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Original article

Studying synergism of methyl linked cyclohexyl thiophenes with triazole: Synthesis and their cdk5/p25 inhibition activity

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ABSTRACT

A novel clubbed triazolyl thiophene 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 to 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 that thiophene 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 thiophene (IC50 = 46 ± 2 nM).

In recent years, environmentally benign synthetic methods have received considerable attention and solvent-free protocols are reported. 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.

2. Result and discussion

2.1. Chemistry

Compounds **1A–D**, **2A–D**, **3A–L**, **4A–L**, **5A–L**, **6A–L**, **7A–L**, **8AA–8AJ**, **8M–8Z**, **9A–L** and **10A–L** were synthesized using reported methods [4,8,9,14–27]. Compounds **1A–D** was converted to thiocarbazate salts by treatment with carbon disulphide and potassium hydroxide, which on treatment with hydrazine hydrate gave **2A–D**. Compounds **2A–D** was treated with 4–chlorobenzoyl chloride at 0 °C to yield **3A–L** (Scheme 1). The transformed compounds **3A–L** on treatment with diiodomethane in the presence of strong alkali i.e. sodium hydroxide gave **4A–L** (Scheme 2). Title compounds **3A–L** were treated with chloroacetonitrile, which

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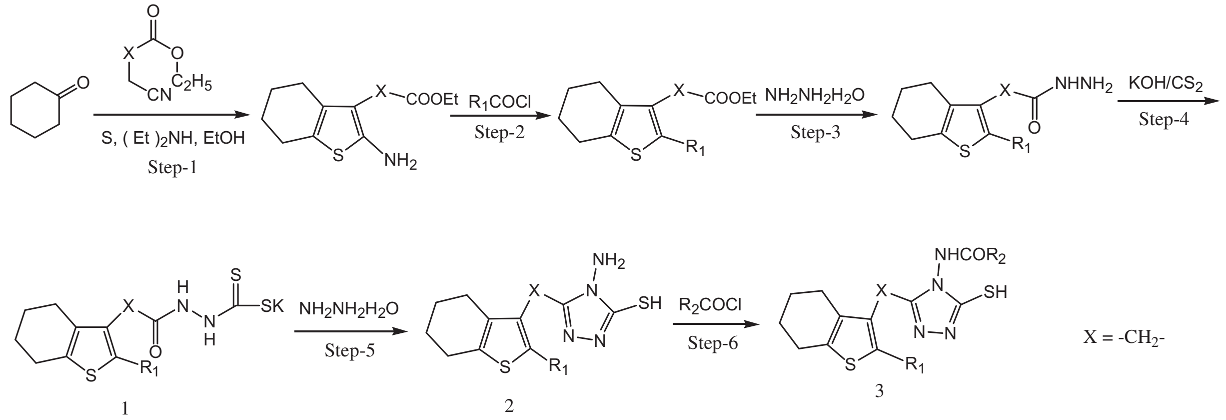


Scheme 1. Synthesis of lead compound/s **3** [4,8,9,14–27].

on neutralization with sodium carbonate gave a precipitates of compounds **5A**–**L** (Scheme 3). Compounds **3A**–**L**, when treated with methyl bromoacetate in basic condition produced **6A**–**L**. Chemical transformation of **6A**–**L** to **7A**–**L** was achieved by treatment it with hydrazine hydrate (Scheme 4). While compounds **7A**–**L**, on treatment with appropriate acid chlorides, furnished **8A**–**L**. Schiff bases, the condensation products of **9A**–**L**, were synthesized by treating **7A**–**L** with benzaldehyde and confirmed by absence of triplet of NH of hydrate. Compounds **7A**–**L** were converted to thiocarbazate salts by treatment with carbon disulphide and potassium hydroxide, which on treatment with hydrazine hydrate gave **10A**–**L** (Scheme 5). The NMR spectra confirmed formation of triazole derivative from hydrazide, which shows presence of sulfhydryl proton at 3.1.

2.2. Cyclic-dependent kinase 5/p25 inhibiting activity

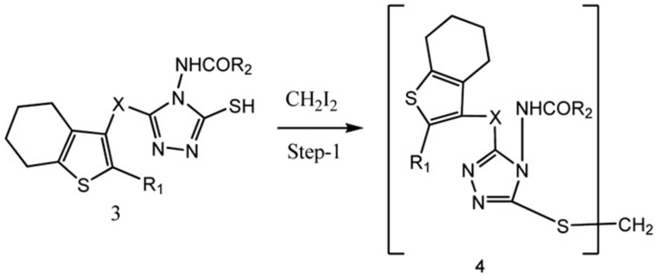
Kinase inhibition was measured by use of scintillation proximity assays (SPA). The results of the assays are reported in Tables 1–4. During the preliminary screening compound **2A** has emerged as hit cdk5/p25 (IC50 = 043 ± 02 nM), with good potency and more opportunities for chemical transformation for the optimization. Testing of **2A** against other cdk's revealed that **2A** was essentially equipotent at inhibiting cdk2/cyclin E (IC50 = 52 ± 5 nM), a cancer target. Thus with an objective to improve cdk5 potency and minimize cdk2 activity, certain chemical modification have been performed. Variation of amine side chain of **2A** with MAOS allowed us to rapidly explore the first arm of the parmacophore. As a first step towards lead optimization amino group was protected to the corresponding compounds **3A**–**L** however, all of these modifications were resulted in a substantial decrease in activity. The next structural modification made was a dimeric product of **4A**–**L** but these changes were also resulted in a substantial loss of biological activity.

s-Alkylation with acetonitrile provided the first analogs **5A** and **5I** that demonstrated with excellent activity, while others exhibited moderate to poor activity. Thus it was decided to modify the structure as SH group. In order to optimize the sulfhydryl component, compounds **6A**–**L** were synthesized and investigated, which revealed loss of activity. A further modification of compounds **6A**–**L** produced compounds **7A**–**L**. The results of the cdk5/p25 inhibitory activity are quite interesting as **7A**–**D** have shown impressive percentage of inhibition. Compounds **7A**–**L** was selected for further studies as it has a free amino group, which opened an area for further modification at this point. Compounds **8A**–**AJ** was obtained by treatment with acid chlorides which ultimately showed decreased activity. Furthermore, compounds **7A**–**L** were converted to Schiff bases with benzaldehyde, and on investigation, **9A**–**E** have shown promising activity while other remained inactive. Compounds **10A**–**L** were found to be inactive.

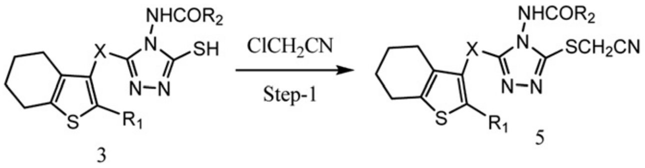
Attention was then turned to optimization of the **9A**–**E** in order to gain the selectivity over cdk2. On comparing, **9E** afforded improved cdk5 potency that is >17.5 -fold selectivity versus cdk2. The compound **9A** was equally selective versus cdk2 and had slightly improved cdk5 IC50. Other derivatives were had noticeably decreased cdk5 activity.

3. Conclusion

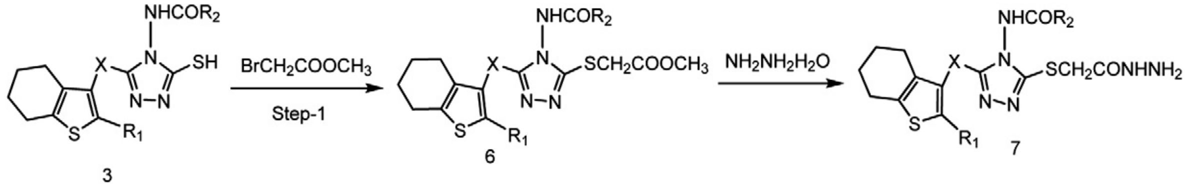
In conclusion, a novel series of clubbed triazolyl thiophene derivatives that inhibit cdk5/p25 has been discovered. It was found that the potency of the screening hit **2A** could be enhanced first by structural transformation to a 2-position of thiophene core and amino and sulfhydryl groups in triazole core and subsequently by the introduction of appropriate constituents on both the heterocyclic rings leading to the most promising compounds **9A** and **9B**. 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.



Scheme 2. Synthesis of Bis derivatives **4**.



Scheme 3. Synthesis of cyano analogs **5**.



Scheme 4. Synthesis of hydrazides **7**.

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 CDCl3 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, d, t, ds, dd, m, and br s. 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 ±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′-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3 yl]-hydrazinecarbodithioic potassium salt (**1A**), N′-[2-(2-Acetylamino-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl)-acetyl]-hydrazinecarbodithioic potassium salt (**1B**), N′-[2-(2-benzoyl-amino-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl)-acetyl]-hydrazine-carbodithioic potassium salt (**1C**), 3-{3-[2-(N′-dithiocarboxy-hydrazino)-2-oxo-ethyl]-4,5,6,7-tetrahydro-benzo[b]thiophen-2-ylamino}-propionic potassium salt (**1D**)*

Above titled compound were prepared as per the reported method [28–33,35].

*4.1.1.1. N′-[2-(2-Chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl]-hydrazine carbodithioic potassium salt (**1A**).* Yield 86%; mp 213–215 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43–1.59 (m, 4H, cyclohexane), 2.45–2.51 (t, 4H, cyclohexane CH_2, $J = 4.4$ Hz), 3.41 (s, 2H, CH_2), 4.19 (s, 2H, CH_2Cl), 4.25–4.50 (dd, 2H, $J_{NH-NH} = 4.21$, $J_{NH-NH} = 4.52$), 8.03 (s, 2H, NH); MS m/z (%) 415 (M^+, 55), 376 (32.4), 341 (53.7), 250 (100), 208 (12.8), 165 (7.2), 151 (31.6), 99 (27), 85 (15.6); Anal. Calcd. for $C_{13}H_{15}ClKN_3O_2S_3$:C, 37.53; H, 3.63; N, 10.10. Found: C, 37.41; H, 3.52; N, 10.05.

*4.1.2. Preparation of N-[3-(4-Amino-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl)-4,5,6,7-tetrahydro-benzo(b)thiophen-2-yl]-2-chloro-acetamide (**2A**), N-[3-(4-Amino-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl)-4,5,6,7-tetrahydro-benzo(b)thiophen-2-yl]-2-acetamide (**2B**), N-[3-(4-Amino-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl)-4,5,6,7-tetrahydro-benzo(b)thiophen-2-yl]-2- benzamide (**2C**), N-[3-(4-Amino-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl)-4,5,6,7-tetrahydrobenzo(b)thiophen-2-yl]-2- propionic acid (**2D**)*

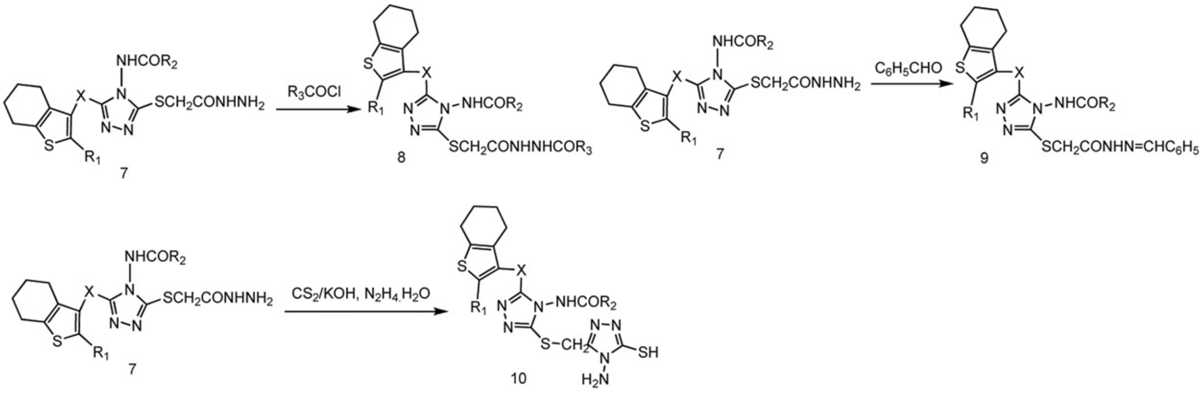
Above titled compound were prepared using cited methods [31,32,35].

*4.1.2.1. N-[3-(4-Amino-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl)-4,5,6,7-tetrahydro-benzo (b) thiophen-2-yl]-2-chloro-acetamide (**2A**).* Yield 76%; mp 254–256 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53–1.68 (m, 4H, cyclohexane), 2.02 (s, 2H, NH_2), 2.20–2.42 (t, 4H, cyclohexane CH_2, $J = 4.9$ Hz), 3.2 (s, 1H, SH), 3.76 (s, 2H, CH_2), 4.15 (s, 2H, CH_2Cl), 8.02 (s, 1H, NH); MS m/z (%) 358 (M^+, 100), 323 (67.8), 290 (19.2), 248 (44.8), 192 (10.1), 177 (23.6); Anal. Calcd. for $C_{13}H_{16}ClN_5OS_2$: C, 43.63; H, 4.51; N, 19.57. Found: C, 43.76; H, 4.47; N, 19.72.

4.1.3. General preparation of 7-chloro-hepta-2,4,6-triynoic acid -{3-[2-(2-substituted-amino) 4, 5, 6, 7-tetrahydro-benzo[b] thiophen-3-ylmethyl]-5-mercapto-[1,2,4] triazol-4-yl}-amide [30,31,35]

The triazole (**2**) (1 mmol) in 20 ml of 10% NaOH was treated drop wise 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.

*4.1.3.1. 7-Chloro-hepta-2,4,6-triynoic acid -{3-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-5-mercapto-[1,2,4]triazol-4-yl}-amide (**3A**).* Yield 71%; mp 284–286 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14–1.61 (m, 4H, cyclohexane), 2.25–2.58 (t, 4H, cyclohexane CH_2, $J = 4.8$ Hz), 3.1 (s, 1H, SH), 3.72 (s, 2H, CH_2), 4.15 (s, 2H, CH_2Cl), 7.12–7.36 (m, 4H, ArH), 8.06 (s, 2H, NH); MS m/z (%) 496 (M^+, 100), 494 (80), 457 (52), 373 (33.8), 296 (17), 268 (9.8),



Scheme 5. Synthesis of triazole-amides **8**, Schiff bases **9** and Triazolo-*s*-triazole **10**.

Table 1
cdk5 IC_{50} values of the compound 1A–5L.

Sr. No.	R1	R2	R3	Cdk5 IC_{50} (nM)[a]
1A	$NHCOCH_2Cl$	–	–	247 ± 37
1B	$NHCOCH_3$	–	–	346 ± 41
1C	$NHCOC_6H_5$	–	–	373 ± 39
1D	$NHCH_2CH_2COOH$	–	–	397 ± 35
2A	$NHCOCH_2Cl$	–	–	043 ± 02
2B	$NHCOCH_3$	–	–	386 ± 85
2C	$NHCOC_6H_5$	–	–	621 ± 23
2D	$NHCH_2CH_2COOH$	–	–	268 ± 31
3A	$NHCOCH_2Cl$	$-4-ClC_6H_4$	–	343 ± 12
3B	$NHCOCH_3$	$-4-ClC_6H_4$	–	460 ± 71
3C	$NHCOC_6H_5$	$-4-ClC_6H_4$	–	183 ± 11
3D	$NHCH_2CH_2COOH$	$-4-ClC_6H_4$	–	457 ± 43
3E	$NHCOCH_2Cl$	$-C_6H_5$	–	751 ± 74
3F	$NHCOCH_3$	$-C_6H_5$	–	572 ± 63
3G	$NHCOC_6H_5$	$-C_6H_5$	–	891 ± 112
3H	$NHCH_2CH_2COOH$	$-C_6H_5$	–	674 ± 67
3I	$NHCOCH_2Cl$	$-CH_3$	–	689 ± 82
3J	$NHCOCH_3$	$-CH_3$	–	584 ± 47
3K	$NHCOC_6H_5$	$-CH_3$	–	618 ± 46
3L	$NHCH_2CH_2COOH$	$-CH_3$	–	433 ± 24
4A	$NHCOCH_2Cl$	$-4-ClC_6H_4$	–	340 ± 22
4B	$NHCOCH_3$	$-4-ClC_6H_4$	–	367 ± 21
4C	$NHCOC_6H_5$	$-4-ClC_6H_4$	–	627 ± 39
4D	$NHCH_2CH_2COOH$	$-4-ClC_6H_4$	–	164 ± 11
4E	$NHCOCH_2Cl$	$-C_6H_5$	–	669 ± 42
4F	$NHCOCH_3$	$-C_6H_5$	–	646 ± 83
4G	$NHCOC_6H_5$	$-C_6H_5$	–	695 ± 47
4H	$NHCH_2CH_2COOH$	$-C_6H_5$	–	577 ± 38
4I	$NHCOCH_2Cl$	$-CH_3$	–	849 ± 85
4J	$NHCOCH_3$	$-CH_3$	–	839 ± 56
4K	$NHCOC_6H_5$	$-CH_3$	–	766 ± 81
4L	$NHCH_2CH_2COOH$	$-CH_3$	–	734 ± 36
5A	$NHCOCH_2Cl$	$-4-ClC_6H_4$	–	058 ± 12
5B	$NHCOCH_3$	$-4-ClC_6H_4$	–	415 ± 75
5C	$NHCOC_6H_5$	$-4-ClC_6H_4$	–	551 ± 72
5D	$NHCH_2CH_2COOH$	$-4-ClC_6H_4$	–	422 ± 12
5E	$NHCOCH_2Cl$	$-C_6H_5$	–	657 ± 64
5F	$NHCOCH_3$	$-C_6H_5$	–	393 ± 61
5G	$NHCOC_6H_5$	$-C_6H_5$	–	486 ± 78
5H	$NHCH_2CH_2COOH$	$-C_6H_5$	–	4874 ± 86
5I	$NHCOCH_2Cl$	$-CH_3$	–	061 ± 06
5J	$NHCOCH_3$	$-CH_3$	–	458 ± 84
5K	$NHCOC_6H_5$	$-CH_3$	–	564 ± 64
5L	$NHCH_2CH_2COOH$	$-CH_3$	–	641 ± 74

[a] Inhibitory concentration; The IC50 for Roscovitine (Positive control) was observed as 280 ± 41.

280 (14.7), 289 (29.8), 246 (9.3), 220 (10.8), 164 (10.1), 98 (15.1), 85 (5.3); Anal. Calcd. for $C_{20}H_{19}Cl_2N_5O_2S_2$: C, 48.39; H, 3.86; N, 14.11. Found: C, 48.55; H, 3.98; N, 14.03.

*4.1.3.2. N-{3-[2-(2-Chloro-acetylamino)-4, 5, 6, 7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-5-mercapto-[1,2,4] triazol-4-yl}-benzamide (**3E**).* Yield 69%; mp 275–277 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.42–1.67 (m, 4H, cyclohexane), 2.42–2.69 (t, 4H, cyclohexane CH_2, $J = 4.4$ Hz), 3.1 (s, 1H, SH), 3.84 (s, 2H, CH_2), 4.08 (s, 2H, CH_2Cl), 7.11–7.68 (m, 5H, ArH), 8.05 (s, 2H, NH); MS *m/z* (%) 462 (M^+, 68), 427 (100), 426 (57), 378 (43.6), 301 (14), 285 (10.1), 246 (28.3), 220 (34.6), 164 (20.3), 98 (9), 85 (12.3); Anal. Calcd. for $C_{20}H_{20}ClN_5O_2S_2$: C, 52.00; H, 4.36; N, 15.16. Found: C, 52.13; H, 4.50; N, 15.20.

*4.1.3.3. N-[3-(4-Acetylamino-5-mercapto-4H-[1,2,4] triazol-3-ylmethyl)-4, 5, 6, 7-tetrahydro-benzo[b]thiophen-2-yl]-2-chloro-acetamide (**3I**).* Yield 77%; mp 282–284 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.38–1.61 (m, 4H, cyclohexane), 2.08 (s, 3H, CH_3), 2.48–2.56 (t,

Table 2
cdk5 IC_{50} values of the compound 6A–8Z.

Sr. No.	R1	R2	R3	Cdk5 IC_{50} (nM)[a]
6A	$NHCOCH_2Cl$	$-4-ClC_6H_4$	–	443 ± 106
6B	$NHCOCH_3$	$-4-ClC_6H_4$	–	389 ± 35
6C	$NHCOC_6H_5$	$-4-ClC_6H_4$	–	293 ± 38
6D	$NHCH_2CH_2COOH$	$-4-ClC_6H_4$	–	541 ± 18
6E	$NHCOCH_2Cl$	$-C_6H_5$	–	681 ± 47
6F	$NHCOCH_3$	$-C_6H_5$	–	578 ± 74
6G	$NHCOC_6H_5$	$-C_6H_5$	–	353 ± 71
6H	$NHCH_2CH_2COOH$	$-C_6H_5$	–	641 ± 119
6I	$NHCOCH_2Cl$	$-CH_3$	–	695 ± 112
6J	$NHCOCH_3$	$-CH_3$	–	389 ± 97
6K	$NHCOC_6H_5$	$-CH_3$	–	567 ± 103
6L	$NHCH_2CH_2COOH$	$-CH_3$	–	733 ± 137
7A	$NHCOCH_2Cl$	$-4-ClC_6H_4$	–	039 ± 02
7B	$NHCOCH_3$	$-4-ClC_6H_4$	–	064 ± 02
7C	$NHCOC_6H_5$	$-4-ClC_6H_4$	–	236 ± 34
7D	$NHCH_2CH_2COOH$	$-4-ClC_6H_4$	–	201 ± 31
7E	$NHCOCH_2Cl$	$-C_6H_5$	–	**037 ± 01**
7F	$NHCOCH_3$	$-C_6H_5$	–	192 ± 47
7G	$NHCOC_6H_5$	$-C_6H_5$	–	264 ± 72
7H	$NHCH_2CH_2COOH$	$-C_6H_5$	–	455 ± 79
7I	$NHCOCH_2Cl$	$-CH_3$	–	061 ± 01
7J	$NHCOCH_3$	$-CH_3$	–	249 ± 36
7K	$NHCOC_6H_5$	$-CH_3$	–	180 ± 27
7L	$NHCH_2CH_2COOH$	$-CH_3$	–	931 ± 24
8A	$NHCOCH_2Cl$	$-4-ClC_6H_4$	$-CH_3$	468 ± 64
8AA	$NHCOC_6H_5$	$-CH_3$	$-CH_3$	639 ± 67
8AB	$NHCH_2CH_2COOH$	$-CH_3$	$-CH_3$	366 ± 48
8AC	$NHCOCH_2Cl$	$-CH_3$	$-C_6H_5$	363 ± 34
8AD	$NHCOCH_3$	$-CH_3$	$-C_6H_5$	436 ± 46
8AE	$NHCOC_6H_5$	$-CH_3$	$-C_6H_5$	411 ± 83
8AF	$NHCH_2CH_2COOH$	$-CH_3$	$-C_6H_5$	476 ± 68
8AG	$NHCOCH_2Cl$	$-CH_3$	CH_2Cl	452 ± 67
8AH	$NHCOCH_3$	$-CH_3$	CH_2Cl	474 ± 74
8AI	$NHCOC_6H_5$	$-CH_3$	CH_2Cl	358 ± 72
8AJ	$NHCH_2CH_2COOH$	$-CH_3$	CH_2Cl	265 ± 38
8B	$NHCOCH_3$	$-4-ClC_6H_4$	$-CH_3$	641 ± 49
8C	$NHCOC_6H_5$	$-4-ClC_6H_4$	$-CH_3$	318 ± 30
8D	$NHCH_2CH_2COOH$	$-4-ClC_6H_4$	$-CH_3$	352 ± 28
8E	$NHCOCH_2Cl$	$-4-ClC_6H_4$	$-C_6H_5$	617 ± 23
8F	$NHCOCH_3$	$-4-ClC_6H_4$	$-C_6H_5$	525 ± 47
8G	$NHCOC_6H_5$	$-4-ClC_6H_4$	$-C_6H_5$	483 ± 41
8H	$NHCH_2CH_2COOH$	$-4-ClC_6H_4$	$-C_6H_5$	271 ± 17
8I	$NHCOCH_2Cl$	$-4-ClC_6H_4$	CH_2Cl	229 ± 67
8J	$NHCOCH_3$	$-4-ClC_6H_4$	CH_2Cl	260 ± 38
8K	$NHCOC_6H_5$	$-4-ClC_6H_4$	CH_2Cl	340 ± 22
8L	$NHCH_2CH_2COOH$	$-4-ClC_6H_4$	CH_2Cl	185 ± 39
8M	$NHCOCH_2Cl$	$-C_6H_5$	$-CH_3$	350 ± 61
8N	$NHCOCH_3$	$-C_6H_5$	$-CH_3$	548 ± 46
8O	$NHCOC_6H_5$	$-C_6H_5$	$-CH_3$	564 ± 77
8P	$NHCH_2CH_2COOH$	$-C_6H_5$	$-CH_3$	467 ± 48
8Q	$NHCOCH_2Cl$	$-C_6H_5$	$-C_6H_5$	468 ± 92
8R	$NHCOCH_3$	$-C_6H_5$	$-C_6H_5$	413 ± 45
8S	$NHCOC_6H_5$	$-C_6H_5$	$-C_6H_5$	466 ± 73
8T	$NHCH_2CH_2COOH$	$-C_6H_5$	$-C_6H_5$	434 ± 80
8U	$NHCOCH_2Cl$	$-C_6H_5$	CH_2Cl	532 ± 57
8V	$NHCOCH_3$	$-C_6H_5$	CH_2Cl	643 ± 74
8W	$NHCOC_6H_5$	$-C_6H_5$	CH_2Cl	547 ± 74
8X	$NHCH_2CH_2COOH$	$-C_6H_5$	CH_2Cl	362 ± 35
8Y	$NHCOCH_2Cl$	$-CH_3$	$-CH_3$	454 ± 49
8Z	$NHCOCH_3$	$-CH_3$	$-CH_3$	327 ± 48

[a] Inhibitory concentration; The IC50 for Roscovitine (Positive control) was observed as 280 ± 41.

Table 3
cdk5 IC_{50} values of the compound **9A**–**10L**.

Sr. No.	R1	R2	R3	Cdk5 IC_{50} (nM)[a]	Sr. No.	R1	R2	R3	Cdk5 IC_{50} (nM)[a]
9A	$NHCOCH_2Cl$	$-4\text{-}ClC_6H_4$	–	035 ± 01	**10A**	$NHCOCH_2Cl$	$-4\text{-}ClC_6H_4$	–	2860 ± 116
9B	$NHCOCH_3$	$-4\text{-}ClC_6H_4$	–	213 ± 76	**10B**	$NHCOCH_3$	$-4\text{-}ClC_6H_4$	–	6380 ± 104
9C	$NHCOC_6H_5$	$-4\text{-}ClC_6H_4$	–	217 ± 23	**10C**	$NHCOC_6H_5$	$-4\text{-}ClC_6H_4$	–	3280 ± 217
9D	$NHCH_2CH_2COOH$	$-4\text{-}ClC_6H_4$	–	258 ± 37	**10D**	$NHCH_2CH_2COOH$	$-4\text{-}ClC_6H_4$	–	5850 ± 158
9E	$NHCOCH_2Cl$	$-C_6H_5$	–	032 ± 1	**10E**	$NHCOCH_2Cl$	$-C_6H_5$	–	6185 ± 144
9F	$NHCOCH_3$	$-C_6H_5$	–	385 ± 28	**10F**	$NHCOCH_3$	$-C_6H_5$	–	6437 ± 149
9G	$NHCOC_6H_5$	$-C_6H_5$	–	435 ± 52	**10G**	$NHCOC_6H_5$	$-C_6H_5$	–	4587 ± 179
9H	$NHCH_2CH_2COOH$	$-C_6H_5$	–	267 ± 52	**10H**	$NHCH_2CH_2COOH$	$-C_6H_5$	–	4985 ± 146
9I	$NHCOCH_2Cl$	$-CH_3$	–	336 ± 48	**10I**	$NHCOCH_2Cl$	$-CH_3$	–	4035 ± 186
9J	$NHCOCH_3$	$-CH_3$	–	453 ± 75	**10J**	$NHCOCH_3$	$-CH_3$	–	5146 ± 230
9K	$NHCOC_6H_5$	$-CH_3$	–	467 ± 44	**10K**	$NHCOC_6H_5$	$-CH_3$	–	6142 ± 201
9L	$NHCH_2CH_2COOH$	$-CH_3$	–	535 ± 52	**10L**	$NHCH_2CH_2COOH$	$-CH_3$	–	6212 ± 251

[a] Inhibitory concentration; The IC50 for Roscovitine (Positive control) was observed as 280 ± 41.

4H, cyclohexane CH_2, $J = 4.6$ Hz), 3.01 (s, 1H, SH), 3.78 (s, 2H, CH_2), 4.23 (s, 2H, CH_2Cl), 8.08 (s, 2H, NH); MS *m/z* (%) 400 (M^+, 71), 365 (73), 316 (54.6), 301 (34.2), 273 (100), 272 (10.4), 246 (25.9), 218 (18.5), 164 (5.8), 98 (42); Anal. Calcd. for $C_{15}H_{18}ClN_5O_2S_2$: C, 45.05; H, 4.54; N, 17.51. Found: C, 45.16; H, 4.34; N, 17.69.

4.1.4. General preparation of N,N′-(methylenebis{sulfanedial-5{3-[(2- substituted-amino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]}-4H-[1,2,4]triazole-3,4-dial})di-4-chloro benzamide [7,35]

The triazole (**3**) (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 vacuos, 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.

*4.1.4.1. N,N′-(Methylenebis {sulfanedial-5{3-[(2-chloroacetylamino)-4, 5, 6, 7-tetrahydro-benzo[b]thiophen-3-ylmethyl]}-4H-[1,2,4] triazole-3,4-dial}) di-4-chlorobenzamide (**4A**).* Yield 84%; mp 266–268 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.31–1.60 (m, 8H, cyclohexane), 2.02–2.24 (t, 8H, cyclohexane CH_2, $J = 4.5$ Hz), 3.81 (s, 4H, CH_2), 4.18 (s, 4H, CH_2Cl), 4.62 (s, 2H, CH_2), 7.15–7.36 (m, 8H, ArH), 8.04 (s, 4H, NH); Anal. Calcd. for $C_{41}H_{38}Cl_4N_{10}O_4S_4$: C, 49.00; H, 3.81; N, 13.94. Found: C, 49.11; H, 3.75; N, 13.99.

*4.1.4.2. N,N′-(Methylenebis{sulfanedial-5{3-[(2-chloro-acetylamino)-4,5,6,7-tetrahydro – benzo [b]thiophen-3-ylmethyl]}-4H-[1,2,4]triazole-3,4-dial})di-benzamide (**4E**).* Yield 79%; mp 278–280 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.20–1.51 (m, 8H, cyclohexane), 2.21–2.39 (t, 8H, cyclohexane CH_2, $J = 4.7$ Hz), 3.61 (s, 4H, CH_2), 4.15 (s, 4H, CH_2Cl), 4.35 (s, 4H, SCH_2), 7.18–7.55 (m, 10H, ArH), 8.05 (s, 4H, NH); Anal. Calcd. for $C_{41}H_{40}Cl_2N_{10}O_4S_4$: C, 52.61; H, 4.31; N, 14.96. Found: C, 52.58; H, 4.41; N, 14.82.

Table 4
Selectivity ratio of most active compounds.

Compound	Cdk5 IC_{50} (nm)[a]	Cdk2 IC_{50} (nm)[a]	Select k2/k5
2A	43 ± 2	52 ± 5	1.2
5A	58 ± 12	1136 ± 154	19.6
5I	61 ± 6	1319 ± 89	21.6
7A	39 ± 2	799 ± 58	20.5
7B	64 ± 2	587 ± 48	9.2
7E	37 ± 1	487 ± 68	13.2
7I	61 ± 1	5986 ± 132	98.1
9A	35 ± 1	48 ± 7	1.4
9E	32 ± 1	560 ± 21	17.5

[a] Inhibitory concentration.

*4.1.4.3. N,N′-(Methylenebis{sulfanedial-5{3-[(2-chloro-acetylamino)-4,5,6,7-tetrahydro- benzo [b]thiophen-3-ylmethyl]}-4H-[1,2,4]triazole-3,4-dial})di-acetamide (**4I**).* Yield 89%; mp 259–261 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.24–1.59 (m, 8H, cyclohexane), 2.08 (s, 6H, $COCH_3$), 2.44–2.56 (t, 8H, cyclohexane CH_2, $J = 4.3$ Hz), 3.61 (s, 4H, CH_2), 4.14 (s, 4H, CH_2Cl), 4.51 (s, 4H, SCH_2), 7.98 (s, 4H, NH); Anal. Calcd. for $C_{31}H_{36}Cl_2N_{10}O_4S_4$: C, 45.86; H, 4.47; N, 17.25. Found: C, 45.78; H, 4.59; N, 17.18.

4.1.5. General preparation of 7-chloro-hepta-2, 4, 6-triynoic acid {3-[2-(2- substituted-amino)-4, 5, 6, 7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-5-cyanomethylsulfanyl-[1,2,4] triazol-4-yl}-amide [34,35]

The triazole (**3**) (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.

*4.1.5.1. 7-Chloro-hepta-2, 4, 6-triynoic acid {3-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-5-cyanomethylsulfanyl-[1,2,4] triazol-4-yl}-amide (**5A**).* Yield 86%; mp 245–247 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.25–1.49 (m, 4H, cyclohexane), 2.51–2.68 (t, 4H, cyclohexane CH_2, $J = 4.8$ Hz), 3.81 (s, 2H, CH_2), 4.12 (s, 2H, CH_2CN), 4.42 (s, 2H, CH_2Cl), 7.17–7.41 (m, 4H, ArH), 8.0 (s, 2H, NH); MS *m/z* (%) 535 (M^+, 100), 480 (21.9), 421 (20.6), 324 (34.2), 310 (40.8), 254 (17.9), 83 (28.9), 69 (16.1), 55 (11.3); Anal. Calcd. for $C_{22}H_{20}Cl_2N_6O_2S_2$: C, 49.35; H, 3.76; N, 15.69. Found: C, 49.23; H, 3.64; N, 15.81.

*4.1.5.2. N-{3-[2-(2-Chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-5-cyanomethylsulfanyl-[1,2,4] triazol-4-yl}-benzamide (**5E**).* Yield 82%; mp 268–270 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.38–1.55 (m, 4H, cyclohexane), 2.44–2.62 (t, 4H, cyclohexane CH_2, $J = 4.4$ Hz), 3.68 (s, 2H, CH_2), 4.09 (s, 2H, CH_2CN), 4.22 (s, 2H, CH_2Cl), 7.13–7.56 (m, 5H, ArH), 8.04 (s, 2H, NH); MS *m/z* (%) 501 (M^+, 54), 484 (36), 425 (100), 324 (9.3), 310 (12), 254 (28), 161 (32), 83 (40), 69 (7.9), 55 (14.6); Anal. Calcd. for $C_{22}H_{21}ClN_6O_2S_2$: C, 52.74; H, 4.22; N, 16.77. Found: C, 52.67; H, 4.31; N, 16.68.

*4.1.5.3. N-[3-(4-Acetylamino-5-cyanomethylsulfanyl-4H-[1,2,4] triazol-3-ylmethyl)-4, 5, 6, 7-tetrahydro-benzo[b]thiophen-2-yl]-2-chloro-acetamide (**5I**).* Yield 81%; mp 271–273 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.34–1.52 (m, 4H, cyclohexane), 2.06 (s, 3H, $COCH_3$), 2.44–2.62 (t, 4H, cyclohexane CH_2, $J = 4.2$ Hz), 3.72 (s, 2H, CH_2), 3.81 (s, 2H, CH_2CN), 4.22 (s, 2H, CH_2Cl), 8.04 (s, 2H, NH); MS *m/z* (%) 439 (M^+, 49), 422 (79), 363 (19), 328 (62.3), 287 (53.4), 272 (100), 258 (19.5), 216 (12.4), 160 (6.8), 83 (29), 69 (9.7), 55 (13); Anal. Calcd. for $C_{17}H_{19}ClN_6O_2S_2$: C, 46.52; H, 4.36; N, 19.15. Found: C, 46.58; H, 4.28; N, 19.07.

4.1.6. General preparation of [5-[2-(2-substituted-amino)-4, 5, 6, 7-tetrahydro-benzo[b] thiophen-3-ylmethyl]-4-(7-chloro-hepta-2, 4, 6-triynoylamino)-4H-[1,2,4] triazol-3-ylsulfanyl]-acetic acid methyl ester [7,32–35]

A solution of triazole (**3**) (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.

*4.1.6.1. [5-[2-(2-Chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b] thiophen-3-ylmethyl]-4-(7-chloro-hepta-2,4,6-triynoylamino)-4H-[1,2,4] triazol-3-ylsulfanyl]-acetic acid methyl ester (**6A**).* Yield 82%; mp 279–281 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41–1.68 (m, 4H, cyclohexane), 2.40–2.61 (t, 4H, cyclohexane CH_2, J = 4.1 Hz), 3.56 (s, 3H, OCH_3), 3.8 (s, 2H, CH_2), 3.9 (s, 2H, SCH_2), 4.28 (s, 2H, CH_2Cl), 7.20–7.46 (m, 4H, ArH), 8.09 (broad, 2H, NH); MS *m/z* (%) 568 (M^+,100), 509 (14), 459 (12.3), 437 (11.3), 423 (7.2), 366 (7.7), 329 (79), 328 (10), 314 (19.5), 286 (7.9), 271 (31.6), 222 (10.5), 219 (5.7); Anal. Calcd. for $C_{23}H_{23}Cl_2N_5O_4S_2$: C, 48.59; H, 4.08; N, 12.32. Found: C, 48.47; H, 4.22; N, 12.16.

*4.1.6.2. {4-Benzoylamino -5-[2-(2-chloro-acetylamino)-4, 5, 6, 7 -tetrahydro-benzo [b] thiophen-3-ylmethyl]-4H-[1,2,4]triazol -3 -ylsulfanyl}-acetic acid methyl ester (**6E**).* Yield 75%; mp 253–255 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61–1.84 (m, 4H, cyclohexane), 2.38–2.51 (t, 4H, cyclohexane CH_2, J = 4.8 Hz), 3.53 (s, 3H, OCH_3), 3.61 (s, 2H, CH_2), 3.83 (s, 2H, SCH_2), 4.02 (s, 2H, CH_2Cl), 6.73–7.22 (m, 5H, ArH), 8.01 (broad, 2H, NH); MS *m/z* (%) 534 (M^+, 100), 476 (21.9), 462 (24.6), 440 (34.2), 426 (39.8), 369 (17.9), 332 (11.3), 288 (5.8), 273 (4), 219 (3), 83 (5.9); Anal. Calcd. for $C_{23}H_{24}ClN_5O_4S_2$: C, 51.73; H, 4.53; N, 13.11. Found: C, 51.63; H, 4.44; N, 13.01.

*4.1.6.3. {4-Acetylamino-5-[2-(2-chloro-acetylamino)-4, 5, 6, 7-tetrahydro-benzo [b] thiophen-3-ylmethyl]-4H-[1,2,4] triazol-3 -ylsulfanyl}- acetic acid methyl ester (**6I**).* Yield 78%; mp 265–267 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34–1.59 (m, 4H, cyclohexane), 2.01 (s, 3H, CH_3), 2.36–2.52 (t, 4H, cyclohexane CH_2, J = 4.1 Hz), 3.63 (s, 3H, OCH_3), 3.69 (s, 2H, CH_2), 3.85 (s, 2H, SCH_2), 4.20 (s, 2H, CH_2Cl), 8.02 (broad, 2H, NH); MS *m/z* (%) 472 (M^+, 92), 414 (67), 401 (37.6), 366 (12.8), 319 (6.3), 291 (27.1), 275 (4.9), 219 (33.2), 161 (100), 95 (55.4), 83 (16.7); Anal. Calcd. for $C_{18}H_{22}ClN_5O_4S_2$: C, 45.81; H, 4.70; N, 14.84. Found: C, 45.75; H, 4.53; N, 14.66.

4.1.7. General preparation of 7-chloro-hepta-2, 4, 6-triynoic acid-{3-[2-(2- substituted-amino)-4, 5, 6, 7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-5-hydrazinocarbonyl methylsulfanyl-[1,2,4] triazol-4-yl}-amide [31,32,35]

A solution of (**6**) (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 vacuos, 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.

*4.1.7.1. 7-Chloro-hepta-2, 4, 6-triynoicacid-{3 -[2 -(2 -chloro-acetylamino)-4,5, 6,7- tetrahydro-benzo[b]thiophen-3-ylmethyl]-5-hydrazinocarbonylmethylsulfanyl-[1,2,4] triazol-4-yl}-amide (**7A**).* Yield 83%; mp 230–232 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41–1.62 (m, 4H, cyclohexane), 2.14 (d, 2H, NH_2, J = 6.5 Hz), 2.45–2.64 (t, 4H, cyclohexane CH_2, J = 4.4 Hz), 3.71 (s, 2H, CH_2), 3.74 (s, 2H, SCH_2), 4.03 (s, 2H, CH_2Cl), 4.07–4.15 (t, 1H, NH, J = 4.3 Hz), 7.22–7.48 (m, 4H, ArH), 8.2 (broad, 2H, NH); MS *m/z* (%) 568 (M^+, 65), 531 (69), 488 (145), 438 (61), 380 (78), 363 (8.4), 286 (100), 258 (13), 242 (18), 210 (51), 155 (33), 123 (25), 89 (49); Anal. Calcd. for $C_{22}H_{23}Cl_2N_7O_3S_2$: C, 46.48; H, 4.08; N, 17.25. Found: C, 46.38; H, 4.11; N, 17.13.

*4.1.7.2. N-{3-[2-(2-Chloro-acetylamino)-4, 5, 6, 7-tetrahydro-benzo [b] thiophen-3-ylmethyl] -5-hydrazinocarbonylmethylsulfanyl-[1,2,4] triazol-4-yl}-benzamide (**7E**).* Yield 79%; mp above 300 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38–1.61 (m, 4H, cyclohexane), 2.06 (d, 2H, NH_2, J = 6.4 Hz), 2.45–2.62 (t, 4H, cyclohexane CH_2, J = 4.2 Hz), 3.67 (s, 2H, CH_2), 3.78 (s, 2H, SCH_2), 4.07 (s, 2H, CH_2Cl), 4.10–4.24 (t, 1H, NH, J = 4.7 Hz), 7.35–7.66 (m, 5H, ArH), 8.06 (broad, 2H, NH); MS *m/z* (%) 534 (M^+, 38.2), 498 (100), 455 (10.4), 439 (17.8), 380 (11.7), 363 (29), 286 (46.4), 258 (31.2), 210 (57), 155 (20.8), 123 (7.7), 89 (10.9); Anal. Calcd. for $C_{22}H_{24}ClN_7O_3S_2$: C, 49.48; H, 4.53; N, 18.36. Found: C, 49.51; H, 4.39; N, 18.17.

*4.1.7.3. N-[3- (4-Acetylamino-5-hydrazinocarbonylmethylsulfanyl-4H -[1,2,4] triazol-3-ylmethyl)-4,5,6,7-tetrahydro-benzo [b] thiophen-2-yl]-2-chloro-acetamide (**7I**).* Yield 67%; mp 249–251 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24–1.48 (m, 4H, cyclohexane), 1.86 (d, 2H, NH_2, J = 6.3 Hz), 2.08 (s, 3H, $COCH_3$), 2.43–2.54 (t, 4H, cyclohexane CH_2, J = 4.9 Hz), 3.43 (s, 2H, CH_2), 3.60 (s, 2H, SCH_2), 4.08 (s, 2H, CH_2Cl), 4.12–4.28 (t, 1H, NH, J = 4.0 Hz), 8.09 (broad, 2H, NH); MS *m/z* (%) 472 (M^+, 93.3), 436 (24), 393 (21), 378 (12.27), 320 (5.86), 303 (17), 271 (100), 229 (15.2), 212 (8.4), 156 (34), 123 (20), 69 (70); Anal. Calcd. for $C_{17}H_{22}ClN_7O_3S_2$: C, 43.26; H, 4.70; N, 20.77. Found: C, 43.20; H, 4.57; N, 20.81.

4.1.8. General preparation of 7-chloro-hepta-2, 4, 6-triynoic acid -{3-(2- substituted -amino-4,5,6,7-tetrahydro-benzo[b]thiophen-3ylmethyl)-5-[2-(N′-acetyl-hydrazino)-2-oxo-ethylsulfanyl] -[1,2,4] triazol-4-yl}-amide [30,31,35]

To a solution of (**7**) (1 mmol) in dichloromethane (excess amount), appropriate acid chloride (1 mmol) was added drop-wise with constant vigorous stirring. After 25 min of stirring, acidic alumina (10 g) was added. Dichloromethane then was evaporated in vacuos, 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.

*4.1.8.1. 7-Chloro-hepta-2, 4, 6-triynoicacid{3-[2-(N′-acetyl-hydrazino) -2-oxo-ethylsulfanyl]-5-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-[1,2,4]triazol-4-yl}-amide (**8A**).* Yield 82%; mp 254–256 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40–1.66 (m, 4H, cyclohexane), 2.08 (s, 3H, $COCH_3$), 2.54–2.68 (t, 4H, cyclohexane CH_2, J = 4.5 Hz), 3.72 (s, 2H, CH_2), 3.90 (s, 2H, SCH_2), 4.08 (s, 2H, CH_2Cl), 4.24–4.64 (dd, 2H, J_{NH-NH} = 4.13, J_{NH-NH} = 4.63), 7.20–7.36 (m, 4H, ArH), 8.04 (s, 2H, NH); MS *m/z* (%) 610 (M^+, 79), 575 (26), 540 (52), 489 (63), 461 (78), 438 (32), 395 (41), 376 (4.8), 326 (19), 240 (18), 223 (7.6), 210 (100), 171 (8.3), 144 (3.3); Anal. Calcd. for $C_{24}H_{25}Cl_2N_7O_4S_2$: C, 47.21; H, 4.13; N, 16.06. Found: C, 47.09; H, 4.03; N, 16.17.

*4.1.8.2. N- (3-{4- Acetylamino-5-[2-(N′ -benzoyl-hydrazino)-2-oxo-ethylsulfanyl]-4H -[1,2,4]triazol-3-ylmethyl}-4,5,6,7-tetrahydro-benzo[b]thiophen-2-yl)-2-chloro-acetamide (**8AC**).* Yield 71%; mp 226–228 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37–1.63 (m, 4H, cyclohexane), 2.02 (s, 3H, $COCH_3$), 2.48–2.62 (t, 4H, cyclohexane CH_2, J = 4.1 Hz), 3.69 (s, 2H, CH_2), 3.78 (s, 2H, SCH_2), 4.11 (s, 2H, CH_2Cl), 4.22–4.64 (dd, 2H, J_{NH-NH} = 4.28, J_{NH-NH} = 4.59), 7.20–7.69 (m, 5H, ArH), 8.05 (s, 2H, NH); MS *m/z* (%) 576 (M^+, 100), 541 (56.2), 496 (30.8), 481 (29.5), 427 (10), 379 (15.7), 325 (13.3), 287 (37), 241 (23.5), 167 (76), 124 (22), 109 (7), 98 (5.5); Anal. Calcd. for $C_{24}H_{26}ClN_7O_4S_2$: C, 50.04; H, 4.55; N, s17.02. Found: C, 50.13; H, 4.43; N, 17.14.

*4.1.8.3. N-[3-(4-Acetylamino-5-{2-[N′-(2-chloro-acetyl)-hydrazino]-2-oxo-ethylsulfanyl}-4H-[1,2,4]triazol-3-ylmethyl)-4,5,6,7-tetrahydro-benzo[b]thiophen-2-yl]-2-chloro-acetamide (**8AG**).* Yield 84%; mp 247–249 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.36–1.51 (m, 4H, cyclohexane), 2.02 (s, 3H, $COCH_3$), 2.41–2.63 (t, 4H, cyclohexane CH_2, $J = 4.4$ Hz), 3.68 (s, 2H, CH_2), 3.76 (s, 2H, SCH_2), 4.17 (s, 4H, CH_2Cl), 4.25–4.69 (dd, 2H, $J_{NH-NH} = 4.33$, $J_{NH-NH} = 4.63$), 8.07 (s, 2H, NH); MS *m/z* (%) 548 (M^+, 14.1), 513 (38), 498 (15.7), 458 (17.3), 423 (100), 377 (23.5), 309 (3.9), 280 (13.2), 229 (5.8), 177 (11), 161 (29.6).; Anal. Calcd. for $C_{19}H_{23}Cl_2N_7O_4S_2$: C, 41.61; H, 4.23; N, 17.88. Found: C, 41.66; H, 4.13; N, 17.80.

*4.1.8.4. 7-Chlorohepta-2, 4, 6-triynoic acid {3- [2- (N′-benzoyl-hydrazino)- 2-oxo-ethylsulfanyl]-5-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-[1,2,4]triazol-4-yl}-amide (**8E**).* Yield 71%; mp 231–233 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.65–1.64 (m, 4H, cyclohexane), 2.51–2.64 (t, 4H, cyclohexane CH_2, $J = 4.2$ Hz), 3.70 (s, 2H, CH_2), 3.79 (s, 2H, SCH_2), 4.23 (s, 2H, CH_2Cl), 4.34–4.62 (dd, 2H, $J_{NH-NH} = 4.23$, $J_{NH-NH} = 4.76$), 6.90–7.38 (m, 9H, ArH), 8.03 (s, 2H, NH); MS *m/z* (%) 672 (M^+, 100), 614 (11.1), 579 (13.2), 534 (32), 480 (3.6), 432 (3.4), 379 (8.2), 325 (8.1), 287 (11.9), 241 (15.4), 167 (23.8), 124 (54.2), 109 (25.7), 98 (22); Anal. Calcd. for $C_{29}H_{27}Cl_2N_7O_4S_2$: C, 51.79; H, 4.05; N, 14.58. Found: C, 51.72; H, 4.10; N, 14.42.

*4.1.8.5. 7-Chloro-hepta- 2,4,6-triynoic acid (3-[2-(2-chloro-acetylamino)-4,5, 6, 7-tetrahydro -benzo[b]thiophen-3-ylmethyl]-5-{2-[N′ -(2-chloro-acetyl)-hydrazino]-2-oxo-ethylsulfanyl}-[1,2,4]triazol-4-yl)-amide (**8I**).* Yield 74%; mp 290–292 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.33–1.55 (m, 4H, cyclohexane), 2.44–2.62 (t, 4H, cyclohexane CH_2, $J = 4.8$ Hz), 3.76 (s, 2H, CH_2), 3.78 (s, 2H, SCH_2), 4.13 (s, 4H, CH_2Cl), 4.26–4.64 (dd, 2H, $J_{NH-NH} = 4.43$, $J_{NH-NH} = 4.68$), 7.17–7.40 (m, 4H, ArH), 8.02 (s, 2H, NH); MS *m/z* (%) 644 (M^+, 35.5), 609 (43), 574 (27.7), 559 (5.5), 519 (40), 471 (26.8), 436 (10.5), 408 (13.2), 390 (8.7), 338 (4.7), 310 (100), 280 (9.1), 229 (20.2), 177 (33.3), 161 (8.8); Anal. Calcd. for $C_{24}H_{24}Cl_3N_7O_4S_2$: C, 44.69; H, 3.75; N, 15.20. Found: C, 44.79; H, 3.63; N, 15.18.

*4.1.8.6. N-{3-[2-(N′-Acetyl-hydrazino)-2-oxo-ethylsulfanyl]-5-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-[1,2,4]triazol-4-yl}-benzamide (**8M**).* Yield 57%; mp 170–172 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.31–1.59 (m, 4H, cyclohexane), 2.06 (s, 3H, $COCH_3$), 2.45–2.64 (t, 4H, cyclohexane CH_2, $J = 4.7$ Hz), 3.71 (s, 2H, CH_2), 3.74 (s, 2H, SCH_2), 4.19 (s, 4H, CH_2Cl), 4.28–4.61 (dd, 2H, $J_{NH-NH} = 4.23$, $J_{NH-NH} = 4.58$), 7.08–7.68 (m, 5H, ArH), 8.04 (s, 2H, NH); MS *m/z* (%) 576 (M^+, 13.6), 540 (13), 489 (40.9), 438 (6), 376 (51.3), 326 (100), 240 (77.4), 223 (15.9), 171 (25), 144 (4.4); Anal. Calcd. for $C_{24}H_{26}ClN_7O_4S_2$: C, 50.04; H, 4.55; N, 17.02. Found: C, 50.19; H, 4.49; N, 17.21.

*4.1.8.7. N-{3 -[2-(N′-Benzoyl-hydrazino)- 2 -oxo-ethylsulfanyl]-5-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-[1,2,4]triazol-4-yl}-benzamide (**8Q**).* Yield 91%; mp 124–126 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.38–1.54 (m, 4H, cyclohexane), 2.44–2.62 (t, 4H, cyclohexane CH_2, $J = 5.2$ Hz), 3.69 (s, 2H, CH_2), 3.79 (s, 2H, SCH_2), 4.16 (s, 4H, CH_2Cl), 4.22–4.62 (dd, 2H, $J_{NH-NH} = 4.46$, $J_{NH-NH} = 4.61$), 7.38–7.74 (m, 10H, ArH), 8.02 (s, 2H, NH); MS *m/z* (%) 638 (M^+, 100), 603 (43), 579 (36), 534 (84), 480 (54.6), 432 (9.9), 392 (12), 379 (37), 325 (40), 298 (32), 287 (26), 241 (6.3), 167 (10), 109 (5.3), 98 (14); Anal. Calcd. for $C_{29}H_{28}ClN_7O_4S_2$: C, 54.58; H, 4.42; N, 15.36. Found: C, 54.39; H, 4.33; N, 15.43.

*4.1.8.8. N-(3-[2-(2-Chloro-acetylamino)- 4, 5, 6, 7-tetrahydro-benzo[b] thiophen -3-ylmethyl]-5-{2-[N′-(2-chloro-acetyl)-hydrazino]-2-oxo-ethylsulfanyl}-[1,2,4]triazol-4-yl)-benzamide (**8U**).* Yield 82%; mp 235–237 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.40–1.64 (m, 4H, cyclohexane), 2.56–2.73 (t, 4H, cyclohexane CH_2, $J = 4.6$ Hz), 3.78 (s, 2H, CH_2), 3.85 (s, 2H, SCH_2), 4.11 (s, 4H, CH_2Cl), 4.21–4.48 (dd, 2H, $J_{NH-NH} = 4.52$, $J_{NH-NH} = 4.74$), 6.90–7.32 (m, 5H, ArH), 8.05 (s, 2H, NH); MS *m/z* (%) 610 (M^+, 100), 575 (85), 533 (49), 472 (14), 431 (29), 402 (11), 353 (7.8), 310 (67), 281 (31), 234 (24), 178 (19), 134 (6.1), 98 (3.4); Anal. Calcd. for $C_{24}H_{25}Cl_2N_7O_4S_2$: C, 47.21; H, 4.13; N, 16.06. Found: C, 47.08; H, 4.24; N, 16.18

*4.1.8.9. N-(3-{4-Acetylamino-5-[2-(N′-acetyl-hydrazino)-2-oxo-ethylsulfanyl]-4H-[1,2,4] triazol-3-ylmethyl}-4,5,6,7-tetrahydro-benzo[b]thiophen-2-yl)-2-chloro-acetamide (**8Y**).* Yield 81%; mp 251–253 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.39–1.65 (m, 4H, cyclohexane), 2.04 (s, 6H, $COCH_3$), 2.47–2.61 (t, 4H, cyclohexane CH_2, $J = 4.4$ Hz), 3.68 (s, 2H, CH_2), 3.72 (s, 2H, SCH_2), 4.09 (s, 4H, CH_2Cl), 4.18–4.49 (dd, 2H, $J_{NH-NH} = 4.26$, $J_{NH-NH} = 4.50$), 8.03 (s, 2H, NH); MS *m/z* (%) 514 (M^+, 100), 479 (62), 438 (15.8), 376 (52), 335 (71), 279 (8.7), 223 (24), 177 (27); Anal. Calcd. for $C_{19}H_{24}ClN_7O_4S_2$: C, 44.40; H, 4.71; N, 19.07. Found: C, 44.34; H, 4.87; N, 19.28.

4.1.9. General preparation of 7-chloro-hepta-2, 4, 6-triynoic acid -{3-(benzylidene-hydrazinocarbonylm ethylsulfanyl)-5-[2-(2-substituted -amino)- 4, 5, 6, 7-tetrahydro-benzo[b]thiophen-3ylmethyl]- [1,2,4] triazol-4-yl}-amide [31,32,35]

A solution of (**7**) (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 vacuos, 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.

*4.1.9.1. 7-Chloro-hepta-2, 4, 6-triynoic acid-{3-(benzylidene-hydrazinocarbonylm ethylsulfanyl)-5-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3ylmethyl]-[1,2,4] triazol-4-yl}-amide (**9A**).* Yield 86%; mp decomposed around 226–228 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.38–1.61 (m, 4H, cyclohexane), 2.51–2.62 (t, 4H, cyclohexane CH_2, $J = 4.4$ Hz), 3.76 (s, 2H, CH_2), 3.94 (s, 2H, SCH_2), 4.24 (s, 2H, CH_2Cl), 6.91–7.40 (m, 9H, ArH), 8.06 (s, 3H, NH), 8.24 (s, 1H, N=CH); MS *m/z* (%) 656 (M^+, 69), 615 (19), 570 (37), 532 (21), 468 (58), 399 (24), 348 (35), 287 (100), 210 (18.4), 157 (11.4), 103 (12.3); Anal. Calcd. for $C_{29}H_{27}Cl_2N_7O_3S_2$: C, 53.05; H, 4.14; N, 14.93. Found: C, 53.14; H, 4.03; N, 14.87.

*4.1.9.2. N-{3-(Benzylidene-hydrazinocarbonylmethylsulfanyl)-5-[2-(2 -chloro -acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-[1,2,4]triazol-4-yl}-benzamide (**9E**).* Yield 78%; mp 182–184 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.66–1.87 (m, 4H, cyclohexane), 2.40–2.51 (t, 4H, cyclohexane CH_2, $J = 4.9$ Hz), 3.64 (s, 2H, CH_2), 3.80 (s, 2H, SCH_2), 4.19 (s, 2H, CH_2Cl), 6.78–7.54 (m, 10H, ArH), 7.8 (d, 2H, benzene CH), 7.96 (s, 3H, NH), 8.13 (s, 1H, N=CH); MS *m/z* (%) 622 (M^+, 6.9), 573 (31), 535 (3), 500 (6), 470 (13.7), 399 (3.4), 348 (39), 319 (16), 287 (100), 224 (67.6), 210 (29), 157 (5), 103 (9.8); Anal. Calcd. for $C_{29}H_{28}ClN_7O_3S_2$: C, 55.98; H, 4.54; N, 15.76. Found: C, 55.98; H, 4.42; N, 15.69.

*4.1.9.3. N-{3-[4-Acetylamino -5-(benzylidene-hydrazinocarbonylmethylsulfanyl)-4H-[1,2,4] triazol-3-ylmethyl]-4,5,6,7-tetrahydro-benzo[b]thiophen-2-yl}-2-chloro-acetamide (**9I**).* Yield 81%; mp decomposed around 221–223 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.42–1.60 (m, 4H, cyclohexane), 2.03 (s, 3H, $COCH_3$), 2.44–2.60 (t, 4H, cyclohexane CH_2, $J = 4.6$ Hz), 3.75 (s, 2H, CH_2), 3.92 (s, 2H, SCH_2), 4.26 (s, 2H, CH_2Cl), 6.91–7.12 (m, 5H, ArH), 8.01 (s, 3H, NH), 8.12 (s, 1H, N=CH); MS *m/z* (%) 560 (M^+, 100), 525 (3.7), 474 (13.5), 432 (9), 363 (12), 322 (17), 305 (60.4), 263 (32), 231 (76), 175 (38.6); Anal. Calcd. for $C_{24}H_{26}ClN_7O_3S_2$: C, 51.47; H, 4.68; N, 17.51. Found: C, 51.55; H, 4.49; N, 17.60.

4.1.10. *General preparation of 7-chloro-hepta-2, 4, 6-triynoic acid -{3-(4- amino-5-mercapto-4H-[1,2,4] triazol-3-ylmethylsulfanyl)-5-[2-(2- substituted -amino)-4, 5, 6, 7-tetrahydro-benzo[b]thiophen-3ylmethyl] – [1,2,4] triazol-4-yl}-amide [31,32,35]*

The (**7**) (1 mmol) was dissolved in alcoholic potassium hydroxide (1 mmol) and kept for stirring. Carbon disulphide (1.5 mmol) was added drop wise to this 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 (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 vacuos, 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.

*4.1.10.1. 7-Chloro-hepta-2,4,6-triynoic acid -{3-(4- amino-5-mercapto-4H-[1,2,4] triazol-3-ylmethylsulfanyl)-5-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3ylmethyl] – [1,2,4] triazol-4-yl}-amide (**10A**).* Yield 72%; mp 232–234 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.31–1.58 (m, 4H, cyclohexane), 2.04 (s, 2H, NH_2), 2.56–2.73 (t, 4H, cyclohexane CH_2, $J = 4.3$ Hz), 3.06 (s, 1H, SH), 3.82 (s, 2H, CH_2), 4.12 (s, 2H, SCH_2), 4.27 (s, 2H, CH_2Cl), 7.14–7.41 (m, 4H, ArH), 8.1 (s, 2H, NH); MS *m/z* (%) 624 (M^+, 82), 570 (43), 517 (31), 461 (26), 384 (31), 326 (13.2), 247 (15), 226 (17), 126 (100); Anal. Calcd. for $C_{23}H_{23}Cl_2N_9O_2S_3$: C, 44.23; H, 3.71; N, 20.18. Found: C, 44.12; H, 3.65; N, 20.02.

*4.1.10.2. N-{3-(4-Amino-5-mercapto-4H-[1,2,4]triazol-3-ylmethylsulfanyl)-5-[2-(2-chloro-acetyl amino)-4,5,6,7-tetrahydro-benzo [b]thiophen-3-ylmethyl]-[1,2,4]triazol-4-yl}-benzamide (**10E**).* Yield 80%; mp 256–258 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.43–1.72 (m, 4H, cyclohexane), 2.03 (s, 2H, NH_2), 2.36–2.62 (t, 4H, cyclohexane CH_2, $J = 4.2$ Hz), 3.03 (s, 1H, SH), 3. 86 (s, 2H, CH_2), 4.07 (s, 2H, SCH_2), 4.20 (s, 2H, CH_2Cl), 7.06–7.62 (m, 5H, ArH), 8.08 (s, 2H, NH); MS *m/z* (%) 590 (M^+, 100), 536 (17), 483 (22), 427 (61), 350 (14), 326 (45.7), 247 (39), 226 (6.6), 126 (22.3), 89 (19.7); Anal. Calcd. for $C_{23}H_{24}ClN_9O_2S_3$: C, 46.81; H, 4.10; N, 21.36. Found: C, 46.93; H, 4.19; N, 21.48.

*4.1.10.3. N-{3-[4-Acetylamino -5 -(4-amino-5- mercapto-4H-[1,2,4] triazol -3 -yl methylsulfanyl)-4H-[1,2,4]triazol-3-ylmethyl]-4,5,6,7-tetrahydro-benzo[b]thiophen-2-yl}-2-chloro-acetamide (**10I**).* Yield 77%; mp 269–271 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.26–1.59 (m, 4H, cyclohexane), 1.97 (s, 2H, NH_2), 2.08 (s, 3H, $COCH_3$), 2.52–2.71 (t, 4H, cyclohexane CH_2, $J = 4.5$ Hz), 3.12 (s, 1H, SH), 3.76 (s, 2H, CH_2), 4.17 (s, 2H, SCH_2), 4.23 (s, 2H, CH_2Cl), 8.04 (s, 2H, NH); MS *m/z* (%) 528 (M^+, 56.3), 474 (52), 421(100), 365 (10), 288 (48), 246 (93), 219 (8.1), 83 (72.3); Anal. Calcd. for $C_{18}H_{22}ClN_9O_2S_3$: C, 40.94; H, 4.20; N, 23.87. Found: C, 40.85; H, 4.07; N, 23.98.

4.2. *Cyclin-dependent kinase 5/p25 inhibiting activity*

Kinase inhibit ion was measured by use of scintillation proximity assays (SPA) [8]. 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 PKTPKKAKKL. 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 [36] 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.

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