yellow microcrystals; mp 261–263 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.17 (s, 3H, $OCH_3$), 3.52 (s, 3H, $COCH_3$), 3.74 (s, 2H, $CH_2$), 3.92 (s, 2H, $SCH_2$), 6.46 (s, 1H, CH of thiazole), 7.17–7.38 (m, 4H, ArH), 8.13 (br, 2H, NH); MS (%) 492 ($M^+$, 9), 387 (31), 337 (1), 323 (2), 309 (1), 273 (100), 272 (8); Anal. calcd for $C_{18}H_{17}N_7O_6S_2$: C, 43.99; H, 3.49; N, 19.95. Found: C, 43.78; H, 3.63; N, 19.74.

**4.6.3. Methyl{[4-(3-nitrobenzoylamino)-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl} methyl)-4*H*-1,2,4-triazol-3-yl]sulfanyl} acetate (5g).** Yield 75%; yellow microcrystals; mp 226–228 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.32 (s, 3H, $OCH_3$), 3.62 (s, 2H, $CH_2$), 3.83 (s, 2H, $SCH_2$), 6.25 (s, 1H, CH of thiazole), 6.93–7.51 (m, 9H, ArH), 8.22 (br, 2H, NH); MS (%) 553 (69.9, $M^+$), 356 (54), 354 (43), 248 (100), 233 (39), 232 (15); Anal. calcd for $C_{23}H_{19}N_7O_6S_2$: C, 49.90; H, 3.46; N, 17.71. Found: C, 49.79; H, 3.33; N, 17.86.

**4.6.4. 3-{*N*-[4-(4-(3-Nitrobenzoylamino)-5-[(2-methoxy-2-oxoethyl)sulfanyl]-4*H*-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino} propanoic acid (5h).** Yield 79%; yellow microcrystals; mp above 300 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.30–2.41 (q, 2H, $CH_2$, $J$ = 4.0 Hz), 3.18–3.23 (t, 2H, $CH_2$, $J$ = 7.3 Hz), 3.46 (s, 3H, $OCH_3$), 3.61 (s, 2H, $CH_2$), 3.93 (s, 2H, $SCH_2$), 4.11–4.18 (t, 1H, NH, $J$ = 7.9 Hz), 6.26 (s, 1H, CH of thiazole), 7.25–7.61 (m, 4H, ArH), 8.29 (br, 1H, NH), 11.13 (br, 1H, OH); MS (%) 521 ($M^+$, 100), 379 (67), 309 (41), 272 (58),131 (16), 120 (60), 117 (44), 94 (27), 91(48), 84 (81); Anal. calcd for $C_{19}H_{19}N_7O_7S_2$: C, 43.76; H, 3.67; N, 18.80. Found: C, 43.57; H, 3.86; N, 18.91.

### 4.7. General preparation of *N*-{3-({2-[(substituted)amino]-1,3-thiazol-4-yl}methyl)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4*H*-1,2,4-triazol-4-yl}-3-nitrobenzamide (6e–h)

A solution of (**5e**–**h**) (0.01 mol) with 5 mL (0.01 mol) hydrazine hydrate (98%) was prepared in 10 mL ethanol. To this, acidic alumina (10 g) was added. Ethanol then was evaporated in vacuo, and mixture was kept inside the alumina bath and irradiated for 5–6 min at the power level of 300 W. The mixture was cooled and the product was extracted with ether. Ether was distilled off and product thus obtained was crystallized from *n*-hexane-carbon tetrachloride mixture.

**4.7.1. *N*-{3-({2-[(Chloroacetyl)amino]-1,3-thiazol-4-yl}-methyl)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4*H*-1,2,4-triazol-4-yl}-3-nitrobenzamide (6e).** Yield 83%; beige powder; mp 245–247 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.13 (d, 2H, $NH_2$, $J$ = 6.5 Hz), 3.64 (s, 2H, $CH_2Cl$), 3.94 (s, 2H, $SCH_2$), 4.07–4.15 (t, 1H, NH, $J$ = 4.3 Hz), 6.65 (s, 1H, CH of thiazole), 7.22–7.48 (m, 4H, ArH), 8.21 (br, 2H, NH); MS (%) 526 (65, $M^+$), 419 (69), 386 (145), 356 (61), 328 (78), 311 (8.4), 269 (24), 235 (100), 201 (13), 184 (18), 156 (53), 124 (25), 89 (49); Anal. calcd for $C_{17}H_{16}N_9O_5S_2$: C, 38.82; H, 3.07; N, 73.97. Found: C, 38.96; H, 3.24; N, 74.11.

**4.7.2. *N*-{3-({2-[(Acetyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4*H*-1,2,4-triazol-4-yl}-3-nitrobenzamide (6f).** Yield 82%; beige powder; mp 250–252 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.16 (d, 2H, $NH_2$, $J$ = 6.5 Hz), 2.32 (s, 3H, $CH_3$), 3.67 (s, 2H, $CH_2$), 3.81 (s, 2H, $SCH_2$), 4.15–4.31 (t, 1H, NH, $J$ = 4.5 Hz), 6.69 (s, 1H, CH of thiazole), 7.45–7.71 (m, 4H, ArH), 8.12 (br, 2H, NH); MS (%) 492 (78, $M^+$), 390 (72), 321 (26.3), 247 (6.3), 215 (18.3), 174 (65.3), 136 (25), 88 (100), 69 (14.3); Anal. calcd for $C_{17}H_{17}N_9O_5S_2$: C, 41.54; H, 3.49; N, 25.65. Found: C, 41.67; H, 3.31; N, 25.87.

**4.7.3. *N*-{3-({2-[(Benzoyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4*H*-1,2,4-triazol-4-yl}-3-nitrobenzamide (6g).** Yield 71%; beige powder; mp 222–224 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm):

2.11 (d, 2H, $NH_2$, $J$ = 6.5 Hz), 3.38 (s, 2H, $CH_2$), 3.76 (s, 2H, $SCH_2$), 4.12–4.39 (t, 1H, NH, $J$ = 4.1 Hz), 6.11 (s, 1H, CH of thiazole), 7.13–7.68 (m, 9H, ArH), 8.10 (br, 2H, NH); MS (%) 553 (89, $M^+$), 486 (31), 421 (60), 378 (14.3), 352 (45), 305 (24), 241 (73), 208 (56), 174 (66), 146 (100), 109 (18), 88 (15); Anal. calcd for $C_{22}H_{19}N_9O_5S_2$: C, 47.73; H, 3.46; N, 22.77. Found: C, 47.86; H, 3.64; N, 22.90.

**4.7.4. 3-{*N*-[4-(4-(3-Nitrobenzoylamino)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4*H*-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino}propanoic acid (6h).** Yield 84%; beige powder; mp 243–245 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.13 (d, 2H, $NH_2$, $J$ = 6.1 Hz), 2.27–2.31 (q, 2H, $CH_2$, $J$ = 4.2 Hz), 3.16–3.27 (t, 2H, $CH_2$, $J$ = 7.1 Hz), 3.46 (s, 2H, $CH_2$), 3.84 (s, 2H, $SCH_2$), 4.23–4.45 (t, 2H, NH, $J$ = 4.1 Hz, $J$ = 8.1 Hz), 6.14 (s, 1H, CH of thiazole), 7.34–7.61 (m, 4H, ArH), 8.03 (br, 1H, NH), 11.09 (br, 1H, OH); MS (%) 521 (93.3, $M^+$), 435 (32), 389 (22), 334 (56.4), 306 (28), 247 (64), 217 (100), 147 (83), 108 (71), 79 (10.2); Anal. calcd for $C_{18}H_{19}N_9O_6S_2$: C, 41.45; H, 3.67; N, 24.77. Found: C, 41.63; H, 3.74; N, 24.91.

### 4.8. General preparation of *N*-[3-{[2-(substituted-hydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(substituted)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitro-benzamide (7m–x)

To a solution of (**6e**–**h**) (0.01 mol) in dichloromethane (excess amount), appropriate acid chloride (0.01 mol) was added dropwise with constant vigorous stirring. After 25 min. of stirring, acidic alumina (10 g) was added. Dichloromethane then was evaporated in vacuo, and mixture was kept inside the alumina bath and irradiated for 5–6 min at the power level of 300 W. The mixture was cooled and the product was extracted with ether. Ether was distilled off and product thus obtained was crystallized from *n*-hexane-carbon tetrachloride mixture.

**4.8.1. *N*-[3-{[2-(2-Acetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (7m).** Yield 86%; yellow microcrystals; mp 241–244 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.12 (s, 3 H, $CH_3$), 3.43 (s, 2H, $CH_2$), 3.74 (s, 2H, $SCH_2$), 4.11–4.20 (dd, 2H, $J_{NH-NH}$ = 4.21 Hz, $J_{NH-NH}$ = 4.48 Hz), 4.54 (s, 2H, $CH_2Cl$), 6.63 (s, 1H, thiazole CH), 7.23–7.53 (m, 4H, ArH), 8.32 (s, 2H, NH); MS (%) 568 (56, $M^+$), 489 (29), 462 (45), 408 (27), 389 (71), 318 (41), 274 (16), 223 (100), 188 (29), 179 (22); Anal. calcd for $C_{19}H_{18}ClN_9O_6S_2$: C, 40.18; H, 3.19; N, 22.19. Found: C, 40.34; H, 3.42; N, 22.27.

**4.8.2. *N*-[3-{[2-(2-Acetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (7n).** Yield 71%; yellow microcrystals; mp 227–229 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.42 (s, 6H, $CH_3$), 3.74 (s, 2H, $CH_2$), 3.87 (s, 2H, $SCH_2$), 4.20–4.28 (dd, 2H, $J_{NH-NH}$ = 4.35 Hz, $J_{NH-NH}$ = 4.76 Hz), 6.27 (s, 1H, thiazole CH), 7.11–7.32 (m, 4H, ArH), 8.18 (s, 2H, NH); MS (%) 533 (97, $M^+$), 408 (62), 398 (17.2), 359 (9.1), 327 (74), 297 (54), 241 (8.3), 223 (100), 174 (24), 146 (27); Anal. calcd for $C_{19}H_{19}N_9O_6S_2$: C, 42.77; H, 3.59; N, 23.63. Found: C, 42.94; H, 3.75; N, 23.84.

**4.8.3. *N*-[3-{[2-(2-Acetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (7o).** Yield 82%; yellow microcrystals; mp 226–231 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.03 (s, 3H, $CH_3$), 3.21 (s, 2H, $CH_2$), 3.45 (s, 2H, $SCH_2$), 4.24–4.31 (dd, 2H, $J_{NH-NH}$ = 4.30 Hz, $J_{NH-NH}$ = 4.70 Hz), 6.76 (s, 1H, thiazole CH), 7.31–7.87 (m, 9H, ArH), 8.42 (s, 2H, NH); MS (%) 595 (11, $M^+$), 507 (56), 467 (35), 423 (16), 374 (52), 329 (47), 276 (74), 223 (100), 164 (35), 151 (08); Anal. calcd for $C_{24}H_{21}N_9O_6S_2$: C, 48.40; H, 3.55; N, 21.16. Found: C, 48.63; H, 3.72; N, 21.30.

**4.8.4. 3-[*N*-(4-[5-[2-(2-Acetylhydrazino)-2-oxoethyl]sulfanyl-4-(-3-nitrobenzoylamino)-4*H*-1,2,4-triazol-3-yl]methyl-1,3-thiazol-2-yl)amino]propanoic acid (7p).** Yield 87%; yellow microcrystals; mp 239–242 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.11 (s, 3H, $CH_3$), 2.31–2.37 (q, 2H, $CH_2$, $J$ = 4.7 Hz), 3.20–3.26 (t, 2H, $CH_2$, $J$ = 7.3 Hz), 3.53 (s, 2H, $CH_2$), 3.79 (s, 2H, $SCH_2$), 4.12-4.20 (dd, 2H, $J_{NH-NH}$ = 4.4 Hz, $J_{NH-NH}$ = 4.6 Hz), 6.39 (s, 1H, thiazole CH), 7.11–7.42 (m, 4H, ArH), 8.27 (s, 2H, NH) 11.13 (br, 1H, OH); MS (%) 563 (64, $M^+$), 476 (33), 438 (19), 387 (43), 341 (51), 317 (21), 264 (36), 224(100), 151 (16), 138 (67); Anal. calcd for $C_{20}H_{21}N_9O_7S_2$: C, 42.62; H, 3.76; N, 22.37. Found: C, 42.76; H, 3.83; N, 22.54.

**4.8.5. *N*-[3-{[2-(2-Benzoylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitro benzamide (7q).** Yield 82%; yellow microcrystals; mp 241–244 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.34 (s, 2H, $CH_2$), 3.67 (s, 2H, $SCH_2$), 4.15–4.23 (dd, 2H, $J_{NH-NH}$ = 4.23 Hz, $J_{NH-NH}$ = 4.46 Hz), 4.36 (s, 2H, $CH_2Cl$), 6.52 (s, 1H, thiazole CH), 7.17–7.72 (m, 9H, ArH), 8.45 (s, 2H, NH); MS (%) 630 (84, $M^+$), 548 (17), 389 (26), 374 (46), 331 (58), 247 (14), 242 (13), 223 (100), 194 (29), 136 (21); Anal. calcd for $C_{24}H_{20}ClN_9O_6S_2$: C, 45.75; H, 3.20; N, 20.01. Found: C, 45.88; H, 3.43; N, 20.18.

**4.8.6. *N*-[3-{[2-(2-Benzoylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (7r).** Yield 74%; yellow powder; mp 286–288 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.38 (s, 3H, $CH_3$), 3.68 (s, 2H, $CH_2$), 3.71 (s, 2H, $SCH_2$), 4.13–4.21 (dd, 2H, $J_{NH-NH}$ = 4.23 Hz, $J_{NH-NH}$ = 4.54 Hz), 6.12 (s, 1H, thiazole CH), 6.94–7.21 (m, 9H, ArH), 8.09 (s, 2H, NH); MS (%) 595 (86, $M^+$), 496 (71), 431 (14), 357 (78), 329 (38), 305 (41), 287 (17), 241 (35), 167 (100), 109 (37), 98 (19); Anal. calcd for $C_{24}H_{21}N_9O_6S_2$: C, 48.40; H, 3.55; N, 21.16. Found: C, 48.58; H, 3.72; N, 21.34.

**4.8.7. *N*-[3-{[2-(2-Benzoylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (7s).** Yield 79%; yellow microcrystals; mp 229–233 °C; $^1$H NMR

(300 MHz, $CDCl_3$), $\delta$ (ppm): 3.16 (s, 2H, $CH_2$), 3.31 (s, 2H, $SCH_2$), 4.32–4.39 (dd, 2H, $J_{NH-NH}$ = 4.37 Hz, $J_{NH-NH}$ = 4.74 Hz), 6.47 (s, 1H, thiazole CH), 7.11–7.82 (m, 14H, ArH), 8.26 (s, 2H, NH); MS (%) 658 (91, $M^+$), 587 (66), 526 (19), 471 (16), 436 (68), 376 (34), 316 (61), 223 (100), 111 (37), 119 (54); Anal. calcd for $C_{29}H_{23}N_9O_6S_2$: C, 52.96; H, 3.52; N, 19.17. Found: C, 52.79; H, 3.67; N, 19.33.

**4.8.8. 3-{*N*-(4-[(4-(3-Nitrobenzoylamino)-5-[2-(2-benzoylhydrazino)-2-oxoethyl]sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl)amino}propionic acid (7t).** Yield 83%; yellow microcrystals; mp 230–235 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.21–2.29 (q, 2H, $CH_2$, $J$ = 4.5 Hz), 3.14–3.21 (t, 2H, $CH_2$, $J$ = 7.7 Hz), 3.62 (s, 2H, $CH_2$), 3.85 (s, 2H, $SCH_2$), 4.25–4.37 (dd, 2H, $J_{NH-NH}$ = 4.7 Hz, $J_{NH-NH}$ = 4.9 Hz), 6.61 (s, 1H, thiazole CH), 7.26–7.35 (m, 9H, ArH), 8.32 (s, 2H, NH) 10.87 (br, 1H, OH) ; MS (%) 625 (97, $M^+$), 542 (17), 505 (47), 486 (53), 451 (68), 364 (24), 307 (37), 223 (100), 197 (41), 164 (46); Anal. calcd for $C_{25}H_{23}N_9O_7S_2$: C, 47.99; H, 3.11; N, 20.15. Found: C, 48.17; H, 3.27; N, 20.41.

**4.8.9. *N*-[3-{[2-(2-chloroacetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitro benzamide (7u).** Yield 77%; yellow microcrystals; mp 233–237 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.12 (s, 2H, $CH_2$), 3.38 (s, 2H, $SCH_2$), 4.21–4.33 (dd, 2H, $J_{NH-NH}$ = 4.10 Hz, $J_{NH-NH}$ = 4.52 Hz), 4.47 (s, 4H, $CH_2Cl$), 6.67 (s, 1H, thiazole CH), 7.25–7.56 (m, 4H, ArH), 8.24 (s, 2H, NH); MS (%) 602 (84, $M^+$), 513 (46), 474 (13), 431 (68), 417 (49), 328 (42), 274 (12), 223 (100), 184 (18), 161 (34); Anal. calcd for $C_{19}H_{17}Cl_2N_9O_6S_2$: C, 37.88; H, 2.84; N, 20.93. Found: C, 37.97; H, 2.98; N, 21.16.

**4.8.10. *N*-[3-{[2-(2-Chloroacetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (7v).** Yield 57%; dark brown microcrystals; mp 166–168 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.53 (s, 3H, $CH_3$), 3.81 (s, 2H, $CH_2$), 3.92 (s, 2H, $SCH_2$), 4.17 (s, 2H, $CH_2Cl$), 4.28–4.34 (dd, 2H, $J_{NH-NH}$ = 4.52 Hz, $J_{NH-NH}$ = 4.92 Hz), 6.41 (s, 1H, thiazole CH), 7.26–7.46 (m, 4H, ArH), 8.32 (s, 2H, NH); MS (%) 568 (54, $M^+$), 487 (36), 437 (84), 369 (54.2), 284 (9.3), 238 (100), 158 (12), 128 (37), 77 (32); Anal. calcd for $C_{19}H_{18}ClN_9O_6S_2$: C, 40.18; H, 3.19; N, 22.19. Found: C, 40.32; H, 3.35; N, 22.36.

**4.8.11. *N*-[3-{[2-(2-Chloroacetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (7w).** Yield 78%; yellow microcrystals; mp 232–235 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.27 (s, 2H, $CH_2$), 3.46(s, 2H, $SCH_2$), 4.13–4.22 (dd, 2H, $J_{NH-NH}$ = 4.11 Hz, $J_{NH-NH}$ = 4.57 Hz), 4.41 (s, 2H, $CH_2Cl$), 6.39 (s, 1H, thiazole CH), 7.29–7.63 (m, 9H, ArH), 8.41 (s, 2H, NH); MS (%) 585 (79, $M^+$), 621 (16), 513 (27), 415 (42), 384 (67), 306 (41), 264 (34), 223 (100), 167 (26), 144 (18); Anal. calcd for $C_{24}H_{20}ClN_9O_6S_2$: C, 45.75; H, 3.20; N, 20.01. Found: C, 45.87; H, 3.39; N, 20.17.

**4.8.12. 3-[*N*-(4-[4-(3-Nitrobenzoylamino)-5-(2-[2-(2-chloroacetyl)hydrazino]-2-oxo-ethylsulfanyl)-4*H*-1,2,4-triazol-3-yl]methyl-1,3-thiazol-2-yl)amino]propanoic acid (7x).** Yield 80%; yellow microcrystals; mp 237–239 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.11–2.18 (q, 2H, $CH_2$, $J$ = 4.68 Hz), 3.23–3.29 (t, 2H, $CH_2$, $J$ = 7.3 Hz), 3.53 (s, 2H, $CH_2$), 3.71 (s, 2H, $SCH_2$), 4.07–4.16 (dd, 2H, $J_{NH-NH}$ = 4.4 Hz, $J_{NH-NH}$ = 4.7 Hz), 4.23 (s, 2H, $CH_2Cl$), 6.49 (s, 1H, thiazole CH), 7.12–7.19 (m, 4H, ArH), 8.54 (s, 2H, NH) 11.17 (br, 1H, OH) ; MS (%) 598 (91, $M^+$), 512 (69), 482 (12), 438 (89), 376 (73), 264 (58), 234 (76), 223 (100), 166 (28), 123 (21); Anal. calcd for $C_{20}H_{20}ClN_9O_7S_2$: C, 40.17; H, 3.37; N, 21.08. Found: C, 40.34; H, 3.56; N, 21.24.

**4.9. General procedure for *N*-[3-({2-[(2*E*)-2-benzylidenehydrazino]-2-oxoethyl}sulfanyl)-5-({2-[(substituted)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (8e–h)**

A solution of (**6e–h**) (0.01 mol) with benzaldehyde (0.01 mol) was prepared in 10 mL ethanol. To this, acidic alumina (10 g) was added. Ethanol then was evaporated in vacuo, and mixture was kept inside the alumina bath and irradiated for 1 min at the power level of 300 W. The mixture was cooled and poured on ice. The solid thus separated was filtered and extracted with ether. Ether was distilled off and product thus obtained was crystallized from hot ethanol.

**4.9.1. *N*-[3-({2-[(2*E*)-2-Benzylidenehydrazino]-2-oxoethyl}sulfanyl)-5-({2-[(chloro-acetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitro benzamide (8e).** Yield 81%; brown powder; mp 222–224 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.77 (s, 2H, $CH_2$), 4.16 (s, 2H, $SCH_2$), 4.13 (s, 2H, $CH_2Cl$), 6.22 (s, 1H, thiazole CH), 7.30–7.62 (m, 9H, ArH), 8.16 (S, 3H, NH), 8.27 (S, 1H, N=CH); MS (%) 614 (69, $M^+$), 502 (19), 457 (37), 379 (21), 315 (58), 246 (24), 195 (35), 134 (100), 107 (11.4), 88 (12.3); Anal. calcd for $C_{24}H_{20}ClN_9O_5S_2$: C, 46.94; H, 3.28; N, 20.53. Found: C, 46.73; H, 3.42; N, 20.65.

**4.9.2. *N*-[3-({2-[(2*E*)-2-Benzylidenehydrazino]-2-oxoethyl}sulfanyl)-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2, 4-triazol-4-yl]-3-nitrobenzamide (8f).** Yield 83%; pale brown powder; mp 178–180 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.32 (s, 3H, $CH_3$), 3.97 (s, 2H, $CH_2$), 4.21 (s, 2H, $SCH_2$), 6.25 (s, 1H, thiazole CH), 7.31–7.65 (m, 9H, ArH), 8.26 (s, 3H, NH), 8.36 (s, 1H, N=CH); MS (%) 580 (94, $M^+$), 519 (41), 487 (9.6), 431 (26), 413 (8.4), 389 (100), 365 (20); Anal. calcd for $C_{24}H_{21}N_9O_5S_2$: C, 49.73; H, 3.65; N, 21.75. Found: C, 49.54; H, 3.77; N, 21.86.

**4.9.3. *N*-[3-({2-[(2*E*)-2-Benzylidenehydrazino]-2-oxoethyl}sulfanyl)-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitro benzamide (8g).** Yield 81%; yellow microcrystals; mp 217–219 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 3.63 (s, 2H, $CH_2$), 4.17 (s, 2H, $SCH_2$), 6.23 (s, 1H, thiazole CH), 7.21–7.64 (m, 14H, ArH), 8.16 (s, 3H, NH), 8.41 (s, 1H, N=CH); MS (%) 642 (79, $M^+$),

521 (25), 455 (52), 413 (63), 368 (9.2), 308 (41), 284 (31), 242 (37), 178 (100), 128 (15.2), 97 (28), 87 (7.4); Anal. calcd for $C_{29}H_{23}N_9O_5S_2$: C, 54.28; H, 3.61; N, 19.65. Found: C, 54.43; H, 3.75; N, 19.80.

**4.9.4. 3-{*N*-[4-(4-(3-Nitrobenzoylamino)-5-[(2-oxo-2-2-[(Z)-1-benzylidene]hydrazino-ethyl)sulfanyl]-4*H*-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino} propanoic acid (8h).** Yield 78%; yellow microcrystals; mp 120–122 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.65–2.71 (t, 2H, $CH_2$, $J$ = 4.7 Hz), 3.23-3.31 (q, 2H, $CH_2$, $J$ = 7.5 Hz), 3.92 (s, 2H, $CH_2$), 4.17 (s, 2H, $SCH_2$), 4.32 (t, 1H, NH, $J$ = 8.3 Hz), 6.23 (s, 1H, Thiazole CH), 7.17–7.63 (m, 9H, ArH), 8.15 (s, 2H, NH), 8.32 (s, 1H, N=CH), 10.87 (br s, 1H, OH); MS (%) 609 (74, $M^+$), 497 (45), 426 (13), 364 (63), 326 (32), 248 (65), 185 (12), 146 (100), 109 (37), 87 (42); Anal. calcd for $C_{25}H_{23}N_9O_6S_2$: C, 49.25; H, 3.80; N, 20.68. Found: C, 49.11; H, 3.74; N, 20.57.

### 4.10. General preparation of *N*-[3-{[(4-Amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]sulfanyl}-5-({2-[(substituted)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]-3-nitrobenzamide (9e–h)

The compounds (**6e**–**h**) (0.01 mol) was dissolved in alcoholic potassium hydroxide (0.01 mol) and kept for stirring. Carbon disulfide (0.015 mol) was added dropwise to the solution with stirring. Thick solid mass was obtained, to which 50 mL of absolute alcohol was added. Stirring was continued for 16 h. At the end of 16th-h dry ether was added to the mixture. The precipitate (thiocarbazate) obtained was taken immediately for the next step.

A solution of thiocarbazate (0.01 mol) with hydrazine hydrate (0.01 mol) was prepared in 10 mL ethanol. To this, acidic alumina (10 g) was added. Ethanol then was evaporated in vacuo, and mixture was kept inside the alumina bath and irradiated for 5–6 min at the power level of 300 W. The mixture was cooled and poured on ice. The solid thus separated was filtered and extracted with ether. Ether was distilled off and product thus obtained was crystallized from hot ethanol.

**4.10.1. *N*-[3-{[(4-Amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]sulfanyl}-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitro-benzamide (9e).** Yield 72%; cream microcrystals; mp 228–230 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.16 (s, 2H, $NH_2$), 3.76 (s, 2H, $CH_2$), 4.07 (s, 2H, $CH_2$), 4.25 (s, 2H, $CH_2Cl$), 6.20 (s, 1H, Thiazole CH), 7.23–7.67 (m, 4H, ArH), 8.11 (s, 2H, NH), 12.49 (s, 1H, SH); MS (%) 582 (82, $M^+$), 467 (31), 431 (26), 384 (31), 326 (13.2), 247 (15), 226 (17), 125 (100); Anal. calcd for $C_{18}H_{16}ClN_{11}O_4S_3$: C, 37.14; H, 2.77; N, 26.47. Found: C, 37.36; H, 2.86; N, 26.55.

**4.10.2. *N*-[3-{[(4-Amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]sulfanyl}-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (9f).** Yield 71%; cream microcrystals; mp 252–254 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.13 (s, 2H, $NH_2$), 2.35 (s, 3H, $CH_3$), 3.79 (s, 2H, $CH_2$), 4.14 (s, 2H, $CH_2$), 6.19 (s, 1H, thiazole CH), 7.11–7.35 (m, 4H, ArH), 8.08 (s, 2H, NH), 12.43 (s, 1H, SH); MS (%) 548 (76, $M^+$), 454 (32), 350 (30), 335 (100), 323 (2.93), 222 (12.26), 220 (3.73), 207 (5.86), 192 (7.07); Anal. calcd for $C_{18}H_{17}N_{11}O_4S_3$: C, 39.48; H, 3.13; N, 28.14. Found: C, 39.64; H, 3.32; N, 28.31.

**4.10.3. *N*-[3-{[(4-Amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]sulfanyl}-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4*H*-1,2,4-triazol-4-yl]-3-nitrobenzamide (9g).** Yield 80%; cream microcrystals; mp 264–266 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.41 (s, 2H, $NH_2$), 3.75 (s, 2H, $CH_2$), 4.34 (s, 2H, $CH_2$), 6.23 (s, 1H, thiazole CH), 7.14–7.74 (m, 9H, ArH), 8.17 (s, 2H, NH), 12.63 (s, 1H, SH); MS (%) 510 (93.3, $M^+$), 513 (24), 476 (21), 432 (16), 421 (12), 323 (65), 308 (41), 289 (27), 230 (38), 207 (100), 142 (35.7), 109 (23); Anal. calcd for $C_{23}H_{19}N_{11}O_4S_3$: C, 45.31; H, 3.14; N, 25.27. Found: C, 45.50; H, 3.30; N, 25.20.

**4.10.4. 3-[(4-[5-[(4-Amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)methyl]sulfanyl-4-(3-nitro benzoylamino)-4*H*-1,2,4-triazol-3-yl]methyl-1,3-thiazol-2-yl)amino] propanoic acid (9h).** Yield 77%; cream microcrystals; mp 237–239 °C; $^1$H NMR (300 MHz, $CDCl_3$), $\delta$ (ppm): 2.13 (s, 2H, $NH_2$), 2.46–2.49 (t, 2H, $CH_2$, $J$ = 4.3 Hz), 3.14–3.20 (q, 2H, $CH_2$, $J$ = 6.7 Hz), 3.98 (s, 2H, $CH_2$), 4.23–4.29 (t, 1H, NH, $J$ = 7.3 Hz), 4.36 (s, 2H, $CH_2$), 6.26 (s, 1H, thiazole CH), 7.27–7.56 (m, 4H, ArH), 8.17 (s, 1H, NH), 10.64 (br s, 1H, OH), 12.35 (s, 1H, SH); MS (%) 578 (84, $M^+$), 497 (23), 450 (47), 438 (38), 371 (25), 370 (75), 354 (10), 235 (100), 220 (22), 207 (68), 192 (70); Anal. calcd for $C_{19}H_{19}N_{11}O_5S_3$: C, 39.51; H, 3.32; N, 26.67. Found: C, 39.36; H, 3.22; N, 26.51.

### 4.11. Pharmacological activity

**4.11.1. Antitubercular activity.** Primary screening was conducted at 6.25 μg mL$^{-1}$ against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[29] Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system.[30] Compounds showing more than/95% inhibition in the primary screening were considered active and then re-tested at lower concentrations against *M. tuberculosis* H37Rv in order to determine the actual MIC, using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 95% with respect to the controls. Rifampin (RMP) was used as the reference compound (RMP MIC = 0.015–0.125 mg mL$^{-1}$). We also have done cytotoxicity analysis of the above-synthesized compounds, using neutral red uptake by using Vero-C-1008 cell line at various concentrations (6.25 μg/mL to 50 μg/mL), none of them were found toxic. Hence the activities of the above-synthesized compounds were not due to cytotoxicity of compounds.

**4.11.2. Antimicrobial activity.** The compounds listed in Tables 1 and 2 were screened for the antimicrobial activity against different microorganisms under the following conditions.

**Table 4.** Antibacterial activity of the compounds **1a–d**, **2a–h**, **3a–h**, **4a–h**, **5a–h**, **6a–h**, **7a–x**, **8a–h** and **9a–h**

| Compound | Organisms | | | |
|---|---|---|---|---|
| | Sa | Pa | Ec | St |
| **1a** | 18 | 17 | 14 | 12 |
| **1b** | 28 | 27 | 22 | 24 |
| **1c** | 22 | 20 | 18 | 14 |
| **1d** | 25 | 22 | 20 | 16 |
| **2a** | 20 | 20 | 18 | 14 |
| **2b** | 16 | 16 | 20 | 16 |
| **2c** | 16 | 10 | 10 | 18 |
| **2d** | 11 | 17 | 15 | 22 |
| **2e** | 22 | 22 | 20 | 16 |
| **2f** | 16 | 16 | 12 | 12 |
| **2g** | 11 | 24 | 16 | 12 |
| **2h** | 28 | 25 | 18 | 19 |
| **3a** | 23 | 28 | 29 | 18 |
| **3b** | 24 | 21 | 24 | 17 |
| **3c** | 29 | 27 | 20 | 12 |
| **3d** | 21 | 29 | 22 | 20 |
| **3e** | 18 | 30 | 27 | 24 |
| **3f** | 27 | 32 | 26 | 26 |
| **3g** | 26 | 24 | 24 | 19 |
| **3h** | 25 | 26 | 21 | 18 |
| **4a** | 24 | 28 | 26 | 16 |
| **4b** | 28 | 20 | 23 | 24 |
| **4c** | 31 | 27 | 27 | 19 |
| **4d** | 30 | 29 | 21 | 25 |
| **4e** | 24 | 24 | 19 | 24 |
| **4f** | 22 | 26 | 20 | 21 |
| **4g** | 28 | 24 | 22 | 17 |
| **4h** | 25 | 23 | 24 | 24 |
| **5a** | 18 | 16 | 10 | 12 |
| **5b** | 20 | 16 | 10 | 10 |
| **5c** | 22 | 22 | 20 | 16 |
| **5d** | 26 | 24 | 22 | 18 |
| **5e** | 26 | 24 | 20 | 20 |
| **5f** | 24 | 22 | 20 | 18 |
| **5g** | 22 | 11 | 24 | 24 |
| **5h** | 22 | 22 | 20 | 20 |
| **6a** | 16 | 18 | 12 | 12 |
| **6b** | 16 | 16 | 12 | 14 |
| **6c** | 32 | 32 | 26 | 24 |
| **6d** | 23 | 26 | 18 | 22 |
| **6e** | 29 | 20 | 19 | 18 |
| **6f** | 19 | 23 | 22 | 17 |
| **6g** | 28 | 24 | 17 | 20 |
| **6h** | 24 | 28 | 16 | 21 |
| **7a** | 25 | 21 | 24 | 22 |
| **7b** | 27 | 19 | 20 | 23 |
| **7c** | 26 | 24 | 21 | 19 |
| **7d** | 20 | 21 | 26 | 18 |
| **7e** | 21 | 26 | 21 | 20 |
| **7f** | 28 | 22 | 20 | 17 |
| **7g** | 19 | 24 | 18 | 18 |
| **7h** | 18 | 25 | 19 | 23 |
| **7i** | 24 | 21 | 18 | 21 |
| **7j** | 25 | 20 | 18 | 20 |
| **7k** | 26 | 23 | 19 | 22 |
| **7m** | 27 | 24 | 18 | 24 |
| **7n** | 33 | 34 | 30 | 29 |
| **7o** | 20 | 18 | 14 | 12 |
| **7p** | 24 | 10 | 14 | 14 |
| **7q** | 21 | 18 | 10 | 18 |
| **7r** | 32 | 30 | 30 | 27 |
| **7s** | 18 | 12 | 14 | 12 |
| **7t** | 12 | 10 | 10 | 14 |
| **7u** | 14 | 16 | 12 | 18 |
| **7v** | 12 | 18 | 18 | 14 |
| **7w** | 14 | 11 | 18 | 12 |
| **7x** | 24 | 26 | 24 | 24 |
| **8a** | 24 | 19 | 19 | 17 |
| **8b** | 25 | 20 | 20 | 20 |
| **8c** | 21 | 24 | 23 | 21 |
| **8d** | 22 | 27 | 18 | 22 |
| **8e** | 31 | 30 | 26 | 25 |
| **8f** | 30 | 31 | 24 | 26 |
| **8g** | 32 | 30 | 25 | 24 |
| **8h** | 30 | 32 | 26 | 27 |
| **9a** | 27 | 20 | 22 | 20 |
| **9b** | 28 | 21 | 23 | 18 |
| **9c** | 26 | 19 | 20 | 19 |
| **9d** | 21 | 24 | 19 | 23 |
| **9e** | 20 | 26 | 18 | 21 |
| **9f** | 18 | 13 | 21 | 18 |
| **9g** | 24 | 27 | 20 | 19 |
| **9h** | 26 | 28 | 17 | 18 |
| Gent | 34 | 35 | 31 | 30 |

Sa: *S. taphylococcus aureus*, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, St: *Salmonella typhosa*, Gent: Gentamycin.

Method: Well diffusion method,[31]
Solvent: chloroform

Condition: 24 h at 24–28 °C,

Medium: the nutrient agar medium,
Concentrations: 50 μM and 100 μM
Standard: the antibiotic gentamycin

The nutrient agar medium, 20 mL, was poured into the sterile petri dishes. To the solidified plates, wells were made using a sterile cork borer 10 mm in diameter. The 24-hour subcultured bacteria were inoculated in the Petri-plates, with a sterile cotton swab dipped in the nutrient broth medium. After inoculating, the compounds were dissolved separately with the chloroform solvent and poured into the wells with varying concentrations ranging from 50 to 100 μM using a micropipette. The plates were left over for 24 h at 24–28 °C. The antibiotic Gentamycin was used as a standard for comparative study Table 4.

The percentage of inhibition was calculated by the formula

$$\% \text{ Inhibition} = \text{Diameter of the inhibition zone} \times 100$$

## Acknowledgments

Authors are thankful to Dr. K.G. Bothara, Principal, AISSMS College of Pharmacy, Pune, India, for providing the facilities and Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) for the antitubercular activity.

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