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Clubbed thiazoles by MAOS: A novel approach to cyclin-dependent kinase 5/p25 inhibitors as a potential treatment for Alzheimer's disease

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Abstract—A novel clubbed triazolyl thiazole series of cdk5/p25 inhibitors, potentially useful for the treatment of Alzheimer's disease, is disclosed. Evaluation of the SAR of substitution within these series has allowed the identification of a range of compounds which significantly reduce brain cdk5/p25 and thus have potential as possible treatments for Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder accompanied by memory decline, cognitive impairment, and visual-spatial disorientation, for which no effective treatment exists today. Postmortem brain analysis of AD patients reveals extensive formation of neurofibrillary tau protein tangles and amyloid plaques. The serine/threonine kinase cdk5 along with its cofactor p25[1] (or the longer cofactor, p35) has been supposed to hyperphosphorylate tau,[2] leading to the formation of paired helical filaments and deposition of cytotoxic neurofibrillary tangles[3] and thus responsible for neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, stroke, or Huntington's disease.[4] cdk5 also phosphorylates Dopamine and Cyclic AMP-Regulated Phosphorprotein (DARPP-32) at threonine 75 and is thus indicated in having a role in dopaminergic neurotransmission.[5] Inhibition of the anomalous cdk5/p25 complex is, therefore, a viable target for treating Alzheimer's disease by preventing tau hyperphosphorylation and neurofibrillary tangle formation. Literature survey reveals 2-aminothiazole derivatives[6] as the potential inhibitors of cdk5/p25 for the treatment of Alzheimer's disease and other neurodegenerative disorders.[7–13]

Based on this hypothesis, we embarked on a cdk5/p25 inhibitor discovery program to find an orally bioavailable, high potency compound/s. Screening of an in-house database provided several hits with modest cdk5/p25 inhibitory activity, one of which was the clubbed triazolyl thiazole 1 ($IC_{50} = 48 \pm 2$ nM).

In recent years, environmentally benign synthetic methods have received considerable attention and solvent free protocols are reported.[14–16] 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 Scheme 1.

2. Results and discussion

2.1. Synthesis

Compounds **1a–d**, **2e–l**, **3e–l**, **4e–l**, **5e–l**, **6e–l**, **7m–aj**, **8e–l**, and **9e–l** were synthesized as per the literature.[17,18]

Keywords: Thiazole; Triazole; cdk5/p25; Alzheimer's disease.
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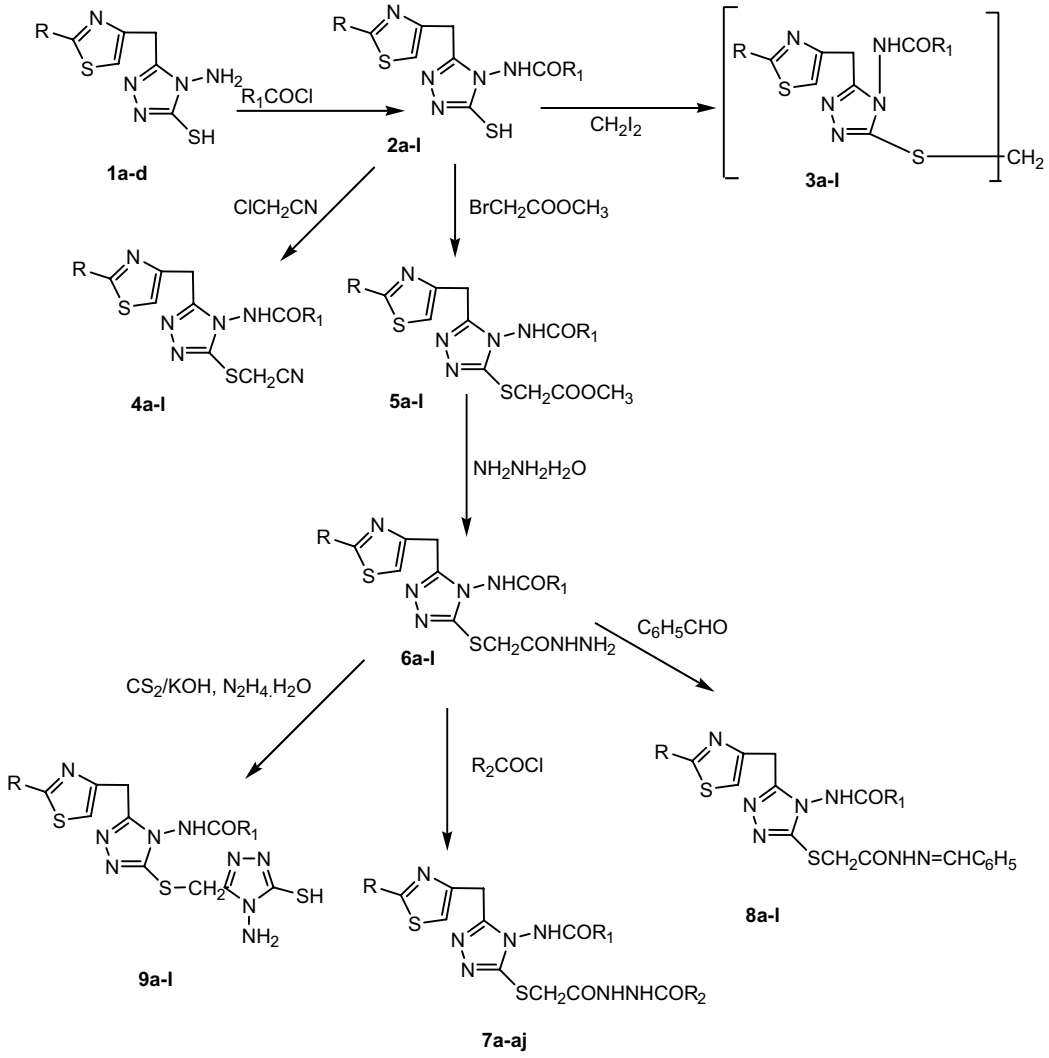

Scheme 1.

Compounds **1a**–**d**, adsorbed on acidic alumina (aluminum oxide, acidic, Brockmann I, ~150 mesh, 58 Å CAMAG 506-C-I, surface area 155 m^2/g, pH 6.0), when treated with 4-chlorobenzoyl chloride at 0 °C to yield **2a**–**d**. The transformed compounds **2a**–**d** on treatment with diiodomethane in the presence of strong alkali, that is, sodium hydroxide, gave **3a**–**d**. Title compounds **2a**–**d** were treated with chloroacetonitrile, which on neutralization with sodium carbonate gave precipitates of compounds **4a**–**d**. Compounds **2a**–**d**, when treated with methyl bromoacetate in basic condition, produced **5a**–**d**. Chemical transformation of **5a**–**d** to **6a**–**d** was achieved by treating it 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 disulfide and potassium hydroxide, which on treatment with hydrazine hydrate gave **9a**–**d**. The NMR spectra confirmed formation of triazole derivative from hydrazide, which shows 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 **6a**–**d** to **9a**–**d** when performed in conventional method, while reaction involving MORE method gave good yield (71–80%).

2.2. Cyclin-dependent kinase 5/p25 inhibiting activity

Kinase inhibition was measured by the use of scintillation proximity assays (SPA).[8]

The results of the assays are reported in Tables 1–4. During the preliminary screening compound **1b** has emerged as hit cdk5/p25 (IC_{50} = 48 ± 2 nM), with good potency and more opportunities for chemical transformation for the optimization. Testing of **1b** against other cdks revealed that **1b** was essentially equipotent at inhibiting cdk2/cyclin E (IC_{50} = 50 ± 3 nM), a cancer target. Thus with an objective to improve cdk5 potency and minimize cdk2 activity, certain chemical modifications have been performed. Variation of the amine side chain of **1b** with MAOS allowed us to rapidly explore the first arm of the pharmacophore. As a first step toward lead

Table 1. SAR of cdk5/p5 inhibitory screening

SN	R	R_1	R_2	cdk5 IC_{50} (nM)
1a	$NHCOCH_2Cl$	—	—	390 ± 89
1b	$NHCOCH_3$	—	—	048 ± 02
1c	$NHCOC_6H_5$	—	—	630 ± 32
1d	$NHCH_2CH_2COOH$	—	—	264 ± 27
2a	$NHCOCH_2Cl$	–4-ClC_6H_4	—	642 ± 11
2b	$NHCOCH_3$	–4-ClC_6H_4	—	462 ± 72
2c	$NHCOC_6H_5$	–4-ClC_6H_4	—	186 ± 12
2d	$NHCH_2CH_2COOH$	–4-ClC_6H_4	—	462 ± 50
2e	$NHCOCH_2Cl$	–C_6H_5	—	761 ± 84
2f	$NHCOCH_3$	–C_6H_5	—	574 ± 65
2g	$NHCOC_6H_5$	–C_6H_5	—	894 ± 114
2h	$NHCH_2CH_2COOH$	–C_6H_5	—	674 ± 68
2i	$NHCOCH_2Cl$	–CH_3	—	684 ± 78
2j	$NHCOCH_3$	–CH_3	—	598 ± 58
2k	$NHCOC_6H_5$	–CH_3	—	614 ± 46
2l	$NHCH_2CH_2COOH$	–CH_3	—	436 ± 28
3a	$NHCOCH_2Cl$	–4-ClC_6H_4	—	340 ± 22
3b	$NHCOCH_3$	–4-ClC_6H_4	—	382 ± 24
3c	$NHCOC_6H_5$	–4-ClC_6H_4	—	640 ± 34
3d	$NHCH_2CH_2COOH$	–4-ClC_6H_4	—	168 ± 11
3e	$NHCOCH_2Cl$	–C_6H_5	—	674 ± 46
3f	$NHCOCH_3$	–C_6H_5	—	647 ± 84
3g	$NHCOC_6H_5$	–C_6H_5	—	698 ± 46
3h	$NHCH_2CH_2COOH$	–C_6H_5	—	587 ± 28
3i	$NHCOCH_2Cl$	–CH_3	—	847 ± 86
3j	$NHCOCH_3$	–CH_3	—	847 ± 64
3k	$NHCOC_6H_5$	–CH_3	—	764 ± 84
3l	$NHCH_2CH_2COOH$	–CH_3	—	743 ± 46
4a	$NHCOCH_2Cl$	–4-ClC_6H_4	—	562 ± 79
4b	$NHCOCH_3$	–4-ClC_6H_4	—	062 ± 11
4c	$NHCOC_6H_5$	–4-ClC_6H_4	—	064 ± 04
4d	$NHCH_2CH_2COOH$	–4-ClC_6H_4	—	426 ± 14
4e	$NHCOCH_2Cl$	–C_6H_5	—	657 ± 67
4f	$NHCOCH_3$	–C_6H_5	—	395 ± 64
4g	$NHCOC_6H_5$	–C_6H_5	—	496 ± 84
4h	$NHCH_2CH_2COOH$	–C_6H_5	—	487 ± 96
4i	$NHCOCH_2Cl$	–CH_3	—	415 ± 78
4j	$NHCOCH_3$	–CH_3	—	463 ± 86
4k	$NHCOC_6H_5$	–CH_3	—	574 ± 64
4l	$NHCH_2CH_2COOH$	–CH_3	—	641 ± 78
5a	$NHCOCH_2Cl$	–4-ClC_6H_4	—	440 ± 102
5b	$NHCOCH_3$	–4-ClC_6H_4	—	380 ± 32
5c	$NHCOC_6H_5$	–4-ClC_6H_4	—	290 ± 41
5d	$NHCH_2CH_2COOH$	–4-ClC_6H_4	—	560 ± 22
5e	$NHCOCH_2Cl$	–C_6H_5	—	674 ± 46
5f	$NHCOCH_3$	–C_6H_5	—	587 ± 76
5g	$NHCOC_6H_5$	–C_6H_5	—	364 ± 74
5h	$NHCH_2CH_2COOH$	–C_6H_5	—	648 ± 124
5i	$NHCOCH_2Cl$	–CH_3	—	695 ± 118
5j	$NHCOCH_3$	–CH_3	—	467 ± 106
5k	$NHCOC_6H_5$	–CH_3	—	598 ± 110
5l	$NHCH_2CH_2COOH$	–CH_3	—	746 ± 148
6a	$NHCOCH_2Cl$	–4-ClC_6H_4	—	044 ± 02
6b	$NHCOCH_3$	–4-ClC_6H_4	—	072 ± 02
6c	$NHCOC_6H_5$	–4-ClC_6H_4	—	034 ± 01
6d	$NHCH_2CH_2COOH$	–4-ClC_6H_4	—	064 ± 01

optimization amino group was protected to the corresponding compounds **2a**–**l** however, all of these modifications resulted in a substantial decrease in activity. The next structural modification made was a dimeric product of **3a**–**l** but these changes also resulted in a substantial loss of biological activity.

s-Alkylation with acetonitrile provided the first analogs **4b** and **4c** that demonstrated excellent activity, while others exhibited moderate to poor activity. Thus it was decided to modify the structure at SH group. In order to optimize the sulfhydryl component, compounds **5a**–**l** were synthesized and investigated, which revealed loss

Table 2. SAR of cdk5/p5 inhibitory screening

SN	R	R_1	R_2	cdk5 IC_{50} (nM)
6e	$NHCOCH_2Cl$	$-C_6H_5$	—	246 ± 38
6f	$NHCOCH_3$	$-C_6H_5$	—	197 ± 49
6g	$NHCOC_6H_5$	$-C_6H_5$	—	268 ± 78
6h	$NHCH_2CH_2COOH$	$-C_6H_5$	—	465 ± 84
6i	$NHCOCH_2Cl$	$-CH_3$	—	201 ± 34
6j	$NHCOCH_3$	$-CH_3$	—	241 ± 36
6k	$NHCOC_6H_5$	$-CH_3$	—	186 ± 29
6l	$NHCH_2CH_2COOH$	$-CH_3$	—	943 ± 23
7a	$NHCOCH_2Cl$	$-4\text{-}ClC_6H_4$	CH_3	464 ± 64
7b	$NHCOCH_3$	$-4\text{-}ClC_6H_4$	CH_3	641 ± 52
7c	$NHCOC_6H_5$	$-4\text{-}ClC_6H_4$	CH_3	340 ± 30
7d	$NHCH_2CH_2COOH$	$-4\text{-}ClC_6H_4$	CH_3	352 ± 24
7e	$NHCOCH_2Cl$	$-4\text{-}ClC_6H_4$	C_6H_5	640 ± 18
7f	$NHCOCH_3$	$-4\text{-}ClC_6H_4$	C_6H_5	534 ± 48
7g	$NHCOC_6H_5$	$-4\text{-}ClC_6H_4$	C_6H_5	484 ± 43
7h	$NHCH_2CH_2COOH$	$-4\text{-}ClC_6H_4$	C_6H_5	268 ± 14
7i	$NHCOCH_2Cl$	$-4\text{-}ClC_6H_4$	CH_2Cl	224 ± 64
7j	$NHCOCH_3$	$-4\text{-}ClC_6H_4$	CH_2Cl	260 ± 44
7k	$NHCOC_6H_5$	$-4\text{-}ClC_6H_4$	CH_2Cl	340 ± 23
7l	$NHCH_2CH_2COOH$	$-4\text{-}ClC_6H_4$	CH_2Cl	180 ± 41
7m	$NHCOCH_2Cl$	$-C_6H_5$	CH_3	356 ± 64
7n	$NHCOCH_3$	$-C_6H_5$	CH_3	542 ± 46
7o	$NHCOC_6H_5$	$-C_6H_5$	CH_3	564 ± 87
7p	$NHCH_2CH_2COOH$	$-C_6H_5$	CH_3	472 ± 46
7q	$NHCOCH_2Cl$	$-C_6H_5$	C_6H_5	467 ± 92
7r	$NHCOCH_3$	$-C_6H_5$	C_6H_5	421 ± 46
7s	$NHCOC_6H_5$	$-C_6H_5$	C_6H_5	463 ± 75
7t	$NHCH_2CH_2COOH$	$-C_6H_5$	C_6H_5	432 ± 80
7u	$NHCOCH_2Cl$	$-C_6H_5$	CH_2Cl	530 ± 64
7v	$NHCOCH_3$	$-C_6H_5$	CH_2Cl	643 ± 84
7w	$NHCOC_6H_5$	$-C_6H_5$	CH_2Cl	542 ± 74
7x	$NHCH_2CH_2COOH$	$-C_6H_5$	CH_2Cl	462 ± 55
7y	$NHCOCH_2Cl$	$-CH_3$	CH_3	354 ± 43
7z	$NHCOCH_3$	$-CH_3$	CH_3	384 ± 48
7aa	$NHCOC_6H_5$	$-CH_3$	CH_3	643 ± 67
7ab	$NHCH_2CH_2COOH$	$-CH_3$	CH_3	463 ± 47
7ac	$NHCOCH_2Cl$	$-CH_3$	C_6H_5	365 ± 36
7ad	$NHCOCH_3$	$-CH_3$	C_6H_5	436 ± 47
7ae	$NHCOC_6H_5$	$-CH_3$	C_6H_5	413 ± 81
7af	$NHCH_2CH_2COOH$	$-CH_3$	C_6H_5	476 ± 73
7ag	$NHCOCH_2Cl$	$-CH_3$	CH_2Cl	462 ± 68
7ah	$NHCOCH_3$	$-CH_3$	CH_2Cl	476 ± 74
7ai	$NHCOC_6H_5$	$-CH_3$	CH_2Cl	356 ± 69
7aj	$NHCH_2CH_2COOH$	$-CH_3$	CH_2Cl	264 ± 46
8a	$NHCOCH_2Cl$	$-4\text{-}ClC_6H_4$	—	042 ± 01
8b	$NHCOCH_3$	$-4\text{-}ClC_6H_4$	—	030 ± 01
8c	$NHCOC_6H_5$	$-4\text{-}ClC_6H_4$	—	246 ± 28
8d	$NHCH_2CH_2COOH$	$-4\text{-}ClC_6H_4$	—	238 ± 34
8e	$NHCOCH_2Cl$	$-C_6H_5$	—	467 ± 52
8f	$NHCOCH_3$	$-C_6H_5$	—	485 ± 30
8g	$NHCOC_6H_5$	$-C_6H_5$	—	496 ± 54
8h	$NHCH_2CH_2COOH$	$-C_6H_5$	—	526 ± 74
8i	$NHCOCH_2Cl$	$-CH_3$	—	536 ± 49
8j	$NHCOCH_3$	$-CH_3$	—	463 ± 86
8k	$NHCOC_6H_5$	$-CH_3$	—	467 ± 44
8l	$NHCH_2CH_2COOH$	$-CH_3$	—	635 ± 64

of activity. A further modification of compounds **5a**–**l** produced compounds **6a**–**l**. The results of the cdk5/p25 inhibitory activity are quite interesting because four **6a**–**d** of these compounds have shown impressive percentage of inhibition. Compounds **6a**–**l** were selected for further studies as they have a free amino group, which opened an area for further modification at this point. Compounds **7a**–**aj** were obtained by treatment with acid chlorides which ultimately showed decreased activity. Furthermore, compounds **6a**–**l** were converted to Schiff bases with benzaldehyde, and on investigation, **8a**–**d** have shown promising activity while others remained inactive. Compounds **9a**–**l** were found to be inactive.

Table 3. SAR of cdk5/p5 inhibitory screening

SN	R	R_1	R_2	cdk5 IC_{50} (nM)
9a	$NHCOCH_2Cl$	–4-ClC_6H_4	—	3260 ± 106
9b	$NHCOCH_3$	–4-ClC_6H_4	—	7480 ± 114
9c	$NHCOC_6H_5$	–4-ClC_6H_4	—	3180 ± 212
9d	$NHCH_2CH_2COOH$	–4-ClC_6H_4	—	6650 ± 142
9e	$NHCOCH_2Cl$	–C_6H_5	—	6985 ± 164
9f	$NHCOCH_3$	–C_6H_5	—	6537 ± 148
9g	$NHCOC_6H_5$	–C_6H_5	—	4653 ± 174
9h	$NHCH_2CH_2COOH$	–C_6H_5	—	4785 ± 156
9i	$NHCOCH_2Cl$	–CH_3	—	4635 ± 186
9j	$NHCOCH_3$	–CH_3	—	5246 ± 210
9k	$NHCOC_6H_5$	–CH_3	—	6342 ± 200
9l	$NHCH_2CH_2COOH$	–CH_3	—	6412 ± 246

Table 4. Selectivity ratio of most active compounds

Compound	cdk5 IC_{50} (nM)	cdk2 IC_{50} (nM)	Select k2/k5
1b	48 ± 2	50 ± 3	1
4b	62 ± 11	1140 ± 162	18.3
4c	64 ± 4	1340 ± 104	21
6a	44 ± 2	890 ± 78	20
6b	72 ± 2	624 ± 62	8.6
6c	34 ± 1	468 ± 43	13.8
6d	64 ± 1	6342 ± 142	99
8a	42 ± 1	51 ± 8	1.2
8b	30 ± 1	512 ± 12	17

Attention was then turned to optimization of the **8a**–**d** in order to gain the selectivity over cdk2. On comparing, **8b** afforded improved cdk5 potency that is >17-fold selectivity versus cdk2. The compound **8a** was equally selective versus cdk2 and had slightly improved cdk5 IC_{50}. Other derivatives were had noticeably decreased cdk5 activity.

3. Conclusion

In conclusion, a novel series of clubbed triazolyl thiazole derivatives that inhibit cdk5/p25 has been discovered. It was found that the potency of the screening hit **1b** could be enhanced first by structural transformation to a 2-position of thiazole core and amino and sulfhydryl groups in triazole core and subsequently by the introduction of appropriate substituents on both the heterocyclic rings leading to the most promising compounds **8a** and **8b**. Finally it can be concluded that an ideal cdk5/p25 inhibitor 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 on a Bruker Avance 300 MHz instrument using $CDCl_3$ as solvent using TMS as internal standard; the chemical shifts (δ) are reported in ppm 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 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 analysis was performed on a Heracus CHN-Rapid Analyser. Analysis indicated by the symbols of the elements of functions was within ±0.4% of the theoretical values. The purity of the compounds was checked on silica gel coated Al plates (Merck).

Kinase inhibition was measured by use of scintillation proximity assays (SPA).[10] Enzyme activities were assayed as the incorporation of [33P] from the gamma phosphate of [33P] ATP (Amersham, cat. no. AH-9968) into biotinylated peptide substrate PKTPK KAKKL. Reactions were carried out in a buffer containing 50 mM Tris–HCI, pH 8.0; 10 mM $MgCl_2$, 0.1 mM Na_3VO_4, and 1 mM DTT. The final concentration of ATP was 0.5 μM (final specific radioactivity of 4 μCi/nmol), and the final concentration of substrate was 0.75 μM. Reactions, initiated by the addition of cdk5 and activator protein p25, were carried out at room temperature for 60 min. Reactions were stopped by addition of 0.6 volume of buffer containing (final concentrations): 2.5 mM EDTA, 0.05% Triton X-100, 100 μM ATP, and 1.25 mg/ml streptavidin coated SPA beads (Amersham cat. No. RPNQ0007). Radioactivity associated with the beads was quantified by scintillation counting. We have also done cytotoxicity analysis of the above-synthesized compounds, using neutral red uptake by using Vero-C-1008 cell line[18] at various concentrations (6.25–50 μg/mL), none of them were found toxic. Hence the activities of the above-synthesized compounds were not due to cytotoxicity of compounds.

4.2. 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), and 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.[17]

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

The triazole (**1**) (1 mmol) in 20 mL of 10% NAOH was treated dropwise with an equimolar amount of the 4-chlorobenzoyl chloride at 0 °C, which was stirred for 30–45 min. At the end of stirring, precipitate was observed. It was then filtered, washed thoroughly with water, and crystallized.

4.3.1. *N*-[3-({2-[(Chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)- 5-sulfanyl-4H-1,2,4-triazol-4-yl]-4-chlorobenzamide (2a). Yield 71%; 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, 4H, ArH), 8.06 (s, 2H, NH), 12.31 (s, 1H, SH); MS *m/z* (%) 443 (M^+, 100), 407 (52), 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 $C_{15}H_{12}Cl_2N_6O_2S_2$: C, 40.64; H, 2.73; N, 18.96. Found: C, 40.36; H, 2.48; N, 18.49.

4.3.2. *N*-[3-({2-[(Acetyl)amino]-1,3-thiazol-4-yl}methyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]-4-chlorobenzamide (2b). Yield 77%; 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, 4H, ArH), 8.12 (s, 2H, NH), 12.53 (s, 1H, SH); MS *m/z* (%) 409 (M^+, 100), 376 (37), 312 (29.6), 251 (9.8), 223 (15.1), 211 (10.8), 107 (14.7), 087 (10.1), 82 (7.6); Anal. Calcd for $C_{15}H_{13}ClN_6O_2S_2$: C, 44.06; H, 3.20; N, 20.55. Found: C, 44.32; H, 3.51; N, 20.62.

4.3.3. *N*-[3-({2-[(Benzoyl)amino]-1,3-thiazol-4-yl}methyl)-5-sulfanyl-4H-1,2,4-triazol-4-yl]-4-chlorobenzamide (2c). Yield 76%; 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, 9H, ArH), 8.13 (s, 2H, NH), 12.13 (s, 1H, SH); MS *m/z* (%) 471 (M^+, 80), 436 (21) 306 (28), 292 (8.4), 251 (20), 214 (100), 195 (15), 154 (7), 106 (65); Anal. Calcd for $C_{20}H_{15}ClN_6O_2S_2$: C, 51.01; H, 3.21; N, 17.84. Found: C, 51.17; H, 3.34; N, 17.54.

4.3.4. 3-{*N*-[4-[(4-(4-Chlorobenzoylamino)-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]-1,3-thiazol-2-yl}-aminopropanoic acid (2d). Yield 69%; 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, 4H, ArH), 8.16 (s, 1H, NH), 10.43 (bs, 1H, OH), 12.43 (s, 1H, SH); MS *m/z* (%) 439 (M^+, 100), 389 (52), 323 (14), 309 (17.1), 280 (6), 134 (35.9); Anal. Calcd for $C_{16}H_{15}ClN_6O_3S_2$: C, 43.78; H, 3.44; N, 19.15. Found: C, 43.93; H, 3.27; N, 19.07.

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

The triazole (**2**) (1 mmol), diiodomethane (1.5 mmol), and 5.6 g (1 mmol) 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 white.

4.4.1. *N*,*N*′-(Methylenebis{sulfanedial-[5-({2-[(2-chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazole-3,4-dial]})di-4-chlorobenzamide (3a). Yield 84%; decomposes around 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, 8H, ArH), 7.84 (s, 4H, NH); MS *m/z* (%) 852 (M^+, 7.1), 714 (43), 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 $C_{31}H_{24}Cl_4N_{12}O_4S_4$: C, 41.43; H, 2.69; N, 18.70. Found: C, 41.64; H, 2.81; N, 18.96.

4.4.2. *N*,*N*′-(Methylenebis{sulfanedial-[5-({2-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazole-3,4-dial]})di-4-chlorobenzamide (3b). Yield 77%; decomposes around 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, 8H, ArH), 8.15 (s, 4H, NH); MS *m/z* (%) 784 (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 $C_{31}H_{26}Cl_2N_{12}O_4S_4$: C, 44.87; H, 3.16; N, 20.26. Found: C, 44.62; H, 3.29; N, 20.04.

4.4.3. *N*,*N*′-(Methylenebis{sulfanedial-[5-({2-[(benzoyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazole-3,4-dial]}) di-4-chlorobenzamide (3c). Yield 79%; decomposes around 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, 18H, ArH), 8.16 (s, 4H, NH); MS *m/z* (%) 908 (M^+, 14.1), 886 (38), 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 $C_{41}H_{30}Cl_2N_{12}O_4S_4$: C, 51.62; H, 3.17; N, 17.62. Found: C, 51.48; H, 3.35; N, 17.82.

4.4.4. 3-[4-(4-(4-Chlorobenzoylamino)-5-[([4-(4-chlorobenzoylamino)-5-(2-[(2-carboxy-ethyl)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%; decomposes around 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, 8H, ArH), 8.21 (s, 4H, NH), 10.65 (br s, 2H, OH); MS *m/z* (%) 844 (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 $C_{33}H_{30}Cl_2N_{12}O_6S_4$: C, 44.54; H, 3.40; N, 18.89. Found: C, 44.71; H, 3.64; N, 18.69.

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

The triazole (**2**) (1 mmol) was mixed with 1.2 mL (2 mmol) 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.

4.5.1. *N*-{3-({2-[(2-Chloroacetyl)amino]-1,3-thiazol-4-yl}-methyl)-5-[(cyanomethyl) sulfanyl]-4H-1,2,4-triazol-4-yl}-4-chlorobenzamide (4a). Yield 86%; 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, 4H, ArH), 8.24 (s, 2H, NH); MS *m/z* (%) 482 (M^+, 100), 428 (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 $C_{17}H_{13}Cl_2N_7O_2S_2$: C, 42.33; H, 2.72; N, 20.33. Found: C, 42.51; H, 2.97; N, 20.06.

4.5.2. *N*-{3-({2-[(Acetyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(cyanomethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}-4-chlorobenzamide (4b). Yield 82%; 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, 4H, ArH), 7.91 (s, 2H, NH); MS *m/z* (%) 448 (M^+, 100), 407 (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 $C_{17}H_{14}ClN_7O_2S_2$: C, 45.58; H, 3.15; N, 21.89. Found: C, 45.79; H, 3.36; N, 21.68.

4.5.3. *N*-{3-({2-[(Benzoyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(cyanomethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}-4-chlorobenzamide (4c). Yield 81%; 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, 9H, ArH), 8.24 (s, 2H, NH); MS *m/z* (%) 510 (M^+, 93.3), 485 (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 $C_{22}H_{16}ClN_7O_2S_2$: C, 51.81; H, 3.16; N, 19.23. Found: C, 51.68; H, 3.43; N, 19.52.

4.5.4. 3-{*N*-[4-(4-(4-Chlorobenzoylamino)-5-[(cyanomethyl)sulfanyl]-4H-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]-amino}propanoic acid (4d). Yield 78%; 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, 4H, ArH), 8.25 (s, 1H, NH), 10.84 (br s, 1H, OH); MS *m/z* (%) 478 (M^+, 56.8), 417 (100) 376 (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 $C_{18}H_{16}ClN_7O_3S_2$: C, 45.23; H, 3.37; N, 20.51. Found: C, 45.37; H, 3.56; N, 20.71.

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

A solution of triazole (**2**) (1 mmol), 0.4 g (1 mmol) of sodium hydroxide, and methyl bromoacetate 1.53 g (1 mmol) 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-(benzoylamino)-5-({2-[(2-chloroacetyl)amino]-1,3-thiazol-4-yl} methyl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetate (5a). Yield 82%; 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, CH of thiazole), 7.20–7.46 (m, 4H, ArH), 8.32 (br, 2H, NH); MS *m/z* (%) 515 (M^+, 100), 457 (14), 407 (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 $C_{18}H_{16}Cl_2N_6O_4S_2$: C, 41.95; H, 3.13; N, 16.31. Found: C, 41.68; H, 3.42; N, 16.51.

4.6.2. 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%; 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, CH of thiazole), 7.17–7.38 (m, 4H, ArH), 8.13 (br, 2H, NH); MS *m/z* (%) 481 (M^+, 9), 426 (31), 367 (1), 323 (2), 309 (1), 273 (100), 272 (8); Anal. Calcd for $C_{18}H_{17}ClN_6O_4S_2$: C, 44.95; H, 3.56; N, 17.47. Found: C, 44.75; H, 3.73; N, 17.69.

4.6.3. 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%; 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, CH of thiazole), 6.93–7.51 (m, 9H, ArH), 8.22 (br, 2H, NH); MS *m/z* (%) 543 (M^+, 69.9), 486 (54), 424 (43), 348 (100), 273 (39), 232 (15); Anal. Calcd for $C_{23}H_{19}ClN_6O_4S_2$: C, 50.87; H, 3.53; N, 15.48. Found: C, 50.56; H, 3.61; N, 15.73.

4.6.4. 3-{*N*-[4-(4-(4-Chlorobenzoylamino)-5-[(2-methoxy-2-oxoethyl)sulfanyl]-4H-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino}propanoic acid (5d). Yield 79%; 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, CH of thiazole), 7.25–7.61 (m, 4H, ArH), 8.29 (br, 1H, NH), 11.13 (br, 1H, OH); MS *m/z* (%) 511 (M^+, 100), 479 (67), 409 (41), 372 (58), 331 (16), 270 (60), 217 (44), 194 (27), 107 (48), 84 (81); Anal. Calcd for $C_{19}H_{19}ClN_6O_5S_2$: C, 44.66; H, 3.75; N, 16.45. Found: C, 44.46; H, 3.59; N, 16.24.

4.7. 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}-4-chlorobenzamide

A solution of (**5**) (1 mmol) with 5 mL (1 mmol) 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-[(2-Chloroacetyl)amino]-1,3-thiazol-4-yl}-methyl)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4H-1,2,4-triazol-4-yl}-4-chlorobenzamide (6a). Yield 83%; 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, CH of thiazole), 7.22–7.48 (m, 4H, ArH), 8.21 (br, 2H,

NH); MS *m/z* (%) 515 (M^+, 65), 479 (69), 436 (145), 386 (61), 328 (78), 311 (8.4), 269 (24), 235 (100), 201 (13), 184 (18), 156 (53), 124 (25), 89 (49); Anal. Calcd for $C_{17}H_{16}Cl_2N_8O_3S_2$: C, 39.62; H, 3.13; N, 21.74. Found: C, 39.82; H, 3.34; N, 21.53.

4.7.2. *N*-{3-({2-[(Acetyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(2-hydrazino-2-oxoethyl) sulfanyl]-4H-1,2,4-triazol-4-yl}-4-chlorobenzamide (6b). Yield 82%; 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, CH of thiazole), 7.45–7.71 (m, 4H, ArH), 8.12 (br, 2H, NH); MS *m/z* (%) 481 (M^+, 78), 418 (72), 371 (26.3), 347 (6.3), 265 (18.3), 224 (65.3), 186 (25), 113 (100), 89 (14.3); Anal. Calcd for $C_{17}H_{17}ClN_8O_3S_2$: C, 42.45; H, 3.56; N, 23.30. Found: C, 42.68; H, 3.36; N, 23.27.

4.7.3. *N*-{3-({2-[(Benzoyl)amino]-1,3-thiazol-4-yl}methyl)-5-[(2-hydrazino-2-oxoethyl) sulfanyl]-4H-1,2,4-triazol-4-yl}-4-chlorobenzamide (6c). Yield 71%; 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, CH of thiazole), 7.13–7.68 (m, 9H, ArH), 8.10 (br, 2H, NH); MS *m/z* (%) 543 (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. Calcd for $C_{22}H_{19}ClN_8O_3S_2$: C, 48.66; H, 3.53; N, 20.64. Found: C, 48.71; H, 3.78; N, 20.53.

4.7.4. 3-{*N*-[4-(4-Chlorobenzoylamino)-5-[(2-hydrazino-2-oxoethyl)sulfanyl]-4H-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino}propanoic acid (6d). Yield 84%; 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, CH of thiazole), 7.34–7.61 (m, 4H, ArH), 8.03 (br, 1H, NH), 11.09 (br, 1H, OH); MS *m/z* (%) 511 (M^+, 93.3), 485 (32), 419 (22), 394 (56.4), 316 (28), 247 (64), 217 (100), 147 (83), 108 (71), 79 (10.2); Anal. Calcd for $C_{18}H_{19}ClN_8O_4S_2$: C, 42.31; H, 3.75; N, 21.93. Found: C, 42.57; H, 3.63; N, 21.82.

4.8. 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]-4-chloro benzamide

To a solution of (**6**) (1 mmol) in dichloromethane (excess amount), appropriate acid chloride (1 mmol) 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-[(acetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]-4-chlorobenzamide (7b). Yield 71%; 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, 4H, ArH), 8.18 (s, 2H, NH); MS *m/z* (%) 523 (M^+, 97), 488 (62), 437 (17.2), 389 (9.1), 327 (74), 297 (54), 241 (8.3), 223 (100), 174 (24), 146 (27); Anal. Calcd for $C_{19}H_{19}ClN_8O_4S_2$: C, 43.63; H, 3.66; N, 21.43. Found: C, 43.42; H, 3.89; N, 21.51.

4.8.2. *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]-4-chlorobenzamide (7f). Yield 74%; 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, 9H, ArH), 8.09 (s, 2H, NH); MS *m/z* (%) 584 (M^+, 86), 526 (71), 481 (14), 427 (78), 379 (38), 325 (41), 287 (17), 241 (35), 167 (100), 109 (37), 98 (19); Anal. Calcd for $C_{24}H_{21}ClN_8O_4S_2$: C, 49.27; H, 3.62; N, 19.15. Found: C, 49.51; H, 3.44; N, 19.34.

4.8.3. *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]-4-chlorobenzamide (7j). Yield 57%; 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, 4H, ArH), 8.32 (s, 2H, NH); MS *m/z* (%) 557 (M^+, 54), 507 (36), 467 (84), 419 (54.2), 384 (9.3), 338 (100), 258 (12), 228 (37), 177 (32); Anal. Calcd for $C_{19}H_{18}Cl_2N_8O_4S_2$: C, 40.94; H, 3.25; N, 20.10. Found: C, 40.76; H, 3.42; N, 20.33.

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)-4H-1,2,4-triazol-4-yl]-4-chlorobenzamide

A solution of (**6**) (1 mmol) with benzaldehyde (1 mmol) 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-[(2-chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]-4-chlorobenzamide (8a). Yield 81%; decomposed around 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, 9H, ArH), 8.16 (S, 3H, NH), 8.27 (S, 1H, N=CH); MS *m/z* (%) 603 (M^+, 69), 562 (19), 517 (37), 479 (21), 415 (58), 346 (24), 295 (35), 234 (100),

157 (11.4), 103 (12.3); Anal. Calcd for $C_{24}H_{20}Cl_2N_8O_3S_2$: C, 47.76; H, 3.34; N, 18.57. Found: C, 47.63; H, 3.57; N, 18.74.

4.9.2. *N*-[3-({2-[(2*E*)-2-Benzylidenehydrazino]-2-oxoethyl}sulfanyl)-5-({2-[(acetyl) amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]-4-chlorobenzamide (8b). Yield 83%; 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, 9H, ArH), 8.26 (s, 3H, NH), 8.36 (s, 1H, N=CH); MS *m*/*z* (%) 569 (M^+, 94), 519 (41), 487 (9.6), 431 (26), 413 (8.4), 389 (100), 365 (20); Anal. Calcd for $C_{24}H_{21}ClN_8O_3S_2$: C, 50.66; H, 3.72; N, 19.69. Found: C, 50.54; H, 3.88; N, 19.50.

4.9.3. *N*-[3-({2-[(2*E*)-2-Benzylidenehydrazino]-2-oxoethyl}sulfanyl)-5-({2-[(benzoyl) amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]-4-chlorobenzamide (8c). Yield 81%; decomposed around 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, 14H, ArH), 8.16 (s, 3H, NH), 8.41 (s, 1H, N=CH); MS *m*/*z* (%) 631 (M^+, 79), 571 (25), 515 (52), 463 (63), 418 (9.2), 358 (41), 314 (31), 272 (37), 208 (100), 168 (15.2), 107 (28), 97 (7.4); Anal. Calcd for $C_{29}H_{23}ClN_8O_3S_2$: C, 55.19; H, 3.67; N, 17.75. Found: C, 55.34; H, 3.88; N, 17.53.

4.9.4. 3-{*N*-[4-(4-(4-Chlorobenzoylamino)-5-[(2-oxo-2-2-[(*Z*)-1-phenylmethylidene] hydrazinoethyl)sulfanyl]-4H-1,2,4-triazol-3-ylmethyl)-1,3-thiazol-2-yl]amino} propanoic acid (8d). Yield 78%; 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, 9H, ArH), 8.15 (s, 2H, NH), 8.32 (s, 1H, N=CH), 10.87 (bs, 1H, OH); MS *m*/*z* (%) 599 (M^+, 74), 537 (45), 486 (13), 464 (63), 376 (32), 318 (65), 285 (12), 246 (100), 209 (37), 187 (42); Anal. Calcd for $C_{25}H_{23}ClN_8O_4S_2$: C, 50.12; H, 3.87; N, 18.70. Found: C, 50.36; H, 3.69; N, 18.49.

4.10. 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]-4-chlorobenzamide

The (**6**) (1 mmol) was dissolved in alcoholic potassium hydroxide (1 mmol) and kept for stirring. Carbon disulfide (1.5 mmol) 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 (1 mmol) with hydrazine hydrate (1 mmol) 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, 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-1H-1,2,4-triazol-3-yl)methyl]sulfanyl}-5-({2-[(2-chloroacetyl)amino]-1,3-thiazol-4-yl}methyl)-4H-1,2,4-triazol-4-yl]-4-chlorobenzamide (9a). Yield 72%; 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, 4H, ArH), 8.11 (s, 2H, NH), 12.49 (s, 1H, SH); MS *m*/*z* (%) 571 (M^+, 82), 517 (31), 461 (26), 384 (31), 326 (13.2), 247 (15), 226 (17), 125 (100); Anal. Calcd for $C_{18}H_{16}Cl_2N_{10}O_2S_3$: C, 37.83; H, 2.82; N, 24.51. Found: C, 37.65; H, 2.59; N, 24.74.

4.10.2. *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]-4-chlorobenzamide (9b). Yield 71%; 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, 4H, ArH), 8.08 (s, 2H, NH), 12.43 (s, 1H, SH); MS *m*/*z* (%) 537 (M^+, 76), 474 (32), 419 (30), 385 (100), 323 (2.93), 272 (12.26), 220 (3.73), 207 (5.86), 192 (7.07); Anal. Calcd for $C_{18}H_{17}ClN_{10}O_2S_3$: C, 40.26; H, 3.19; N, 26.08. Found: C, 40.53; H, 3.46; N, 26.34.

4.10.3. *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]-4-chlorobenzamide (9c). Yield 80%; 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, 9H, ArH), 8.17 (s, 2H, NH), 12.63 (s, 1H, SH); MS *m*/*z* (%) 599 (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. Calcd for $C_{23}H_{19}ClN_{10}O_2S_3$: C, 46.11; H, 3.20; N, 23.38. Found: C, 46.34; H, 3.46; N, 23.51.

4.10.4. 3-[(4-[5-[(4-Amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)methyl]sulfanyl-4-(4-chlorobenzoylamino)-4H-1,2,4-triazol-3-yl]methyl-1,3-thiazol-2-yl)amino]propanoic acid (9d). Yield 77%; 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, 4H, ArH), 8.17 (s, 1H, NH), 10.64 (br s, 1H, OH), 12.35 (s, 1H, SH); MS *m*/*z* (%) 567 (M^+, 84), 497 (23), 450 (47), 438 (38), 371 (25), 370 (75), 354 (10), 235 (100), 220 (22), 207 (68), 192 (70); Anal. Calcd for $C_{19}H_{19}ClN_{10}O_3S_3$: C, 40.24; H, 3.38; N, 24.70. Found: C, 40.41; H, 3.63; N, 24.84.

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