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Original article

Clubbed triazoles: A novel approach to antitubercular drugs

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Abstract

In last few decades, though significant progress has been made in the treatment and control strategies of tubercular infections by introducing new diagnostic and monitoring tools and combination therapy, it still continues to be a severe problem. Thus with the aim of developing novel molecules with improved potency for treating *Mycobacterium tuberculosis* H37Rv strain infections and with decreased probability of developing drug resistance, herein we report the synthesis of thiazolyl triazole derivatives, starting from ethyl acetoacetate, by microwave organic reaction enhancement method (MORE) and results of investigations of their antimycobacterial and antimicrobial activities. Many compounds have shown promising activity while others were inactive.
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1. Introduction

The development of resistance to current antimycobacterial therapy endorses the search for more effective agents. In addition, primary and opportunistic microbial infections continue to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants). Several reviews have appeared illustrating the problems encountered by today's infectious disease clinicians [1–3]. As known, the easily gained resistance is the main problem encountered in developing safe and efficient antitubercular agents. Therefore, there is an urgent demand for a new class of antitubercular agent with a different mode of action.

The azole antituberculars 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]. Novel emerging major chemical groups as antimicrobials are triazole and thiazole derivatives [6–11]. 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 [12–22]. Substitutions including thio [23,24], alkylthio and alkenylthio [25,26] derivatives have been carried out primarily at the 3-position of the 1,2,4-triazole ring, as potential antimicrobial and antimycobacterial agents those will overcome the above mentioned resistance problems. Thiazole moiety has already been reported for its antimicrobial activity [27–30]. Thus in continuation of our earlier work [31–33], we herein report the synthesis of new 1,2,4-triazole derivatives clubbed with thiazole moiety as novel antimycobacterials. The synthesized compounds were tested for their antimicrobial activity.

Microwave assisted reactions [34] using dry media [35] have attracted much interest because of the simplicity in

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operation, greater selectivity and rapid synthesis of variety of heterocyclic compounds [36]. Thus it was thought worthwhile to synthesize titled compounds using the green route.

2. Results and discussion

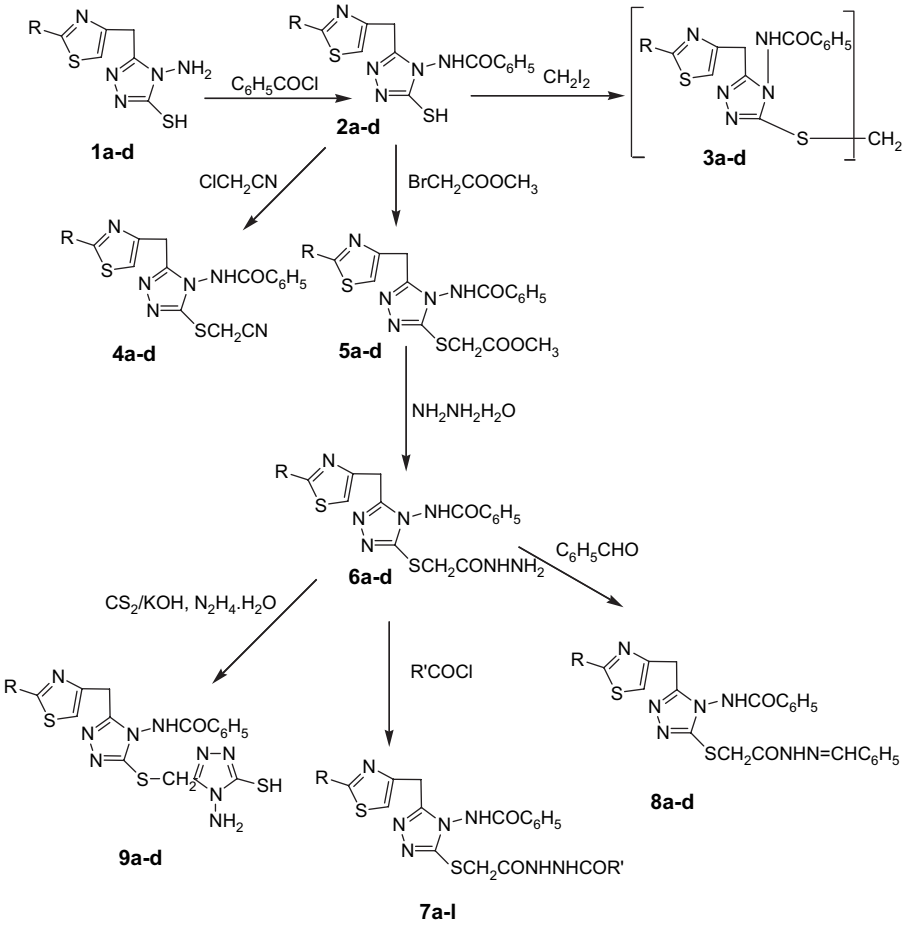
2.1. Chemistry

Compounds **1a–d** were synthesized as per the literature [37]. Compounds **1a–d**, adsorbed on acidic alumina [38], were treated with benzoyl chloride at 0 °C to yield **2a–d**. The transformed compounds **2a–d** on treatment with diiodomethane in the presence of strong alkali i.e. sodium hydroxide gave **3a–d**. Title compounds **2a–d** were treated with chloroacetonitrile, which on neutralization with sodium carbonate gave a precipitates of compounds **4a–d**. Compounds **2a–d**, when treated with methyl bromoacetate under basic conditions produced **5a–d**. Chemical transformation of **5a–d** to **6a–d** was achieved by treating with hydrazine hydrate. While compounds **6a–d**, on treatment with appropriate acid chlorides, furnished **7a–l**. Schiff bases, the condensation products of **8a–d**, were synthesized by treating **6a–d** with benzaldehyde and confirmed by absence of triplet of NH of hydrazide. Compounds **6a–d** were converted to thiocarbazate salts by treatment with carbon disulphide and potassium hydroxide, which on treatment with hydrazine hydrate gave **9a–d** (Scheme 1). The NMR spectra confirmed formation of triazole derivative from hydrazide, which shows the presence of sulfhydryl proton at δ value 12.5. It was observed that there is remarkable loss of product (44% yield) in the second step for the conversion of **6** to **9** when performed in conventional method, while reaction involving MORE method gave good yield (71–80%).

2.2. Antitubercular activity

The results of the in vitro evaluation of antituberculosis activity are reported in Tables 1 and 2. During the preliminary screening four compounds **1a–d** were tested (Table 1) for their antimycobacterial activity, one of the compounds **1b** have exhibited 100% inhibition at this concentration while other compounds exhibited less than 90% inhibition at the same concentration.

Thus we have considered **1b** as a lead molecule and subsequent structural modifications were carried out. As a first step towards lead optimization amino group was protected in the corresponding compounds **2a–d**, however, all of these modifications resulted in a substantial decrease in activity. The



Scheme 1.

Table 1
Result of first antituberculosis screening

Compound	R	R′	MIC (μg ml^{-1})[a]	GI (%)[b]	Compound	R	R′	MIC (μg ml^{-1})[a]	GI (%)[b]
1a	$NHCOCH_2Cl$	–	<6.25	–	**6c**	$NHCOC_6H_5$	–	<6.25	100
1b	$NHCOCH_3$	–	<6.25	100	**6d**	$NHCH_2CH_2COOH$	–	<6.25	96
1c	$NHCOC_6H_5$	–	<6.25	–	**7a**	$NHCOCH_2Cl$	CH_3	<6.25	–
1d	$NHCH_2CH_2COOH$	–	<6.25	–	**7b**	$NHCOCH_3$	CH_3	<6.25	–
2a	$NHCOCH_2Cl$	–	<6.25	–	**7c**	$NHCOC_6H_5$	CH_3	<6.25	–
2b	$NHCOCH_3$	–	<6.25	–	**7d**	$NHCH_2CH_2COOH$	CH_3	<6.25	–
2c	$NHCOC_6H_5$	–	<6.25	–	**7e**	$NHCOCH_2Cl$	C_6H_5	<6.25	–
2d	$NHCH_2CH_2COOH$	–	<6.25	–	**7f**	$NHCOCH_3$	C_6H_5	<6.25	–
3a	$NHCOCH_2Cl$	–	<6.25	–	**7g**	$NHCOC_6H_5$	C_6H_5	<6.25	–
3b	$NHCOCH_3$	–	<6.25	–	**7h**	$NHCH_2CH_2COOH$	C_6H_5	<6.25	–
3c	$NHCOC_6H_5$	–	<6.25	–	**7i**	$NHCOCH_2Cl$	CH_2Cl	<6.25	–
3d	$NHCH_2CH_2COOH$	–	<6.25	–	**7j**	$NHCOCH_3$	CH_2Cl	<6.25	–
4a	$NHCOCH_2Cl$	–	<6.25	–	**7k**	$NHCOC_6H_5$	CH_2Cl	<6.25	–
4b	$NHCOCH_3$	–	<6.25	97	**7l**	$NHCH_2CH_2COOH$	CH_2Cl	<6.25	–
4c	$NHCOC_6H_5$	–	<6.25	100	**8a**	$NHCOCH_2Cl$	–	<6.25	98
4d	$NHCH_2CH_2COOH$	–	<6.25	–	**8b**	$NHCOCH_3$	–	<6.25	96
5a	$NHCOCH_2Cl$	–	<6.25	–	**8c**	$NHCOC_6H_5$	–	<6.25	98
5b	$NHCOCH_3$	–	<6.25	–	**8d**	$NHCH_2CH_2COOH$	–	<6.25	98
5c	$NHCOC_6H_5$	–	<6.25	–	**9a**	$NHCOCH_2Cl$	–	<6.25	–
5d	$NHCH_2CH_2COOH$	–	<6.25	–	**9b**	$NHCOCH_3$	–	<6.25	–
6a	$NHCOCH_2Cl$	–	<6.25	98	**9c**	$NHCOC_6H_5$	–	<6.25	–
6b	$NHCOCH_3$	–	<6.25	100	**9d**	$NHCH_2CH_2COOH$	–	<6.25	–

[a] 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*.

next structural modification made was a dimeric product of **3a–d** but these changes also resulted in a substantial loss of biological activity.

Compounds **4b** and **4c** have shown 97% and 100% inhibition which was obtained by S-alkylation with acetonitrile. Thus looking at the activity, it was decided to modify the structure at SH group. In order to optimize the sulfhydryl component, four compounds **5a–d** were synthesized and investigated, which revealed loss of activity. A further modification of compounds **5a–d** produced compounds **6a–d**. The results of the antimycobacterial activity are quite interesting because all of these compounds have shown inhibition above 90%. Compounds **6a–d** were selected for further studies as it has a protected and free amino group, which opened an area for further modification at this point. Compounds **7a–l** were obtained by treatment with acid chlorides which ultimately showed decreased antimycobacterial activity. Furthermore, compounds **6a–d** were converted to Schiff bases with benzaldehyde, and on investigation all **8a–d** have shown more than 95% inhibition. More interestingly, compounds **8a–c** were the only which have shown good activity in secondary screening. Compounds **9a–d** were found to be inactive.

Table 2
Result of second level antituberculosis assays

SN	MIC (μM)[a]	SN	MIC (μM)[a]
1b	6.25	**6d**	6.25
4b	6.25	**8a**	0.78
4c	3.13	**8b**	1.56
6a	3.13	**8c**	0.39
6b	3.13	**8d**	3.13
6c	6.25		

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

All the compounds that were active in the first level screening were then tested to determine the actual minimum inhibitory concentration (MIC). Therefore, compounds **8a–c** have been proven to be the most active, with MIC values ranging from 0.39 to 1.56 μM.

2.3. Antimicrobial activity

From the antibacterial screening it was observed that all the compounds exhibited activity against all the organisms employed (Table 3). Looking at the structure–activity relationship, marked inhibition in bacteria was observed in the compounds **6b**, **6c**, and **8a–c**, whereas **1d**, **3a**, and **4b–d** have shown moderate activity and others showed least activity.

3. Conclusion

Screening of the in vitro antimycobacterial activity of this novel series has evidenced that derivatives with highly electronegative part at sulfhydryl group have emerged as new compounds endowed with antitubercular activity. Specifically compounds **8a–c**, i.e. 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 **8a–c** were the best choice for the preparations of new derivatives in order to improve its effectiveness on intracellular mycobacteria (macrophage) or in infected animal. Also, improvements are required to obtain new derivatives of these compounds which able to achieve more

Table 3
Antibacterial activity of the synthesized compounds

Compound	Organisms				Compound	Organisms				Compound	Organisms			
	Sa	Pa	Ec	St		Sa	Pa	Ec	St		Sa	Pa	Ec	St
1a	18	17	14	12	**3d**	18	16	10	12	**6c**	33	34	30	29
1b	18	16	15	14	**4a**	20	16	10	10	**6d**	20	18	14	12
1c	22	20	18	14	**4b**	22	22	20	16	**7a**	24	10	14	14
1d	25	22	20	16	**4c**	26	24	22	18	**7b**	21	18	10	18
2a	20	20	18	14	**4d**	26	24	20	20	**8c**	32	30	30	27
2b	16	16	20	16	**5a**	24	22	20	18	**8d**	18	12	14	12
2c	16	10	10	18	**5b**	22	11	24	24	**9a**	12	10	10	14
2d	11	17	15	22	**5c**	22	22	20	20	**9b**	14	16	12	18
3a	22	22	20	16	**5d**	16	18	12	12	**9c**	12	18	18	14
3b	16	16	12	12	**6a**	16	16	12	14	**9d**	14	11	18	12
3c	11	24	16	12	**6b**	32	32	26	24	Gent	34	35	31	30

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

effective antimycobacterial activity with lower toxicity to the mammalian cells.

4. Experimental protocols

4.1. Chemistry

The melting points were recorded on electrothermal apparatus and are uncorrected. ^{1}H NMR spectra on a Bruker Avance 300 MHz instrument using $CDCl_3$ as solvent using TMS as internal standard; the chemical shifts (δ) are reported in parts per million and coupling constants (J) are given in hertz. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet) and bs (broad singlet). Mass spectra were recorded on a Finnigan LCQ mass spectrometer. Microwave irradiation was carried out in Raga Scientific Microwave Systems, Model RG31L at 2450 MHz. Elemental analysis was performed on a Heracus CHN-rapid analyser. Analysis indicated by the symbols of the elements of functions was within $\pm$0.4% of the theoretical values.

The purity of the compounds was checked on silica gel coated Al plates (Merck).

*4.1.1. Preparation of N-{4-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-2-chloroacetamide (**1a**), N-{4-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-acetamide (**1b**), N-{4-[(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-benzamide (**1c**), 3-{N-(4-[(4-amino-5-sulfanyl-4H-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 [37].

4.1.2. General preparation of N-[3-({2-[(substituted)-amino]-1,3-thiazol-4-yl}methyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]benzamide

The triazoles **1a–d** (0.01 mol) in 20 ml of 10% NaOH was treated dropwise with an equimolar amount of the benzoyl chloride at 0 °C, which was stirred for 30–45 min. At the end of stirring a buff colored precipitate was obtained. The precipitate was then filtered, washed thoroughly with water and crystallized.

*4.1.2.1. N-[3-({2-[(2-Chloroacetyl)amino]-1,3-thiazol-4-yl}-methyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]benzamide (**2a**).* Yield 71%; brown; mp 241–243 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.74 (s, 2H, CH_2), 4.13 (s, 2H, CH_2), 6.21 (s, 1H, Thiazole CH), 7.12–7.36 (m, 5H, ArH), 8.06 (s, 2H, NH), 12.31 (s, 1H, SH); MS (%) 408 (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. $C_{15}H_{13}N_6O_2S_2Cl$ (C, H, N, O).

*4.1.2.2. N-[3-({2-[Acetyl-amino]-1,3-thiazol-4-yl}methyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]benzamide (**2b**).* Yield 77%; buff white; mp 250–252 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 5H, ArH), 8.12 (s, 2H, NH), 12.53 (s, 1H, SH); MS (%) 374 (M^+, 100), 312 (29.6), 251 (9.8), 223 (15.1), 211 (10.8), 107 (14.7), 087 (10.1), 82 (7.6); Anal. $C_{15}H_{14}N_6O_2S_2$ (C, H, N, O).

*4.1.2.3. N-[3-({2-[Benzoylamino]-1,3-thiazol-4-yl}methyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]benzamide (**2c**).* Yield 76%; brown; mp 280–282 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.32 (s, 2H, CH_2), 4.18 (s, 2H, CH_2), 6.17 (s, 1H, Thiazole CH), 7.21–7.86 (m, 10H, ArH), 8.13 (s, 2H, NH), 12.13 (s, 1H, SH); MS (%) 436 (M^+, 80), 306 (28), 292 (8.4), 251 (20), 214 (100), 195 (15), 154 (7), 106 (65); Anal. $C_{20}H_{16}N_6O_2S_2$ (C, H, N, O).

*4.1.2.4. 3-{N-[(4-[(4-(Benzoylamino)-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-aminopropanoic acid (**2d**).* Yield 69%; brown; mp 271–273 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 5H, ArH), 8.16 (s, 1H, NH), 10.43 (bs, 1H, OH), 12.43 (s, 1H, SH); MS (%) 404 (M^+, 100), 323 (14), 309 (17.1), 280 (6), 134 (35.9); Anal. $C_{16}H_{16}N_6O_3S_2$ (C, H, N, O).

4.1.3. General preparation of N,N′-(methylenebis{sulfanedial-[5-({2-[(substituted)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazole-3,4-dial]})dibenzamide

The triazoles **2a–d** (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 the 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 white crystals.

*4.1.3.1. N,N′-(Methylenebis{sulfanedial-[5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazole-3,4-dial]})dibenzamide (**3a**).* Yield 84%; brown; mp 278–280 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 10H, ArH), 7.84 (s, 4H, NH); MS (%) 829 (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. $C_{31}H_{26}N_{12}O_4S_4Cl_2$ (C, H, N, O).

*4.1.3.2. N,N′-(Methylenebis{sulfanedial-[5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazole-3,4-dial]})dibenzamide (**3b**).* Yield 77%; brown; mp 262–264 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 10H, ArH), 8.15 (s, 4H, NH); MS (%) 761 (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. $C_{31}H_{28}N_{12}O_4S_4$ (C, H, N, O).

*4.1.3.3. N,N′-(Methylenebis{sulfanedial-[5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazole-3,4-dial]})dibenzamide (**3c**).* Yield 79%; brown; mp 274–276 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.73 (s, 4H, CH_2), 4.43 (s, 2H, CH_2), 6.27 (s, 2H, Thiazole CH), 7.13–7.80 (m, 20H, ArH), 8.16 (s, 4H, NH); MS (%) 885 (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. $C_{41}H_{32}N_{12}O_4S_4$ (C, H, N, O).

*4.1.3.4. 3-[4-(4-(Benzoylamino)-5-[([4-(benzoylamino)-5-(2-[(2-carboxyethyl)amino]-1,3-thiazol-4-ylmethyl)-4H-1,2,4-triazol-3-yl]sulfanylmethyl)sulfanyl]-4H-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]aminopropanoic acid (**3d**).* Yield 89%; yellow; mp 255–257 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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), 6.26 (s, 2H, Thiazole CH), 7.32–7.76 (m, 10H, ArH), 8.21 (s, 4H, NH), 10.65 (bs, 2H, OH); MS (%) 821 (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. $C_{33}H_{32}N_{12}O_6S_4$ (C, H, N, O).

4.1.4. General preparation of N-{3-({2-[(substituted)-amino]-1,3-thiazol-4-yl}methyl)-5-[(cyanomethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}benzamide

The triazoles **2a–d** (0.01 mol) were mixed with 1.2 ml (0.02 mol) of chloroacetonitrile and dissolved in 25 ml of water. Neutralization with sodium carbonate gave a precipitate, which was filtered, washed with cold water (2 × 20 ml), and crystallized.

*4.1.4.1. N-{3-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(cyanomethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}benzamide (**4a**).* Yield 86%; yellow; mp 241–243 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 5H, ArH), 8.24 (s, 2H, NH); MS (%) 448 (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. $C_{17}H_{14}N_7O_2S_2Cl$ (C, H, N, O).

*4.1.4.2. N-{3-({2-[(Acetyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(cyanomethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}benzamide (**4b**).* Yield 82%; yellow; mp 264–266 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 5H, ArH), 7.91 (s, 2H, NH); MS (%) 413 (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. $C_{17}H_{15}N_7O_2S_2$ (C, H, N, O).

*4.1.4.3. N-{3-({2-[(Benzoyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(cyanomethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}benzamide (**4c**).* Yield 81%; yellow; mp 267–269 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.73 (s, 2H, CH_2), 4.14 (s, 2H, CH_2), 6.24 (s, 1H, Thiazole CH), 7.41–7.83 (m, 10H, ArH), 8.24 (s, 2H, NH); MS (%) 475 (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. $C_{22}H_{17}N_7O_2S_2$ (C, H, N, O).

*4.1.4.4. 3-{N-[4-(4-(Benzoylamino)-5-[(cyanomethyl)sulfanyl]-4H-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino}propanoic acid (**4d**).* Yield 78%; yellow; mp 275–277 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 5H, ArH), 8.25 (s, 1H, NH), 10.84 (bs, 1H, OH); MS (%) 443 (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. $C_{18}H_{17}N_7O_3S_2$ (C, H, N, O).

4.1.5. General preparation of methyl{[4-(benzoylamino)-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate

A solution of triazoles **2a–d** (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 the 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 and filtered. After cooling, the filtrate gave almost pure product.

*4.1.5.1. Methyl{[4-(benzoylamino)-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate (**5a**).* Yield 82%; yellow; mp 259–251 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, Thiazole CH), 7.20–7.46 (m, 5H, ArH), 8.32 (broad, 2H, NH); MS (%) 481 (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. $C_{18}H_{17}N_6O_4S_2Cl$ (C, H, N, O).

*4.1.5.2. Methyl{[4-(benzoylamino)-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate (**5b**).* Yield 78%; yellow; mp 261–263 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, Thiazole CH), 7.17–7.38 (m, 5H, ArH), 8.13 (broad, 2H, NH); MS (%) 447 (M^+, 9), 387 (31), 337 (1), 323 (2), 309 (1), 273 (100), 272 (8); Anal $C_{18}H_{18}N_6O_4S_2$ (C, H, N, O).

*4.1.5.3. Methyl{[4-(benzoylamino)-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate (**5c**).* Yield 75%; brown; mp 226–228 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.32 (s, 3H, OCH_3), 3.62 (s, 2H, CH_2), 3.83 (s, 2H, SCH_2), 6.25 (s, 1H, Thiazole CH), 6.93–7.51 (m, 10H, ArH), 8.22 (broad, 2H, NH); MS (%) 508 (M^+, 69.9), 356 (54), 354 (43), 248 (100), 233 (39), 232 (15); Anal. $C_{23}H_{20}N_6O_4S_2$ (C, H, N, O).

*4.1.5.4. 3-{N-[4-(4-(Benzoylamino)-5-[(2-methoxy-2-oxoethyl)-sulfanyl]-4H-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino}-propanoic acid (**5d**).* Yield 79%; pale green; mp above 300 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, Thiazole CH), 7.25–7.61 (m, 5H, ArH), 8.29 (broad, 1H, NH), 11.13 (broad, 1H, OH); MS (%) 476 (M^+, 100), 379 (67), 309 (41), 272 (58), 131 (16), 120 (60), 117 (44), 94 (27), 91 (48), 84 (81); Anal. $C_{19}H_{20}N_6O_5S_2$ (C, H, N, O).

4.1.6. General preparation of N-{3-({2-[(substituted)-amino]-1,3-thiazol-4-yl}methyl)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}benzamide

A solution of **5a–d** (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 was then evaporated in vacuo, and the 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.

*4.1.6.1. N-{3-({2-[(Chloroacetyl)amino]-1,3-thiazol-4-yl}-methyl)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}benzamide (**6a**).* Yield 83%; brown; mp 245–247 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, Thiazole CH), 7.22–7.48 (m, 5H, ArH), 8.21 (broad, 2H, NH); MS (%) 481 (M^+, 65), 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. $C_{17}H_{17}N_8O_3S_2Cl$ (C, H, N, O).

*4.1.6.2. N-{3-({2-[(Acetyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}benzamide (**6b**).* Yield 82%; brown; mp 250–252 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, Thiazole CH), 7.45–7.71 (m, 5H, ArH), 8.12 (broad, 2H, NH); MS (%) 447 (M^+, 78), 390 (72), 321 (26.3), 247 (6.3), 215 (18.3), 174 (65.3), 136 (25), 88 (100), 69 (14.3); Anal. $C_{17}H_{18}N_8O_3S_2$ (C, H, N, O).

*4.1.6.3. N-{3-({2-[(Benzoyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}-benzamide (**6c**).* Yield 71%; brown; mp 222–224 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, Thiazole CH), 7.13–7.68 (m, 10H, ArH), 8.10 (broad, 2H, NH); MS (%) 508 (M^+, 89), 486 (31), 421 (60), 378 (14.3), 352 (45), 305 (24), 241 (73), 208 (56), 174 (66), 146 (100), 109 (18), 88 (15); Anal. $C_{22}H_{20}N_8O_3S_2$ (C, H, N, O).

*4.1.6.4. 3-{N-[4-(4-(Benzoylamino)-5-[(2-hydrazino-2-oxoethyl)-sulfanyl]-4H-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino}-propanoic acid (**6d**).* Yield 84%; brown; mp 243–245 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, Thiazole CH), 7.34–7.61 (m, 5H, ArH), 8.03 (broad, 1H, NH), 11.09 (broad, 1H, OH); MS (%) 476 (M^+, 93.3), 435 (32), 389 (22), 334 (56.4), 306 (28), 247 (64), 217 (100), 147 (83), 108 (71), 79 (10.2); Anal. $C_{18}H_{20}N_8O_4S_2$ (C, H, N, O).

4.1.7. General preparation of N-[3-{[2-(substituted-hydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(substituted)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide

To a solution of **6a–d** (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 was then evaporated in vacuo, and the 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.

*4.1.7.1. N-[3-{[2-(2-Acetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**7a**).* Yield 86%; yellow; mp 241–244 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.12 (s, 3H, CH_3), 3.43 (s, 2H, CH_2), 3.74 (s, 2H, SCH_2), 4.11–4.20 (dd, 2H, $J_{NH-NH} = 4.21$, $J_{NH-NH} = 4.48$), 4.54 (s, 2H, CH_2Cl), 6.63 (s, 1H, Thiazole CH), 7.23–7.53 (m, 5H, ArH), 8.32 (s, 2H, NH); MS (%) 523 (M^+, 56), 489 (29), 462 (45), 408 (27), 389 (71), 318 (41), 274 (16), 223 (100), 188 (29), 179 (22); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

*4.1.7.2. N-[3-{[2-(2-Acetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**7b**).* Yield 71%; yellow; mp 227–229 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.42 (s, 6H, CH_3), 3.74 (s, 2H, CH_2), 3.87 (s, 2H, SCH_2), 4.20–4.28 (dd, 2H, $J_{NH-NH} = 4.35$, $J_{NH-NH} = 4.76$), 6.27 (s, 1H, Thiazole CH), 7.11–7.32 (m, 5H, ArH), 8.18 (s, 2H, NH); MS (%) 488 (M^+, 97), 408 (62), 398 (17.2), 359 (9.1), 327 (74), 297 (54), 241 (8.3), 223 (100), 174 (24), 146 (27); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

*4.1.7.3. N-[3-{[2-(2-Acetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**7c**).* Yield 82%; yellow; mp 226–231 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03 (s, 3H, CH_3), 3.21 (s, 2H, CH_2), 3.45 (s, 2H, SCH_2), 4.24–4.31 (dd, 2H, $J_{NH-NH} = 4.30$, $J_{NH-NH} = 4.70$), 6.76 (s, 1H, Thiazole CH), 7.31–7.87 (m, 10H, ArH), 8.42 (s, 2H, NH); MS (%) 550 (M^+, 11), 507 (56), 467 (35), 423 (16), 374 (52), 329 (47), 276 (74), 223 (100), 164 (35), 151 (08); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

*4.1.7.4. 3-[N-(4-[5-[2-(2-Acetylhydrazino)-2-oxoethyl]sulfanyl-4-(benzoylamino)-4H-1,2,4-triazol-3-yl]methyl-1,3-thiazol-2-yl)-amino]propanoic acid (**7d**).* Yield 87%; yellow; mp 239–242 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, $J_{NH-NH} = 4.4$, $J_{NH-NH} = 4.6$), 6.39 (s, 1H, Thiazole CH), 7.11–7.42 (m, 5H, ArH), 8.27 (s, 2H, NH), 11.13 (broad, 1H, OH); MS (%) 518 (M^+, 64), 476 (33), 438 (19), 387 (43), 341 (51), 317 (21), 264 (36), 224 (100), 151 (16), 138 (67); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

*4.1.7.5. N-[3-{[2-(2-Benzoylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**7e**).* Yield 82%; yellow; mp 241–244 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.34 (s, 2H, CH_2), 3.67 (s, 2H, SCH_2), 4.15–4.23 (dd, 2H, $J_{NH-NH} = 4.23$, $J_{NH-NH} = 4.46$), 4.36 (s, 2H, CH_2Cl), 6.52 (s, 1H, Thiazole CH), 7.17–7.72 (m, 10H, ArH), 8.45 (s, 2H, NH); MS (%) 585 (M^+, 84), 548 (17), 389 (26), 374 (46), 331 (58), 247 (14), 242 (13), 223 (100), 194 (29), 136 (21); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

*4.1.7.6. N-[3-{[2-(2-Benzoylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(acetyl) amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**7f**).* Yield 74%; yellow; mp 286–288 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.38 (s, 3H, CH_3), 3.68 (s, 2H, CH_2), 3.71 (s, 2H, SCH_2), 4.13–4.21 (dd, 2H, $J_{NH-NH} = 4.23$, $J_{NH-NH} = 4.54$), 6.12 (s, 1H, Thiazole CH), 6.94–7.21 (m, 10H, ArH), 8.09 (s, 2H, NH); MS (%) 550 (M^+, 86), 496 (71), 431 (14), 357 (78), 329 (38), 305 (41), 287 (17), 241 (35), 167 (100), 109 (37), 98 (19); Anal. $C_{24}H_{22}N_8O_4S_2$ (C, H, N, O).

*4.1.7.7. N-[3-{[2-(2-Benzoylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**7g**).* Yield 79%; yellow; mp 229–233 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.16 (s, 2H, CH_2), 3.31 (s, 2H, SCH_2), 4.32–4.39 (dd, 2H, $J_{NH-NH} = 4.37$, $J_{NH-NH} = 4.74$), 6.47 (s, 1H, Thiazole CH), 7.11–7.82 (m, 15H, ArH), 8.26 (s, 2H, NH); MS (%) 613 (M^+, 91), 587 (66), 526 (19), 471 (16), 436 (68), 376 (34), 316 (61), 223 (100), 111 (37), 119 (54); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

*4.1.7.8. 3-{N-(4-[(4-(Benzoylamino)-5-[2-(2-benzoylhydrazino)-2-oxoethyl]sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl)amino}propionic acid (**7h**).* Yield 83%; yellow; mp 230–235 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, $J_{NH-NH} = 4.7$, $J_{NH-NH} = 4.9$), 6.61 (s, 1H, Thiazole CH), 7.26–7.35 (m, 10H, ArH), 8.32 (s, 2H, NH), 10.87 (broad, 1H, OH); MS (%) 580 (M^+, 97), 542 (17), 505 (47), 486 (53), 451 (68), 364 (24), 307 (37), 223 (100), 197 (41), 164 (46); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

*4.1.7.9. N-[3-{[2-(2-Chloroacetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**7i**).* Yield 77%; yellow; mp 233–237 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.12 (s, 2H, CH_2), 3.38 (s, 2H, SCH_2), 4.21–4.33 (dd, 2H, $J_{NH-NH} = 4.10$, $J_{NH-NH} = 4.52$), 4.47 (s, 4H, CH_2Cl), 6.67 (s, 1H, Thiazole CH), 7.25–7.56 (m, 5H, ArH), 8.24 (s, 2H, NH); MS (%) 557 (M^+, 84), 513 (46), 474 (13), 431 (68), 417 (49), 328 (42), 274 (12), 223 (100), 184 (18), 161 (34); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

*4.1.7.10. N-[3-{[2-(2-Chloroacetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**7j**).* Yield 57%; dark brown; mp 166–168 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, $J_{NH-NH} = 4.52$, $J_{NH-NH} = 4.92$), 6.41 (s, 1H, Thiazole CH), 7.26–7.46 (m, 5H, ArH), 8.32 (s, 2H, NH); MS (%) 523 (M^+, 54), 487 (36), 437 (84), 369 (54.2), 284 (9.3), 238 (100), 158 (12), 128 (37), 77 (32); Anal. $C_{19}H_{19}N_8O_4S_2Cl$ (C, H, N, O).

*4.1.7.11. N-[3-{[2-(2-Chloroacetylhydrazino)-2-oxoethyl]sulfanyl}-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**7k**).* Yield 78%; yellow; mp 232–235 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27 (s, 2H, CH_2), 3.46 (s, 2H, SCH_2), 4.13–4.22 (dd, 2H, J_{NH-NH} = 4.11, J_{NH-NH} = 4.57), 4.41 (s, 2H, CH_2Cl), 6.39 (s, 1H, Thiazole CH), 7.29–7.63 (m, 10H, ArH), 8.41 (s, 2H, NH); MS (%) 585 (M^+, 79), 576 (16), 513 (27), 415 (42), 384 (67), 306 (41), 264 (34), 223 (100), 167 (26), 144 (18); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

*4.1.7.12. 3-[N-(4-[4-(Benzoylamino)-5-(2-[2-(2-chloroacetyl)-hydrazino]-2-oxo-ethylsulfanyl)-4H-1,2,4-triazol-3-yl]methyl-1,3-thiazol-2-yl)amino]propanoic acid (**7l**).* Yield 80%; yellow; mp 237–239 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, J_{NH-NH} = 4.7), 4.23 (s, 2H, CH_2Cl), 6.49 (s, 1H, Thiazole CH), 7.12–7.19 (m, 5H, ArH), 8.54 (s, 2H, NH), 11.17 (broad, 1H, OH); MS (%) 553 (M^+, 91), 512 (69), 482 (12), 438 (89), 376 (73), 264 (58), 234 (76), 223 (100), 166 (28), 123 (21); Anal. $C_{19}H_{20}N_8O_4S_2$ (C, H, N, O).

4.1.8. General procedure for N-[3-({2-[(2E)-2-benzylidenehydrazino]-2-oxoethyl}sulfanyl)-5-({2-[(substituted)-amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide

A solution of **6a–d** (0.01 mol) with benzaldehyde (0.01 mol) was prepared in 10 ml ethanol. To this acidic alumina (10 g) was added. Ethanol was then evaporated in vacuo, and the 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.

*4.1.8.1. N-[3-({2-[(2E)-2-Benzylidenehydrazino]-2-oxoethyl}sulfanyl)-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**8a**).* Yield 81%; brown; mp 222–224 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 10H, ArH), 8.16 (s, 3H, NH), 8.27 (s, 1H, N=CH); MS (%) 569 (M^+, 69), 502 (19), 457 (37), 379 (21), 315 (58), 246 (24), 195 (35), 134 (100), 107 (11.4), 88 (12.3); Anal. $C_{24}H_{21}N_8O_3S_2Cl$ (C, H, N, O).

*4.1.8.2. N-[3-({2-[(2E)-2-Benzylidenehydrazino]-2-oxoethyl}-sulfanyl)-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**8b**).* Yield 83%; pale brown; mp 178–180 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 10H, ArH), 8.26 (s, 3H, NH), 8.36 (s, 1H, N=CH); MS (%) 535 (M^+, 94), 519 (41), 487 (9.6), 431 (26), 413 (8.4), 389 (100), 365 (20); Anal. $C_{24}H_{22}N_8O_3S_2$ (C, H, N, O).

*4.1.8.3. N-[3-({2-[(2E)-2-Benzylidenehydrazino]-2-oxoethyl}-sulfanyl)-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**8c**).* Yield 81%; pale brown; mp 217–219 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.63 (s, 2H, CH_2), 4.17 (s, 2H, SCH_2), 6.23 (s, 1H, Thiazole CH), 7.21–7.64 (m, 15H, ArH), 8.16 (s, 3H, NH), 8.41 (s, 1H, N=CH); MS (%) 597 (M^+, 79), 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. $C_{29}H_{24}N_8O_3S_2$ (C, H, N, O).

*4.1.8.4. 3-{N-[4-(4-(Benzoylamino)-5-[(2-oxo-2-2-[(Z)-1-phenylmethylidene]hydrazinoethyl)sulfanyl]-4H-1,2,4-triazol-3-yl-methyl)-1,3-thiazol-2-yl]amino}propanoic acid (**8d**).* Yield 78%; brown; mp 120–122 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 10H, ArH), 8.15 (s, 2H, NH), 8.32 (s, 1H, N=CH), 10.87 (bs, 1H, OH); MS (%) 564 (M^+, 74), 497 (45), 426 (13), 364 (63), 326 (32), 248 (65), 185 (12), 146 (100), 109 (37), 87 (42); Anal. $C_{25}H_{24}N_8O_4S_2$ (C, H, N, O).

4.1.9. General preparation of N-[3-{[(4-amino-5-thioxo-4,5-dihydro-1H-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]benzamide

Compounds **6a–d** (0.01 mol) were dissolved in alcoholic potassium hydroxide (0.01 mol) and kept for stirring. Carbon disulphide (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 hour 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 was then evaporated in vacuo, and the 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.

*4.1.9.1. N-[3-{[(4-Amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]sulfanyl}-5-({2-[(chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**9a**).* Yield 72%; brown; mp 228–230 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 5H, ArH), 8.11 (s, 2H, NH), 12.49 (s, 1H, SH); MS (%) 537 (M^+, 82), 467 (31), 431 (26), 384 (31), 326 (13.2), 247 (15), 226 (17), 125 (100); Anal. $C_{18}H_{17}N_{10}O_2S_3Cl$ (C, H, N, O).

*4.1.9.2. N-[3-{[(4-Amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]sulfanyl}-5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}-methyl)-4H-1,2,4-triazol-4-yl]benzamide (**9b**).* Yield 71%; brown; mp 252–254 °C; ^{1}H NMR (300 MHz, $CDCl_3$):

δ 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, 5H, ArH), 8.08 (s, 2H, NH), 12.43 (s, 1H, SH); MS (%) 503 (M^+, 76), 454 (32), 350 (30), 335 (100), 323 (2.93), 222 (12.26), 220 (3.73), 207 (5.86), 192 (7.07); Anal. $C_{18}H_{18}N_{10}O_2S_3$ (C, H, N, O).

*4.1.9.3. N-[3-{[(4-Amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]sulfanyl}-5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]benzamide (**9c**).* Yield 80%; brown; mp 264–266 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 10H, ArH), 8.17 (s, 2H, NH), 12.63 (s, 1H, SH); MS (%) 565 (M^+, 93.3), 513 (24), 476 (21), 432 (16), 421 (12), 323 (65), 308 (41), 289 (27), 230 (38), 207 (100), 142 (35.7), 109 (23); Anal. $C_{23}H_{20}N_{10}O_2S_3$ (C, H, N, O).

*4.1.9.4. 3-[(4-[5-[(4-Amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)-methyl]sulfanyl-4-(benzoylamino)-4H-1,2,4-triazol-3-yl]methyl-1,3-thiazol-2-yl)amino]propanoic acid (**9d**).* Yield 77%; brown; mp 237–239 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 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, 5H, ArH), 8.17 (s, 1H, NH), 10.64 (bs, 1H, OH), 12.35 (s, 1H, SH); MS (%) 533 (M^+, 84), 497 (23), 450 (47), 438 (38), 371 (25), 370 (75), 354 (10), 235 (100), 220 (22), 207 (68), 192 (70); Anal. $C_{19}H_{20}N_{10}O_3S_3$ (C, H, N, O).

4.2. Pharmacology

4.2.1. Antiutubercular 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) [39]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system [40]. Compounds showing more than 95% inhibition in the primary screening were considered as 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 (6.25–50 μg ml^{-1}), none of them were found toxic. Hence the activities of the above-synthesized compounds were not due to cytotoxicity of compounds.

4.2.2. Antimicrobial activity

Compounds listed in Table 1 were screened for the antimicrobial activity against different microorganisms under the following conditions.

Method: Well diffusion method [41];
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-h subcultured bacteria was 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.

The percentage of inhibition was calculated by the formula: % Inhibition = Diameter of the inhibition zone × 100.

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