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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 6397–6406
Bioorganic & Medicinal Chemistry

A novel approach to cyclin-dependent kinase 5/p25 inhibitors: A potential treatment for Alzheimer's disease

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Received 25 April 2007; revised 25 June 2007; accepted 27 June 2007
Available online 4 July 2007

Abstract—Based on the earlier results of our in-house database and compound library, a series of novel clubbed thienyl triazoles was designed which may emerge as potential cdk5/p25 inhibitors, for the treatment of Alzheimer's disease. A benign synthesis was planned so as to take an advantage of MAOS (Microwave Assisted Organic Synthesis) method. Evaluation of the SAR of this series has allowed the identification of compounds **4**, **5**, **7** and **8** from series I while **13**, **14**, **16** and **17** from series II as significant cdk5/p25 inhibitors and thus have potential as possible treatments for Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a progressive brain disorder that gradually destroys a person's memory and ability to learn, reason, make judgments, communicate and carry out daily activities. As Alzheimer's disease progresses, individuals may also experience changes in personality and behaviour, such as anxiety, suspiciousness or agitation, as well as delusions or hallucinations. There are now more than five million people in the United States living with AD. This number includes 4.9 million people over the age of 65 and between 200,000 and 500,000 people under age 65 with early-onset AD, for which no effective treatment exists today (Schemes 1 and 2).

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 Phosphoprotein (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-aminothienyl derivatives[6] as the potential inhibitors of cdk5/p25 for the treatment of Alzheimer's disease and other neurodegenerative disorders.[7–13]

In continuation with our discovery programme[14–19] on triazoles, we embarked on a cdk5/p25 inhibiting activity to find high potency compound/s. Screening of an in-house database provided several hits with modest

Keywords: Thienyl; Triazole; cdk5/p25; Alzheimer's disease.
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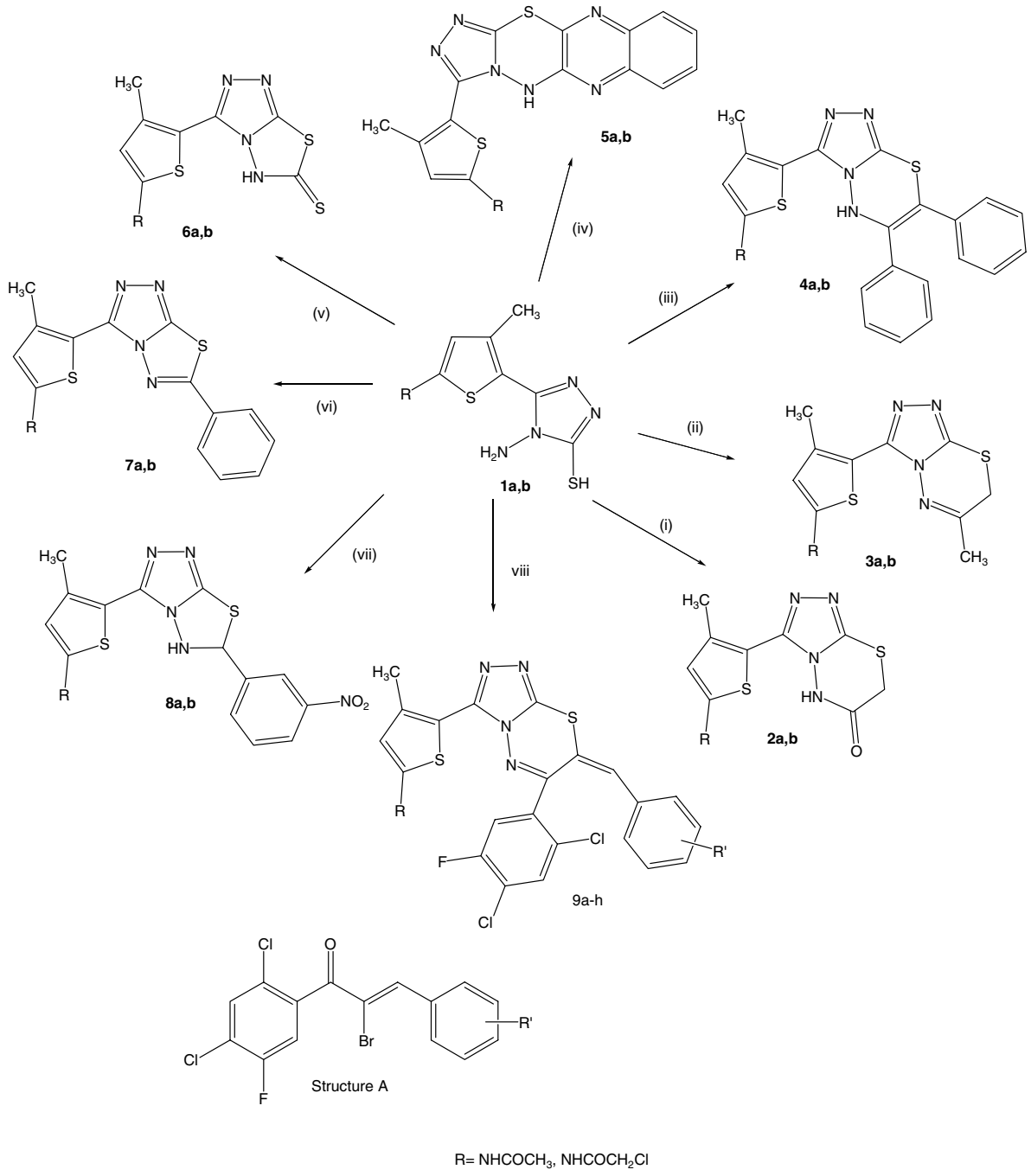
doi:10.1016/j.bmc.2007.06.053



Scheme 1. Reagents and conditions. (i) $ClCH_2COOH$, NaOAc; (ii) CH_3COCH_2Br, K_2CO_3; (iii) $C_6H_5CHOHCOC_6H_5$, KOH; (iv) 2,3-Dichloroquinoxaline, NaOAc; (v) CS_2, KOH; (vi) C_6H_5COOH, $POCl_3$; (vii) 3-NO_2-C_7H_5O; (viii) Ethanolic KOH/reflux; structure A.

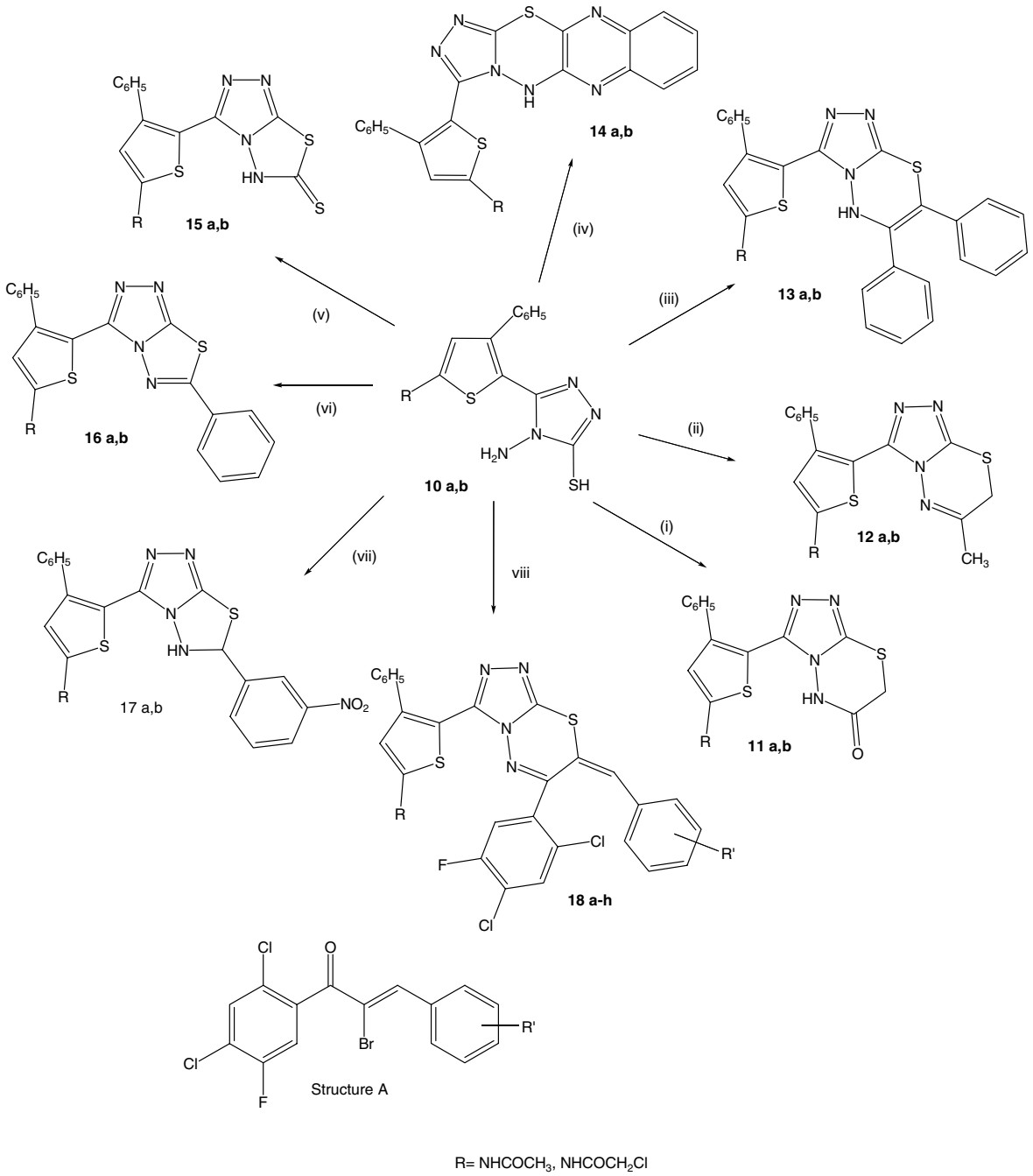
cdk5/p25 inhibitory activity, one of which was the clubbed triazolyl thienyl **1b** ($IC_{50} = 46 \pm 2$ nM), while the other was from **10b** ($IC_{50} = 42 \pm 2$ nM).

In recent years, environmentally benign synthetic methods have received considerable attention and solvent-free protocols are reported.[20–22] 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. Results and discussion

2.1. Synthesis

Compounds **1a**,**b**, **10a**,**b** and structure A[23] were synthesized as per the literature.[24–26] Compounds **1a**,**b**, adsorbed on acidic alumina (Aluminium oxide, acidic, Brockmann I, ~150 mesh, 58 Å CAMAG 506-C-I, Surface area 155 m^2/g. pH = 6.0), when treated with chloroacetic acid in presence of its salt with strong base, yielded **2a**–**b**. The second transformation to the



Scheme 2. Reagents and conditions. (i) $ClCH_2COOH$, NaOAc; (ii) CH_3COCH_2Br, K_2CO_3; (iii) $C_6H_5CHOHCOC_6H_5$, KOH; (iv) 2,3-Dichloroquinoxaline, NaOAc; (v) CS_2, KOH; (vi) C_6H_5COOH, $POCl_3$; (vii) 3-NO_2-C_7H_5O; (viii) Ethanolic KOH/reflux; structure A.

compounds **3a**,**b** was achieved by treatment with halogenated ketone in the presence of inorganic base, i.e., potassium carbonate. Title compounds **4a**,**b** were obtained by treatment of **1a**,**b** with benzoin in basic condition. Usually, just dumping the reaction mixture in water will separate product, but herein, it did not work. A separation of the product was achieved by extraction of product into acetone and then evaporating the acetone layer until dryness. Compounds **1a**,**b** when treated with 2,3-dichloro quinoxaline, a nucleophilic substitution reaction takes place, in basic condition, to yield **5a**,**b**. Chemical transformation of **1a**,**b** to **6a**,**b** was achieved by treating it with carbon disulfide and potassium hydroxide. While compounds **1a**,**b**, on treatment with benzoic acid, in presence of $POCl_3$, furnished **7a**,**b**. Compounds **8a**,**b**, the condensation products of **1a**,**b**, were synthesized by treating it with *m*-nitrobenzaldehyde which was confirmed by absence of peak in IR spectrum as well as in NMR for SH of triazole. The final modifications in the present series are performed, by refluxing compound from the series of compound termed here as structure A with **1a** and **1b** in the presence of ethanolic KOH, to give less active moieties of the series **9a**–**h**. A similar set of reactions was followed using **10a**,**b** as starting material for the conversion to **11a**,**b**–**18a**–**h**.

Table 1. SAR of cdk5/p25 inhibitory screening for compounds **1a**,**b**, **2a**,**b**, **3a**,**b**, **4**,**a**,**b**, **5a**,**b**, **6a**,**b** **7a**,**b**, **8a**,**b**, **9a-h**, **10a**,**b**, **11a**,**b**, **12a**,**b**, **13a**,**b**, **14a**,**b**, **15a**,**b**, **16a**,**b**, **17a**,**b**, **18a-h** and selectivity ratio of most active compounds

Compound	R	R′	Cdk5 IC_{50} (nM)	Cdk2 IC_{50} (nM)	Select k2/k5
1a	$NHCOCH_3$	—	58 ± 2	53 ± 3	1
1b	$NHCOCH_2Cl$	—	46 ± 2	99 ± 7	2.2
2a	$NHCOCH_3$	—	630 ± 32	—	—
2b	$NHCOCH_2Cl$	—	820 ± 52	—	—
3a	$NHCOCH_3$	—	642 ± 11	—	—
3b	$NHCOCH_2Cl$	—	462 ± 72	—	—
4a	$NHCOCH_3$	—	44 ± 2	89 ± 8	2
4b	$NHCOCH_2Cl$	—	72 ± 2	154 ± 62	2
5a	$NHCOCH_3$	—	34 ± 1	70 ± 4	2
5b	$NHCOCH_2Cl$	—	64 ± 1	142 ± 12	2.2
6a	$NHCOCH_3$	—	3260 ± 106	—	
6b	$NHCOCH_2Cl$	—	7480 ± 114	—	
7a	$NHCOCH_3$	—	42 ± 1	51 ± 8	1.2
7b	$NHCOCH_2Cl$	—	30 ± 1	95 ± 11	3.2
8a	$NHCOCH_3$	—	64 ± 18	78 ± 17	1
8b	$NHCOCH_2Cl$	—	53 ± 14	64 ± 14	1
9a	$NHCOCH_3$	H	2370 ± 78	—	—
9b	$NHCOCH_2Cl$	H	3420 ± 117	—	—
9c	$NHCOCH_3$	2-Cl	3284 ± 89	—	—
9d	$NHCOCH_2Cl$	2-Cl	3340 ± 94	—	—
9e	$NHCOCH_3$	4-Cl	3386 ± 84	—	—
9f	$NHCOCH_2Cl$	4-Cl	2968 ± 96	—	—
9g	$NHCOCH_3$	3-NO_2	3046 ± 102	—	—
9h	$NHCOCH_2Cl$	3-NO_2	3074 ± 98	—	—
10a	$NHCOCH_3$	—	54 ± 2	56 ± 3	1
10b	$NHCOCH_2Cl$	—	42 ± 2	120 ± 7	3
11a	$NHCOCH_3$	—	418 ± 24	—	—
11b	$NHCOCH_2Cl$	—	634 ± 28	—	—
12a	$NHCOCH_3$	—	448 ± 18	—	—
12b	$NHCOCH_2Cl$	—	368 ± 20	—	—
13a	$NHCOCH_3$	—	40 ± 2	82 ± 8	2
13b	$NHCOCH_2Cl$	—	62 ± 2	126 ± 22	2
14a	$NHCOCH_3$	—	54 ± 1	64 ± 4	1.2
14b	$NHCOCH_2Cl$	—	30 ± 1	102 ± 10	3.2
15a	$NHCOCH_3$	—	2058 ± 96	—	—
15b	$NHCOCH_2Cl$	—	5896 ± 102	—	—
16a	$NHCOCH_3$	—	38 ± 1	44 ± 8	1.2
16b	$NHCOCH_2Cl$	—	28 ± 1	92 ± 12	3.2
17a	$NHCOCH_3$	—	60 ± 12	64 ± 8	1
17b	$NHCOCH_2Cl$	—	48 ± 9	52 ± 10	1
18a	$NHCOCH_3$	H	1452 ± 68	—	—
18b	$NHCOCH_2Cl$	H	2486 ± 98	—	—
18c	$NHCOCH_3$	2-Cl	2464 ± 76	—	—
18d	$NHCOCH_2Cl$	2-Cl	2458 ± 74	—	—
18e	$NHCOCH_3$	4-Cl	2482 ± 75	—	—
18f	$NHCOCH_2Cl$	4-Cl	2448 ± 72	—	—
18g	$NHCOCH_3$	3-NO_2	2436 ± 80	—	—
18h	$NHCOCH_2Cl$	3-NO_2	2684 ± 82	—	—

2.2. Cyclin-dependent kinase 5/p25 inhibiting activity

Kinase inhibition was measured by the use of scintillation proximity assays (SPA).[27] The results of the assays are reported in Table 1. During the preliminary screening compound **1b** has emerged as hit cdk5/p25 (IC_{50} = 46 ± 2 nM), with good potency and more opportunities for chemical transformation for the optimization (Preliminary screening results for **1a** were comparable, IC_{50} = 58 ± 2 nM). Testing of **1b** against other cdks revealed that **1b** was essentially equipotent at inhibiting cdk2/cyclin E (IC_{50} = 49 ± 3 nM), a cancer target. Thus with an objective to improve cdk5 potency and minimize cdk2 activity, certain chemical modifications have been performed. Cyclization of the amine side chain with the sulfhydryl group of **1b** with MAOS allowed us to rapidly explore the pharmacophore. As a first step towards lead optimization, treatment with chloroacetic acid gave a 6-oxo-thiadiazin ring formation **2a**,**b** however, this modification resulted in a substantial decrease in activity. The next structural modification made was a replacement of 6-oxo by 6-methyl to furnish **3a**–**d** but this change also resulted in a substantial loss of biological activity.

Substitution of 6-methyl and 5*H* by phenyl rings provided the first analogues **4a** and **4b** that demonstrated excellent activity. Thus looking at the activity, it was decided to increase the aromatic nature and bulkiness of the triazole ring. In order to optimize this component, two compounds **5a**,**b** were synthesized and investigated, which revealed quite interesting results of the cdk5/p25 inhibitory activity, as both of the compounds have shown impressive percentage of inhibition. A further modification to thiadiazoles, **6a**,**b** with lesser aromaticity, exhibited loss of activity. Attention was then turned to optimization of the **6a** and **b**. Changing the S with bulkier phenyl improved cdk5 potency and for the first time afforded >threefold selectivity versus cdk2, in case of **7b**. The compound **7a** was equally selective versus cdk2 and had slightly improved cdk5 IC_{50}. A further similar structural modification was a successful attempt, with **8a**,**b** as potential candidate. Further, cdk5/p25 inhibitory evaluations of compounds **9a**–**h** lead to less potent compounds.

A series, wherein methyl group was replaced by phenyl, was designed on a conclusion that increase in aromaticity is directly leading to potentiation of the cdk5/p25 inhibitory activity. Thus the said series was synthesized and evaluated for the cdk5/p25 inhibitory action. Encouraging results were obtained when compared with the compounds with methyl group. Compounds **10a**,**b**, **13a**,**b**, **14a**,**b**, **16a**,**b** and **17a**,**b** were observed with a comparatively improved activity, as illustrated in Table 1.

3. Conclusion

In conclusion, a novel series of clubbed triazolyl-thienyl 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 thienyl core and amino and sulfhydryl groups in triazole core and subsequently by the introduction of appropriate substituents, specially aromatic ring, leading to the most promising compounds **4**, **5**, **7** and **8** from series I while **13**, **14**, **16** and **17** from series II. 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 were recorded on a Bruker Avance 300 MHz instrument using $CDCl_3$ as solvent and TMS as internal standard; the chemical shifts (δ) are reported in ppm and coupling constants (J) are given in Hz. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet) and bs (broad singlet). Mass spectra were recorded on a Finning LCQ mass spectrometer. Microwave irradiation was carried out in Raga Scientific Microwave Systems, Model RG31L at 2450 MHz. Elemental analyses were performed on a Heracus CHN-Rapid Analyser. 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^1-[5-(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)-4-methyl-2-thienyl]acetamide (1a), N^1-[5-(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)-4-methyl-2-thienyl]-2-chloroacetamide (1b), N^1-[5-(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)-4-phenyl-2-thienyl]acetamide (10a), N^1-[5-(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)-4-phenyl-2-thienyl]-2-chloroacetamide (10b). Above titled compounds were prepared as per the literature.[24–26]

4.1.2. General preparation of N^1-[4-substituted-5-(6-oxo-6,7-dihydro-5H-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2-thienyl]-2-substitutedacetamide. A solution of **1** or **10** (0.01 mol) in dichloromethane (20 mL), chloroacetic acid (0.01 mol) and freshly prepared fused sodium acetate (0.01 mol) was prepared. Acidic alumina (Aluminium oxide, acidic, Brockmann I, ~150 mesh, 58 Å CAMAG 506-C-I, Surface area 155 m^2/g. pH = 6.0) about 5 g was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath[28] and irradiated for 40–80 s at a power level of 300 W. The mixture was cooled and then product was extracted with dry methanol and poured onto crushed ice. The solid thus separated was filtered, washed thoroughly with water and recrystallized from aq ethanol.

4.1.2.1. N^1-[4-Methyl-5-(6-oxo-6,7-dihydro-5*H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2-thienyl]-acetamide (2a). Yield 76%; yellow needles; mp 207–212 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.94 (s, 3H, CH_3CO), 2.62 (s, 3H, CH_3), 3.92 (s, 2H, CH_2 of Thiadiazine), 6.12 (s, 1H, CH of Thiophene), 8.06 (s, 2H, NH); MS (%) 309 (M^+, 100); Calcd (%) for $C_{11}H_{11}N_5O_2S_2$: C; 42.71, H; 3.58, N; 22.64. Found: C; 42.90, H; 3.72, N; 22.77.

4.1.2.2. N^1-[4-Methyl-5-(6-oxo-6,7-dihydro-5*H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2-thienyl]-2-chloroacetamide (2b). Yield 82%; brown needles; mp 192–196 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.51 (s, 3H, CH_3), 3.77 (s, 2H, CH_2 of Thiadiazine), 4.38 (s, 2H, CH_2Cl), 5.95 (s, 1H, CH of Thiophene), 8.12 (s, 2H, NH); MS (%) 343 (56, M^+); Calcd (%) for $C_{11}H_{10}N_5O_2S_2Cl$: C; 38.43, H; 2.93, N; 20.37. Found: C; 38.57, H; 2.76, N; 20.53.

4.1.2.3. N^1-[4-Phenyl-5-(6-oxo-6,7-dihydro-5*H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2-thienyl]-acetamide (11a). Yield 74%; white powder; mp 221–225 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.97 (s, 3H, CH_3CO), 3.90 (s, 2H, CH_2 of Thiadiazine), 6.15 (s, 1H, CH of Thiophene), 7.27–7.51 (m, 5H, ArH), 8.12 (s, 2H, NH); MS (%) 371 (M^+, 100); Calcd (%) for $C_{16}H_{13}N_5O_2S_2$: C; 51.74, H; 3.53, N; 18.85. Found: C; 51.91, H; 3.67, N; 18.64.

4.1.2.4. N^1-[4-Phenyl-5-(6-oxo-6,7-dihydro-5*H*-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2-thienyl]-2-chloroacetamide (11b). Yield 88%; white needles; mp 201–206 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.70 (s, 2H, CH_2 of Thiadiazine), 4.29 (s, 2H, CH_2Cl), 5.74 (s, 1H, CH of Thiophene), 7.15–7.46 (m, 5H, ArH), 8.03 (s, 2H, NH); MS (%) 406 (78, M^+); Calcd (%) for $C_{16}H_{12}N_5O_2S_2Cl$: C; 47.35, H; 2.98, N; 17.25. Found: C; 47.22, H; 2.74, N; 17.13.

4.1.3. General preparation of N^1-[4-substituted-5-(6-methyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)-2-thienyl]-2-substituted acetamide. Solution of **1** or **10** (0.01 mol) and *p*-bromophenacyl bromide (0.01 mol) was added to acidic alumina (5 g) at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40–80 s at a power level of 300 W. The mixture was cooled and then product was extracted with dry methanol, followed by neutralization with potassium carbonate. The solid thus separated was filtered, washed thoroughly with water and recrystallized from ethanol.

4.1.3.1. N^1-[4-Methyl-5-(6-methyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2-thienyl]-acetamide (3a). Yield 73%; yellow precipitate; mp 213–218 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (s, 3H, CH_3), 2.06 (s, 3H, CH_3CO), 2.38 (s, 3H, CH_3), 3.02 (s, 2H, CH_2 of Thiadiazine), 6.11 (s, 1H, CH of Thiophene), 8.06 (s, 2H, NH); MS (%) 307 (64, M^+); Calcd (%) for $C_{12}H_{13}N_5OS_2$: C; 46.89, H; 4.26, N; 22.78. Found: C; 46.96, H; 4.38, N; 22.94.

4.1.3.2. N^1-[4-Methyl-5-(6-methyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2-thienyl]-2-chloroacetamide (3b). Yield 88%; brown needles; mp 220–224 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 3.11 (s, 2H, CH_2 of Thiadiazine), 4.42 (s, 2H, CH_2Cl), 5.82 (s, 1H, CH of Thiophene), 8.23 (s, 2H, NH); MS (%) 342 (91, M^+); Calcd (%) for $C_{12}H_{12}ClNO_5S_2$: C; 42.16, H; 3.54, N; 20.49. Found: C; 42.31, H; 3.66, N; 20.63.

4.1.3.3. N^1-[4-Phenyl-5-(6-methyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2-thienyl]-acetamide (12a). Yield 76%; yellow precipitate; mp 219–224 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.17 (s, 3H, CH_3CO), 2.16 (s, 3H, CH_3), 3.11 (s, 2H, CH_2 of Thiadiazine), 6.16 (s, 1H, CH of Thiophene), 7.25–7.55 (m, 5H, ArH), 8.23 (s, 2H, NH); MS (%) 370 (86, M^+); Calcd (%) for $C_{17}H_{15}N_5OS_2$: C; 55.26, H; 4.09, N; 18.96. Found: C; 55.51, H; 4.24, N; 18.85.

4.1.3.4. N^1-[4-Phenyl-5-(6-methyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-2-thienyl]-2-chloroacetamide (12b). Yield 81%; yellow needles; mp 224–227 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 3H, CH_3), 3.42 (s, 2H, CH_2 of Thiadiazine), 4.51 (s, 2H, CH_2Cl), 5.73 (s, 1H, CH of Thiophene), 7.11–7.48 (m, 5H, ArH), 8.52 (s, 2H, NH); MS (%) 404 (90, M^+); Calcd (%) for $C_{17}H_{14}ClN_5OS_2$: C; 50.55, H; 3.49, N; 17.34. Found: C; 50.71, H; 3.64, N; 17.57.

4.1.4. General preparation of N^1-[5-(6,7-diphenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-4-substituted-2-thienyl]-2-substituted acetamide. A solution of **1** or **10** (0.01 mol), benzoin (0.01 mol) and 2*N* KOH solution was prepared. Acidic alumina (5 g) was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 80–120 s at a power level of 300 W. The mixture was cooled and then product was extracted with acetone and was evaporated to dryness. The solid thus separated was washed thoroughly with water and recrystallized from ethanol.

4.1.4.1. N^1-[5-(6,7-Diphenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-3-yl)-4-methyl-2-thienyl]-acetamide (4a). Yield 69%; white crystals; mp 254–258 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.11 (s, 3H, CH_3CO), 2.43 (s, 3H, CH_3), 5.87 (s, 1H, CH of Thiophene), 7.11–7.46 (m, 10H, ArH), 8.17 (s, 2H, NH); MS (%) 446 (79, M^+); Calcd (%) for $C_{23}H_{19}N_5OS_2$: C; 62.00, H; 4.30, N; 15.72. Found: C; 62.23, H; 4.47, N; 15.89.

4.1.4.2. N^1-[5-(6,7-Diphenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)-4-methyl-2-thienyl]-2-chloroacetamide (4b). Yield 73%; white crystals; mp 243–247 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.40 (s, 3H, CH_3), 4.16 (s, 2H, CH_2Cl), 5.90 (s, 1H, CH of Thiophene), 7.07–7.23 (m, 10H, ArH), 8.15 (s, 2H, NH); MS (%) 480 (69, M^+); Calcd (%) for $C_{23}H_{18}ClNO_5S_2$: C; 57.55, H; 3.78, N; 14.59. Found: C; 57.67, H; 3.91, N; 14.70.

4.1.4.3. N^1-[5-(6,7-Diphenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-3-yl)-4-phenyl-2-thienyl]-acetamide (13a). Yield 65%; white crystals; mp 245–249 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.09 (s, 3H, CH_3CO), 5.77 (s, 1H, CH of Thiophene), 7.13–7.63 (m, 15H, ArH), 8.26 (s, 2H, NH); MS (%) 508 (100, M^+); Calcd (%) for $C_{28}H_{21}N_5OS_2$: C; 66.25, H; 4.17, N; 13.80. Found: C; 66.41, H; 4.34, N; 13.97.

4.1.4.4. N^1-[5-(6,7-Diphenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-3-yl)-4-phenyl-2-thienyl]-2-chloroacetamide (13b). Yield 77%; white crystals; mp 251–255 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.08 (s, 2H, CH_2Cl), 5.73 (s, 1H, CH of Thiophene), 7.19–7.59 (m, 15H, ArH), 8.19 (s, 2H, NH); MS (%) 542 (89, M^+); Calcd (%) for $C_{28}H_{20}ClN_5OS_2$: C; 62.04, H; 13.72, N; 12.92. Found: C; 62.22, H; 13.86, N; 12.75.

4.1.5. General preparation of N^1-[4-substituted-5-(5*H*-[1,2,4]triazolo[3′,4′:2,3][1,3,4]thiadiazino[5,6-*b*]quinoxalin-3-yl)-2-thienyl]-2-substituted acetamide. Solution of **1** or **10** (0.01 mol), 2,3-dichloro quinoxaline (0.01 mol) and fused sodium acetate (0.02 mol) was added to acidic alumina (5 g) at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 90–130 s at a power level of 300 W. The mixture was cooled and then product was extracted with dry methanol, concentrated and cooled. The solid thus separated was filtered, washed thoroughly with water and recrystallized from ethanol.

4.1.5.1. N^1-[4-Methyl-5-(5*H*-[1,2,4]triazolo[3′,4′:2,3][1,3,4]-thiadiazino[5,6-*b*]quinoxalin-3-yl)-2-thienyl]-acetamide (5a). Yield 75%; buff precipitate; mp 211–216 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01 (s, 3H, CH_3CO), 2.47 (s, 3H, CH_3), 6.17 (s, 1H, CH of Thiophene), 7.3–7.41 (t, 2H, ArH, J = 7.8 Hz), 7.83–7.95 (d, 2H, ArH, J = 8.9 Hz), 8.24 (s, 2H, NH); MS (%) 395 (79, M^+); Calcd (%) for $C_{17}H_{13}N_7OS_2$: C; 51.63, H; 3.31, N; 24.79. Found: C; 51.77, H; 3.54, N; 24.88.

4.1.5.2. N^1-[4-Methyl-5-(5*H*-[1,2,4]triazolo[3′,4′:2,3]-[1,3,4]thiadiazino[5,6-*b*]quinoxalin-3-yl)-2-thienyl]-2-chloroacetamide (5b). Yield 81%; buff precipitate; mp 221–225 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H, CH_3), 4.27 (s, 2H, CH_2Cl), 6.04 (s, 1H, CH of Thiophene), 7.27–7.36 (t, 2H, ArH, J = 7.8 Hz), 7.87–7.95 (d, 2H, ArH, J = 8.9 Hz), 8.05 (s, 2H, NH); MS (%) 430 (76, M^+); Calcd (%) for $C_{17}H_{12}N_7OS_2Cl$: C; 47.49, H; 2.81, N; 22.81. Found: C; 47.62, H; 2.93, N; 22.97.

4.1.5.3. N^1-[4-Phenyl-5-(5*H*-[1,2,4]triazolo[3′,4′:2,3][1,3,4]-thiadiazino[5,6-*b*]quinoxalin-3-yl)-2-thienyl]-acetamide (14a). Yield 78%; buff precipitate; mp 243–247 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.07 (s, 3H, CH_3CO), 6.22 (s, 1H, CH of Thiophene), 7.11–7.51 (m, 9H, ArH), 8.31 (s, 2H, NH); MS (%) 458 (100, M^+); Calcd (%) for $C_{22}H_{15}N_7OS_2$: C; 57.75, H; 3.30, N; 21.43. Found: C; 57.88, H; 3.52, N; 21.58.

4.1.5.4. N^1-[4-Phenyl-5-(5*H*-[1,2,4]triazolo[3′,4′:2,3][1,3,4]-thiadiazino[5,6-*b*]quinoxalin-3-yl)-2-thienyl]-2-chloroacetamide (14b). Yield 84%; buff precipitate; mp 226–231 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.33 (s, 2H, CH_2Cl), 6.04 (s, 1H, CH of Thiophene), 7.28–7.85 (m, 9H, ArH), 8.17 (s, 2H, NH); MS (%) 492 (100, M^+); Calcd (%) for $C_{22}H_{14}ClN_7OS_2$: C; 53.71, H; 2.87, N; 19.93. Found: C; 53.84, H; 2.72, N; 19.76.

4.1.6. General preparation of N^1-[4-substituted-5-(6-thioxo-5,6-dihydro[1,2,4]triazole [3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-2-substituted acetamide. Carbon disulfide (0.015 mol) was added dropwise with constant stirring to the solution of **1** or **10** (0.01 mol) in methanolic potassium hydroxide solution. Acidic alumina (5 g) was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 150–160 s at a power level of 300 W. The mixture was cooled and then product was extracted with dry methanol, which was then poured onto ice and acidified with dil. hydrochloric acid. The solid thus separated was filtered, washed thoroughly with water and recrystallized from aq Ethanol (70:30).

4.1.6.1. N^1-[4-Methyl-5-(6-thioxo-5,6-dihydro[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-acetamide (6a). Yield 68%; yellow precipitate; mp 187–192 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.13 (s, 3H, CH_3CO), 2.49 (s, 3H, CH_3), 5.82 (s, 1H, CH of Thiophene), 8.34 (s, 2H, NH); MS (%) 311 (78, M^+); Calcd (%) for $C_{10}H_9N_5OS_3$: C; 38.57, H; 2.91, N; 22.49. Found: C; 38.71, H; 2.78, N; 22.62.

4.1.6.2. N^1-[4-Methyl-5-(6-thioxo-5,6-dihydro[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-2-chloroacetamide (6b). Yield 71%; yellow precipitate; mp 190–194 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.38 (s, 3H, CH_3), 4.11 (s, 2H, CH_2Cl), 6.10 (s, 1H, CH of Thiophene), 8.09 (s, 2H, NH); MS (%) 346 (65, M^+); Calcd (%) for $C_{10}H_8N_5OS_3Cl$: C; 34.73, H; 2.33, N; 20.25. Found: C; 34.89, H; 2.51, N; 20.33.

4.1.6.3. N^1-[4-Phenyl-5-(6-thioxo-5,6-dihydro[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-acetamide (15a). Yield 69%; brown precipitate; mp 234–239 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.21 (s, 3H, CH_3CO), 5.82 (s, 1H, CH of Thiophene), 7.36–7.66 (m, 5H, ArH), 8.31 (s, 2H, NH); MS (%) 374 (90, M^+); Calcd (%) for $C_{15}H_{11}N_5OS_3$: C; 48.24, H; 2.97, N; 18.75. Found: C; 48.39, H; 3.17, N; 18.86.

4.1.6.4. N^1-[4-Phenyl-5-(6-thioxo-5,6-dihydro[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-2-chloroacetamide (15b). Yield 76%; buff precipitate; mp 204–209 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.09 (s, 2H, CH_2Cl), 6.10 (s, 1H, CH of Thiophene), 7.22–7.64 (m, 5H, ArH), 8.17 (s, 2H, NH); MS (%) 408 (82, M^+); Calcd (%) for $C_{15}H_{10}ClN_5OS_3$: C; 44.17, H; 2.47, N; 17.17. Found: C; 44.35, H; 2.51, N; 17.35.

4.1.7. General preparation of N^1-[4-substituted-5-(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-2-substituted acetamide. A solution of **1** or **10** (0.01 mol) and benzoic acid (0.01 mol) in $POCl_3$ (5 mL) was prepared. Acidic alumina (5 g) was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40–80 s at a power level of 300 W. The mixture was cooled and then poured onto ice and neutralized with aq potassium carbonate solution. The solid thus separated was filtered, washed thoroughly with water and recrystallized from hexane.

4.1.7.1. N^1-[4-Methyl-5-(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-acetamide (7a). Yield 77%; yellow precipitate; mp 183–187 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.11 (s, 3H, CH_3CO), 2.43 (s, 3H, CH_3), 6.07 (s, 1H, CH of Thiophene), 7.06–7.32 (m, 5H, ArH), 8.07 (s, 1H, NH); MS (%) 355 (100, M^+); Calcd (%) for $C_{16}H_{13}N_5OS_2$: C; 54.07, H; 3.69, N; 19.70. Found: C; 54.21, H; 3.84, N; 19.86.

4.1.7.2. N^1-[4-Methyl-5-(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-2-chloroacetamide (7b). Yield 70%; yellow precipitate; mp 191–195 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.50 (s, 3H, CH_3), 4.16 (s, 2H, CH_2Cl), 6.18 (s, 1H, CH of Thiophene), 7.13–7.39 (m, 5H, ArH), 8.14 (s, 1H, NH); MS (%) 390 (93.3, M^+); Calcd (%) for $C_{16}H_{12}N_5OS_2Cl$: C; 49.29, H; 3.10, N; 17.96. Found: C; 49.42, H; 3.26, N; 18.14.

4.1.7.3. N^1-[4-Phenyl-5-(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-acetamide (16a). Yield 77%; yellow precipitate; mp 194–198 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.18 (s, 3H, CH_3CO), 6.07 (s, 1H, CH of Thiophene), 7.12–7.67 (m, 10H, ArH), 8.12

(s, 1H, NH); MS (%) 417 (100, M^+); Calcd (%) for $C_{21}H_{15}N_5OS_2$: C; 60.41, H; 3.62, N; 16.77. Found: C; 60.62, H; 3.77, N; 16.86.

4.1.7.4. N^1-[4-Phenyl-5-(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-thienyl]-2-chloroacetamide (16b). Yield 78%; yellow precipitate; mp 211–216 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.21 (s, 2H, CH_2Cl), 6.18 (s, 1H, CH of Thiophene), 7.25–7.58 (m, 10H, ArH), 8.17 (s, 1H, NH); MS (%) 451 (100, M^+); Calcd (%) for $C_{21}H_{14}ClN_5OS_2$: C; 55.81, H; 3.12, N; 15.50. Found: C; 55.97, H; 3.28, N; 15.66.

4.1.8. General preparation of N^1-4-substituted-5-[6-(3-nitrophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-2-thienyl-2-substituted acetamide. A solution of **1** or **10** (0.01 mol) and *m*-nitrobenzaldehyde (0.01 mol) was prepared. Acidic alumina (5 g) was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40–80 s at a power level of 300 W. The mixture was cooled and then product was extracted with dry toluene, concentrated and cooled. The solid thus separated was filtered, washed thoroughly with water and recrystallized from ethanol:ethylacetate (2:8) mixture.

4.1.8.1. N^1-4-Methyl-5-[6-(3-nitrophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-thienyl-acetamide (8a). Yield 85%; yellow precipitate; mp 206–210 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.06 (s, 3H, CH_3CO), 2.56 (s, 3H, CH_3), 4.62 (s, 1H, CH of Thiadiazole), 5.93 (s, 1H, CH of Thiophene), 7.63–7.84 (m, 4H, ArH), 8.21 (s, 2H, NH); MS (%) 402 (100, M^+); Calcd (%) for $C_{16}H_{14}N_6O_3S_2$: C; 47.75, H; 3.51, N; 20.88. Found: C; 47.97, H; 3.67, N; 20.98.

4.1.8.2. N^1-4-Methyl-5-[6-(3-nitrophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-thienyl-2-chloroacetamide (8b). Yield 87%; yellow precipitate; mp 223–227 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.46 (s, 3H, CH_3), 3.61 (s, 1H, CH of Thiadiazole), 4.69 (s, 2H, CH_2Cl), 6.03 (s, 1H, CH of Thiophene), 7.69–7.92 (m, 4H, ArH), 8.26 (s, 2H, NH); MS (%) 437 (100, M^+); Calcd (%) for $C_{16}H_{13}N_6O_3S_2Cl$: C; 43.99, H; 3.00, N; 19.24. Found: C; 44.23, H; 3.18, N; 19.46.

4.1.8.3. N^1-4-Phenyl-5-[6-(3-nitrophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-thienyl-acetamide (17a). Yield 89%; yellow precipitate; mp 231–236 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.16 (s, 3H, CH_3CO), 4.73 (s, 1H, CH of Thiadiazole), 6.08 (s, 1H, CH of Thiophene), 7.11–7.56 (m, 9H, ArH), 8.26 (s, 2H, NH); MS (%) 465 (100, M^+); Calcd (%) for $C_{21}H_{16}N_6O_3S_2$: C; 54.30, H; 3.47, N; 18.09. Found: C; 54.52, H; 3.63, N; 18.22.

4.1.8.4. N^1-4-Phenyl-5-[6-(3-nitrophenyl)-5,6-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]-2-thienyl-2-chloroacetamide (17b). Yield 82%; buff precipitate; mp 214–219 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.75 (s, 1H, CH of Thiadiazole), 4.55 (s, 2H, CH_2Cl), 6.17 (s, 1H, CH of Thiophene), 7.24–7.60 (m, 9H, ArH), 8.11 (s, 2H, NH); MS (%) 499 (100, M^+); Calcd (%) for $C_{21}H_{15}ClN_6O_3S_2$: C; 50.55, H; 3.03, N; 16.84. Found: C; 50.68, H; 3.24, N; 16.92.

4.1.9. General preparation of N^1-{5-[7-[(Z)-1-(substitutedphenyl)methylidene]-6-(2,4-dichloro-5-fluorophenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl]-4-methyl/phenyl-2-thienyl}acetamide. A mixture of 2-Bromo-1-(2,4-Dichloro-5-fluoro-phenyl)-3-(4-substituted-phenyl)-propenone (structure A) (0.01 mol), compound **1** or **10** (0.01 mol) and solution of potassium hydroxide (10%, 2.5 mL) in ethanol (25 mL) was under reflux on a water bath for about 5 h. The reaction mixture was cooled and the precipitated solid was filtered, washed with water, dried and recrystallized.

4.1.9.1. N^1-(5-6-(2,4-Dichloro-5-fluorophenyl)-7-[(*Z*)-1-phenylmethylidene]-7*H*-[1,2,4]triazole[3,4-*b*][1,3,4]thiadiazin-3-yl-4-methyl-2-thienyl)acetamide (9a). Yield 88%; buff precipitate; mp 246–251 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.11 (s, 3H, $COCH_3$), 2.42 (s, 3H, CH_3), 6.23 (s, 1H, CH of Thiophene), 6.58 (s, 1H, =CH), 7.11–7.60 (m, 7H, ArH), 8.14 (s, 1H, NH); MS (%) 545 (100, M^+); Calcd (%) for $C_{24}H_{16}Cl_2FN_5OS_2$: C; 52.94, H; 2.96, N; 12.86. Found: C; 53.14, H; 3.19, N; 12.98.

4.1.9.2. N^1-(5-6-(2,4-Dichloro-5-fluorophenyl)-7-[(*Z*)-1-phenylmethylidene]-7*H*-[1,2,4] triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl-4-methyl-2-thienyl)-2-chloroacetamide (9b). Yield 81%; yellow precipitate; mp 239–244 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.13 (s, 3H, CH_3), 4.34 (s, 2H, CH_2Cl), 6.16 (s, 1H, CH of Thiophene), 6.47 (s, 1H, =CH), 7.18–7.55 (m, 7H, ArH), 8.19 (s, 1H, NH); MS (%) 579 (100, M^+); Calcd (%) for $C_{24}H_{15}Cl_3FN_5OS_2$: C; 49.79, H; 2.61, N; 12.10. Found: C; 49.94, H; 2.86, N; 12.38.

4.1.9.3. N^1-{5-[7-[(*Z*)-1-(2-Chlorophenyl)methylidene]-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]-4-methyl-2-thienyl}acetamide (9c). Yield 76%; buff precipitate; mp 227–232 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.07 (s, 3H, $COCH_3$), 2.31 (s, 3H, CH_3), 6.11 (s, 1H, CH of Thiophene), 6.47 (s, 1H, =CH), 7.17–7.51 (m, 6H, ArH), 8.17 (s, 1H, NH); MS (%) 579 (100, M^+); Calcd (%) for $C_{24}H_{15}Cl_3FN_5OS_2$: C; 49.79, H; 2.61, N; 12.10. Found: C; 49.82, H; 2.83, N; 12.44.

4.1.9.4. N^1-{5-[7-[(*Z*)-1-(2-Chlorophenyl)methylidene]-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*]-[1,3,4]thiadiazin-3-yl]-4-methyl-2-thienyl}-2-chloroacetamide (9d). Yield 79%; white crystals; mp 238–243 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.23 (s, 3H, CH_3), 4.20 (s, 2H, CH_2Cl), 6.12 (s, 1H, CH of Thiophene), 6.46 (s, 1H, =CH), 7.18–7.48 (m, 6H, ArH), 8.21 (s, 1H, NH); MS (%) 613 (100, M^+); Calcd (%) for $C_{24}H_{14}Cl_4FN_5OS_2$: C; 47.00, H; 2.30, N; 11.42. Found: C; 47.26, H; 2.42, N; 11.58.

4.1.9.5. N^1-{5-[7-[(*Z*)-1-(4-Chlorophenyl)methylidene]-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]-4-methyl-2-thienyl}acetamide (9e). Yield 78%; white crystals; mp 223–228 °C; ^{1}H

NMR (300 MHz, $CDCl_3$): δ 2.18 (s, 3H, $COCH_3$), 2.46 (s, 3H, CH_3), 6.27 (s, 1H, CH of Thiophene), 6.61 (s, 1H, =CH), 7.24–7.58 (m, 6H, ArH), 8.20 (s, 1H, NH); MS (%) 579 (100, M^+); Calcd (%) for $C_{24}H_{15}Cl_3FN_5OS_2$: C; 49.79, H; 2.61, N; 12.10. Found: C; 49.87, H; 2.74, N; 12.33.

4.1.9.6. N^1-{5-[7-[(*Z*)-1-(4-Chlorophenyl)methylidene]-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*]-[1,3,4]thiadiazin-3-yl]-4-methyl-2-thienyl}-2-chloroacetamide (9f). Yield 80%; white needles; mp 232–237 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.08 (s, 3H, CH_3), 4.18 (s, 2H, CH_2Cl), 6.08 (s, 1H, CH of Thiophene), 6.47 (s, 1H, =CH), 7.10–7.52 (m, 6H, ArH), 8.10 (s, 1H, NH); MS (%) 613 (100, M^+); Calcd (%) for $C_{24}H_{14}Cl_4FN_5OS_2$: C; 47.00, H; 2.30, N; 11.42. Found: C; 47.24, H; 2.46, N; 11.57.

4.1.9.7. N^1-(5-6-(2,4-Dichloro-5-fluorophenyl)-7-[(*Z*)-1-(3-nitrophenyl)methylidene]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl-4-methyl-2-thienyl)acetamide (9g). Yield 75%; buff precipitate; mp 229–234 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.03 (s, 3H, $COCH_3$), 2.25 (s, 3H, CH_3), 6.15 (s, 1H, CH of Thiophene), 6.43 (s, 1H, =CH), 7.17–7.66 (m, 6H, ArH), 8.24 (s, 1H, NH); MS (%) 589 (100, M^+); Calcd (%) for $C_{24}H_{15}Cl_2FN_6O_3S_2$: C; 48.90, H; 2.56, N; 14.26. Found: C; 49.04, H; 2.67, N; 14.38.

4.1.9.8. N^1-(5-6-(2,4-Dichloro-5-fluorophenyl)-7-[(*Z*)-1-(3-nitrophenyl)methylidene]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl-4-methyl-2-thienyl)-2-chloroacetamide (9h). Yield 84%; yellow precipitate; mp 235–240 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.14 (s, 3H, CH_3), 4.32 (s, 2H, CH_2Cl), 6.13 (s, 1H, CH of Thiophene), 6.48 (s, 1H, =CH), 7.21–7.58 (m, 6H, ArH), 8.25 (s, 1H, NH); MS (%) 624 (100, M^+); Calcd (%) for $C_{24}H_{14}Cl_3FN_6O_3S_2$: C; 46.20, H; 2.26, N; 13.47. Found: C; 46.20, H; 2.26, N; 13.47.

4.1.9.9. N^1-(5-6-(2,4-Dichloro-5-fluorophenyl)-7-[(*Z*)-1-phenylmethylidene]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl-4-phenyl-2-thienyl)acetamide (18a). Yield 88%; white precipitate; mp 217–222 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.27 (s, 3H, $COCH_3$), 6.17 (s, 1H, CH of Thiophene), 6.35 (s, 1H, =CH), 7.22–7.83 (m, 12H, ArH), 8.09 (s, 1H, NH); MS (%) 607 (100, M^+); Calcd (%) for $C_{29}H_{18}Cl_2FN_5OS_2$: C; 57.43, H; 2.99, N; 11.55. Found: C; 57.66, H; 3.18, N; 11.68.

4.1.9.10. N^1-(5-6-(2,4-Dichloro-5-fluorophenyl)-7-[(*Z*)-1-phenylmethylidene]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl-4-methyl-2-thienyl)-2-chloroacetamide (18b). Yield 83%; white precipitate; mp 225–229 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 4.13 (s, 2H, CH_2Cl), 6.24 (s, 1H, CH of Thiophene), 6.51 (s, 1H, =CH), 7.26–7.79 (m, 12H, ArH), 8.13 (s, 1H, NH); MS (%) 641 (100, M^+); Calcd (%) for $C_{29}H_{17}Cl_3FN_5OS_2$: C; 54.34, H; 2.67, N; 10.93. Found: C; 54.52, H; 2.76, N; 11.09.

4.1.9.11. N^1-{5-[7-[(*Z*)-1-(2-Chlorophenyl)methylidene]-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]-4-phenyl-2-thienyl}-acetamide (18c). Yield 78%; white precipitate; mp 231–236 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.13 (s, 3H, $COCH_3$), 6.19 (s, 1H, CH of Thiophene), 6.51 (s, 1H, =CH), 7.23–7.75 (m, 11H, ArH), 8.31 (s, 1H, NH); MS (%) 641 (100, M^+); Calcd (%) for $C_{29}H_{17}Cl_3FN_5OS_2$: C; 54.34, H; 2.67, N; 10.93. Found: C; 54.57, H; 2.84, N; 10.74.

4.1.9.12. N^1-{5-[7-[(*Z*)-1-(2-Chlorophenyl)methylidene]-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-3-yl]-4-phenyl-2-thienyl}-2-chloroacetamide (18d). Yield 76%; white precipitate; mp 242–246 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 4.28 (s, 2H, CH_2Cl), 6.17 (s, 1H, CH of Thiophene), 6.38 (s, 1H, =CH), 7.21–7.73 (m, 11H, ArH), 8.17 (s, 1H, NH); MS (%) 675 (100, M^+); Calcd (%) for $C_{29}H_{16}Cl_4FN_5OS_2$: C; 51.57, H; 2.39, N; 10.37. Found: C; 51.69, H; 2.48, N; 10.47.

4.1.9.13. N^1-{5-[7-[(*Z*)-1-(4-Chlorophenyl)methylidene]-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-3-yl]-4-phenyl-2-thienyl}acetamide (18e). Yield 73%; buff precipitate; mp 238–242 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.03 (s, 3H, $COCH_3$), 6.15 (s, 1H, CH of Thiophene), 6.58 (s, 1H, =CH), 7.11–7.74 (m, 11H, ArH), 8.15 (s, 1H, NH); MS (%) 641 (100, M^+); Calcd (%) for $C_{29}H_{17}Cl_3FN_5OS_2$: C; 54.34, H; 2.67, N; 10.93. Found: C; 54.46, H; 2.78, N; 10.89.

4.1.9.14. N^1-{5-[7-[(*Z*)-1-(4-Chlorophenyl)methylidene]-6-(2,4-dichloro-5-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-3-yl]-4-phenyl-2-thienyl}-2-chloroacetamide (18f). Yield 70%; buff precipitate; mp 232–236 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 4.22 (s, 2H, CH_2Cl), 6.15 (s, 1H, CH of Thiophene), 6.51 (s, 1H, =CH), 7.24–7.72 (m, 11H, ArH), 8.16 (s, 1H, NH); MS (%) 675 (100, M^+); Calcd (%) for $C_{29}H_{16}Cl_4FN_5OS_2$: C; 51.57, H; 2.39, N; 10.37. Found: C; 51.72, H; 2.53, N; 10.50.

4.1.9.15. N^1-(5-6-(2,4-Dichloro-5-fluorophenyl)-7-[(*Z*)-1-(3-nitrophenyl)methylidene]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl-4-phenyl-2-thienyl)acetamide (18g). Yield 87%; yellow precipitate; mp 226–231 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.17 (s, 3H, $COCH_3$), 6.25 (s, 1H, CH of Thiophene), 6.55 (s, 1H, =CH), 7.23–7.82 (m, 11H, ArH), 8.18 (s, 1H, NH); MS (%) 652 (100, M^+); Calcd (%) for $C_{29}H_{17}Cl_2FN_6O_3S_2$: C; 53.46, H; 2.63, N; 12.90. Found: C; 53.57, H; 2.76, N; 12.97.

4.1.9.16. *N*1-(5-6-(2,4-Dichloro-5-fluorophenyl)-7-[(*Z*)-1-(3-nitrophenyl)methylidene]-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl-4-phenyl-2-thienyl)-2-chloroacetamide (18h). Yield 88%; yellow precipitate; mp 228–235 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 4.18 (s, 2H, CH_2Cl), 6.21 (s, 1H, CH of Thiophene), 6.56 (s, 1H, =CH), 7.10–7.84 (m, 11H, ArH), 8.17 (s, 1H, NH); MS (%) 686 (100, M^+); Calcd (%) for $C_{29}H_{16}Cl_3FN_6O_3S_2$: C; 50.78, H; 2.35, N; 12.25. Found: C; 50.92, H; 2.49, N; 12.38.

4.2. Pharmacology

4.2.1. Cyclin-dependent kinase 5/p25 inhibiting activity. Kinase inhibition was measured by use of scintillation proximity assays (SPA).[27] Enzyme activities were as-

sayed as the incorporation of [33P] from the gamma phosphate of [33P] ATP (Amersham, cat. no. AH-9968) into biotinylated peptide substrate PKTPKKA KKL. 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 uCi/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[29] 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.

Acknowledgment

Authors are thankful to Dr. K.G. Bothara, Principal, AISSMS College of Pharmacy, Pune, India, for providing the facilities.

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