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A CYCLIN DEPENDENT KINASE-5 INHIBITORS: SYNTHESIS AND SAR OF CLUBBED TRIAZOLE.

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ABSTRACT

The present study, a series of clubbed triazole generated by microwave organic reaction enhancement (MORE) method and examine for their cyclin dependent kinase-5 (cdk5) inhibitors, potentially useful for treatment of Alzheimer disease. Evaluation of the structure active relationship (SAR) of substitution with-in these series has allowed the identification of a range of compounds which significantly reduce brain cdk5/p25 by scintillation proximate assay method. The cdk5/p25 inhibitor data of the tested compounds indicated that **3E, 4E, 6E, 8E** and **8I** showed better activity out of which **8E** and **8I** shows equally selective versus cdk2.

Key words: Cyclohexyl thiophene, triazole, cdk5/p25, Alzheimer's disease, MORE.

INTRODUCTION:

Cyclin-dependent kinase 5 (CDK5) plays an essential role in the development of the central nervous system. Its deregulation has profound cytotoxic effects and has been implicated in the development of neurodegenerative diseases such as Alzheimer's disease and amyotrophic lateral sclerosis, for which no effective treatment exists today[1]. Cdk5 is a member of a family of proline-directed serine/threonine kinases[1, 2]. The serine/threonine kinase cdk5 along with its cofactor p25[3] (or the longer cofactor, p35) has been supposed to hyperphosphorylate tau[4], leading to the formation of paired helical filaments and deposition of cytotoxic neurofibrillary

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tangles[5] and thus responsible to neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, stroke[6] 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[7] 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[8] as the potential inhibitors of cdk5/p25 for the treatment of Alzheimer's disease and other neurodegenerative disorders.[9-12]

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 (IC_{50} = 46 ± 2 nM).

In recent years, environmentally benign synthetic methods have received considerable attention and solvent free protocols are reported[13]. A fast, highly efficient and eco-friendly solvent-free chemical transformation, for the synthesis of title compounds with its derivatives[14-28], by MORE (Microwave organic reaction enhancement), using acidic alumina is designed.

MATERIALS AND METHODS

General Instrumentation

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. 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 Analyzer. 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).

Experimental

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-Benzoylamino-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl)-acetyl]-hydrazinecarbodithioi c 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 compounds were prepared according the literature[14, 24, 28].

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-tetrahydro-benzo(b)thiophen-2-yl]-2-propionicacid **(2D).** Above titled compounds were prepared according the literature[14, 24, 28].

Preparation of 2-Chloro-N-{3-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b] thophen-3-ylmethyl]-5-mercapto-[1,2,4]triazol-4-yl}-benzamide (3I).

The triazole **2A** in 10% NaOH (20 ml, 1 mmol) was treated drop wise with an equimolar amount of the 2-chlorobenzoyl chloride at (0°C), which was stirred for 30-45 min. At the end of stirring a buff colored precipitate was observed. It was then filtered, washed thoroughly with water and crystallized from ethanol **3I.** Buff white crystals, yield (78 %), mp 285–287 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.38-1.61 (m, 4H, cyclohexane), 2.48-2.56 (t, 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), 7.45-7.62 (m, 4H, ArH), 8.08 (s, 2H, NH); MS (%) 496 (M^+, 71); Anal. Calcd for $C_{20}H_{19}Cl_2N_5O_2S_2$: C, 48.39; H, 3.86; N, 14.11. Found: C, 48.46; H, 3.93; N, 14.17. Other compounds in this series were prepared in similar way.

2,4,6-Trichloro-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).

Brown crystals, yield (65 %), mp 276–278°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 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), 7.34 (d, 2H, ArH), 8.05 (s, 2H, NH); MS (%) 565 (M^+, 78); Anal. Calcd for $C_{20}H_{17}Cl_4N_5O_2S_2$: C, 42.49; H, 3.03; N, 12.39. Found: C, 42.57; H, 3.12; N, 12.44.

Preparation of 2-Chloro-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 (4I).

The triazole **3I** (1 mmol) was mixed with chloroacetonitrile (1.2 ml, 2 mmol) and dissolved in water (25 ml). Neutralization with sodium carbonate gave a precipitate, which was filtered, washed with cold water (2 x 20 ml), and crystallized from ethanol **4I.** Yellow crystals, yield (79 %), mp 247–249 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.34-1.52 (m, 4H, cyclohexane), 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), 7.45-7.62 (m, 4H, ArH), 8.04 (s, 2H, NH); MS (%) 536 (M^+, 43); 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, 3.67; N, 15.59. Other compounds in this series were prepared in similar way.

2,4,6-Trichloro-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 (4E).

Yellow crystals, yield (87 %), mp 261–263 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 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), 7.35 (d, 2H, ArH), 8.04 (s, 2H, NH); MS (%) 604 (M^+, 74.2); Anal. Calcd for $C_{22}H_{18}Cl_4N_6O_2S_2$: C, 43.72; H, 3.00; N, 13.91. Found: C, 43.80; H, 3.14; N, 14.01.

Preparation of [5-[2-(2-Chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-4-(2-chloro-benzoylamino)-4H-[1,2,4]triazol-3-ylsulfanyl]-acetic acid methyl ester (5I).

A solution of triazole **3I** (1 mmol), sodium hydroxide (0.4 g, 1 mmol) 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 300W. 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 **5I.** Pale green crystals, yield (77 %), mp 260–262 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.34-1.59 (m, 4H, cyclohexane), 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), 7.34-7.56 (m, 4H, ArH), 8.02 (broad, 2H, NH); MS (%) 569 (M^+, 82); Anal. Calcd for $C_{23}H_{23}Cl_2N_5O_4S_2$: C, 48.59; H, 4.08; N, 12.32. Found: C, 48.49; H, 3.97; N, 12.17. Other compounds in this series were prepared in similar way.

[5-[2-(2-Chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-4-(2,4,6 -trichloro-benzoylamino)-4H-[1,2,4]triazol-3-ylsulfanyl]-acetic acid methyl ester (5E).

Brown crystals, yield (68 %), mp 274–276 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 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), 7.33 (d, 2H, ArH), 8.01 (broad, 2H, NH); MS (%) 637 (M^+, 56.8); Anal. Calcd for $C_{23}H_{21}Cl_4N_5O_4S_2$: C, 43.34; H, 3.32; N, 10.99. Found: C, 43.51; H, 3.42; N, 11.08.

Preparation of 2-Chloro-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(6I).

A solution of **5I** (1 mmol) with hydrazine hydrate (98%) (5 ml, 1 mmol) was prepared in ethanol (10 ml). To this acidic alumina (10 g) was added. Ethanol then was evaporated invacuo, and mixture was kept inside the alumina bath and irradiated for 5-6 min at the power level of 300W. 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 **6I**.Brown crystals, yield (69 %), mp 248–250 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.24-1.48 (m, 4H, cyclohexane), 1.86 (d, 2H, NH_2, J = 6.3 Hz), 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), 7.48-7.64 (m, 4H, ArH), 8.09 (broad, 2H, NH); MS (%) 569 (M^+, 89.3); Anal. Calcd for $C_{22}H_{23}Cl_2N_7O_3S_2$: C, 46.48; H, 4.08; N, 17.25. Found: C, 46.61; H, 4.22; N, 17.38. Other compounds in this series were prepared in similar way.

2,4,6-Trichloro-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 (6E).

Brown crystals, yield (63 %), mp above 300 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 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), 7.31 (d, 2H, ArH), 8.06 (broad, 2H, NH); MS (%) 637 (M^+, 47.3); Anal. Calcd for $C_{22}H_{21}Cl_4N_7O_3S_2$: C, 41.46; H, 3.32; N, 15.38. Found: C, 41.53; H, 3.41; N, 15.50.

Preparation of 3-Chloro-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) -propionamide (7I).

To a solution of **6I (**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 (10g) was added. Dichloromethane then was evaporated invacuo, and mixture was kept inside the alumina bath and irradiated for 5-6 min at the power level of 300W. 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 **7I.** Dark brown crystals, yield (76 %), mp 289–291 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.33-1.55 (m, 4H, cyclohexane), 2.44-2.62 (t, 4H, cyclohexane CH_2, J = 4.5 Hz), 2.47-2.51 (t, 2H, CH_2CH_2Cl, J = 4.9 Hz), 3.58-

3.71 (t, 2H, CH_2CH_2Cl, J = 6.7 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), 8.02 (s, 2H, NH); MS (%) 597 (M^+, 45.5); Anal. Calcd for $C_{20}H_{24}Cl_3N_7O_4S_2$: C, 40.24; H, 4.05; N, 16.42. Found: C, 40.28; H, 4.16; N, 16.57. Other compounds in this series were prepared in similar way.

2-Chloro-N-(3-[2-(2-chloro-acetylamino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl methyl]-5-{2-[N'-(2-chloro-acetyl)-hydrazino]-2-oxo-ethylsulfanyl}-[1,2,4]triazol-4-yl)-benzamide (7AG).

Yellow crystals, Yield (79 %); mp 249–251 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.36-1.51 (m, 4H, cyclohexane), 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), 7.27-7.55 (m, 4H, ArH), 8.07 (s, 2H, NH); MS (%) 645 (M^+,100); Anal. Calcd. for $C_{24}H_{24}Cl_3N_7O_4S_2$: C, 44.69; H, 3.75; N, 15.20. Found: C, 44.81; H, 3.85; N, 15.34.

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}-3-chloro propionamide(7E).

Yellow crystals, yield (64 %), mp 227–229 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.65-1.64 (m, 4H, cyclohexane), 2.31-2.45 (t, 4H, cyclohexane CH_2, J = 4.2 Hz), 2.46-2.54 (t, 2H, CH_2CH_2Cl, J = 4.5 Hz), 3.47-3.66 (t, 2H, CH_2CH_2Cl, J = 7.2 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, 5H, ArH), 8.03 (s, 2H, NH); MS (%) 625 (M^+,89.6); Anal. Calcd for $C_{25}H_{27}Cl_2N_7O_4S_2$: C, 48.08; H, 4.36; N, 15.70. Found: C, 48.17; H, 4.44; N, 15.89.

2,4,6-Trichloro-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 (7U).

Pale brown crystals, yield (78 %), mp 225–227 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 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), 7.30 (d, 2H, ArH), 8.05 (s, 2H, NH); MS (%) 714 (M^+,100); Anal. Calcd for $C_{24}H_{22}Cl_5N_7O_4S_2$: C, 40.38; H, 3.11; N, 13.73. Found: C, 40.41; H, 3.26; N, 13.88.

Preparation of N-{3-(Benzylidene-hydrazinocarbonylmethylsulfanyl)-5-[2-(2-chloro-acetyl amino)-4,5,6,7-tetrahydro-benzo[b]thiophen-3-ylmethyl]-[1,2,4]triazol-4-yl}-2-chloro-benzamide (8I).

A solution of **6I** (1mmol) with aromatic aldehyde (1 mmol) was prepared in ethanol (10 ml). To this acidic alumina (10 g) was added. Ethanol then was evaporated invacuo, and mixture was kept inside the alumina bath and irradiated for 1 min at the power level of 300W. 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 **8I**. Dark brown crystals, yield (66 %), mp decomposed around 226–228 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.42-1.60 (m, 4H, cyclohexane), 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), 7.12-7.52 (m, 9H, ArH), 8.01 (s, 3H, NH), 8.12 (s, 1H, N=CH); MS (%) 657 (M^+, 100); Anal. Calcd for $C_{29}H_{27}Cl_2N_7O_3S_2$: C, 53.05; H, 4.14; N, 14.93. Found: C, 53.11; H, 4.24; N, 15.04. Other compounds in this series were prepared in similar way.

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}-2,4,6-trichloro-benzamide (8E).

Brown crystals, yield (74 %), mp 177–179 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 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, 5H, ArH), 7.33 (d, 2H, ArH), 7.96 (s, 3H, NH), 8.13 (s, 1H, N=CH); MS (%) 726 (M^+,100); Anal. Calcd for $C_{29}H_{25}Cl_4N_7O_3S_2$: C, 48.01; H, 3.47; N, 13.51. Found: C, 47.89; H, 3.34; N, 13.41.

Cyclic-Dependent Kinase 5/p25 inhibiting activity

Kinase inhibit ion 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 PKTPKKAKKL. Reactions were carried out in a buffer containing Tris-HCI (50 mM), pH 8.0; $MgCl_2$ (10mM), Na_3VO_4 (0.1mM), and DTT (1mM). The final concentration of ATP was (0.5µM) (final specific radioactivity of 4uCi/nmol), and the final concentration of substrate was (0.75µM). Reactions, initiated by the addition of cdk5 and activator protein p25, were carried out at room temperature for 60 minutes. Reactions were stopped by addition of 0.6 volume of buffer containing (final concentrations): EDTA (2.5mM), 0.05 %Triton-X 100, ATP (100µM), and streptavidin coated SPA beads (1.25 mg/mL) (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[24] 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.

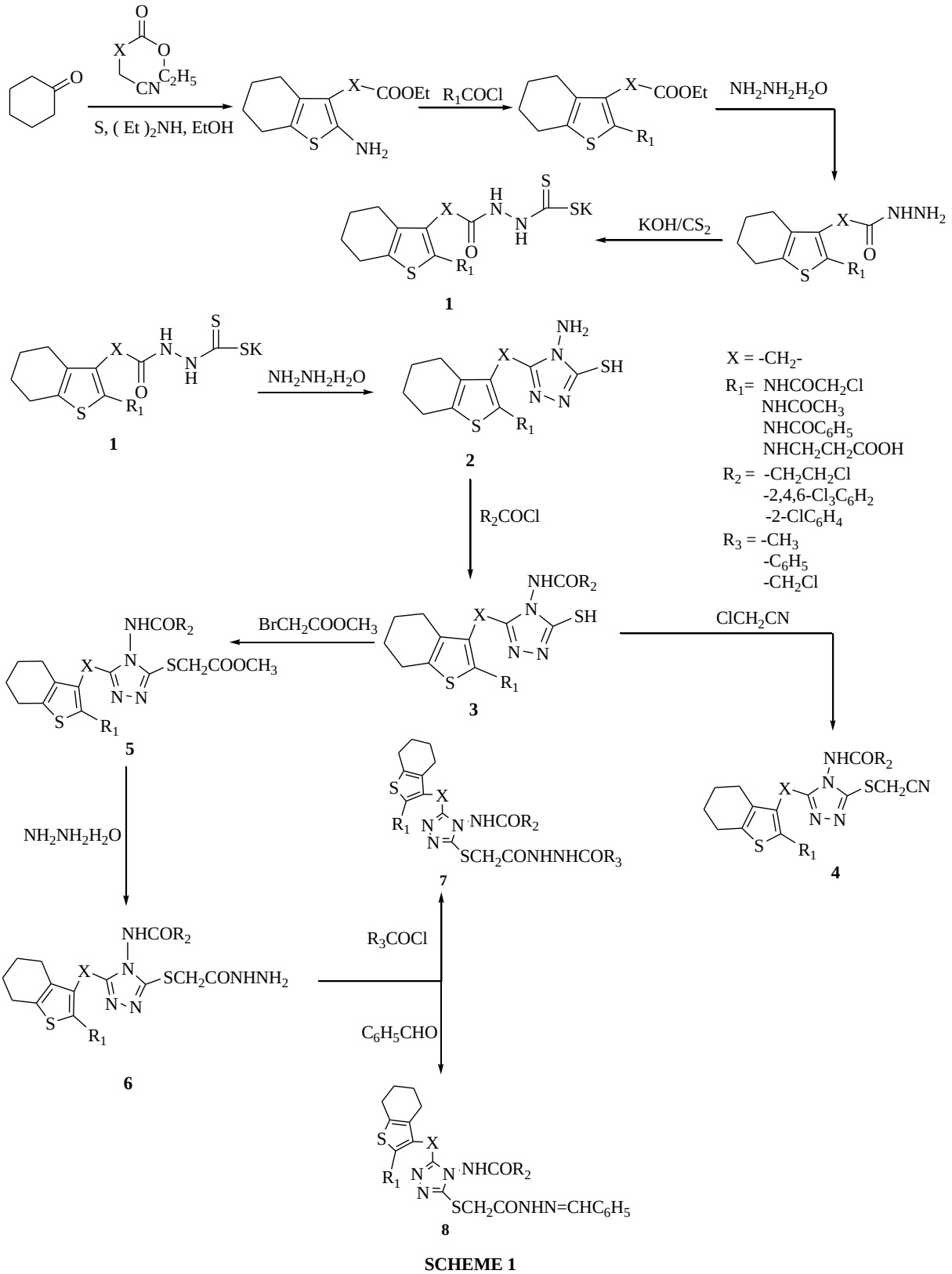
RESULT AND DISCUSSION

Synthesis

Focusing on synthetic efforts on varying the amide side group of cyclohexyl thiophene, triazole and sulfhydryl side group of triazole, compound **1A-D**, **2A-D**, **3A-L**, **4A-L**, **5A-L**, **6A-L**, **7AA-AJ**, **7M-7Z** and **8A-L** were synthesized as per literature. The cyclization of compounds having thiocarbazate structure has shown to be an excellent strategy for synthesis of triazole. Thiacarbazate compound **1A-D** adsorbed on acidic alumina (Aluminium oxide, acidic, Brockmann I, ~150 mesh, 58Å CAMAG 506-C-I, surface area 155m^2/g. pH = 6.0), reacting with hydrazine hydrate to yield **2A-D**. When **2A-D** subjected to Schotten Baumann reaction by using 4-chlorobenzoyl chloride at (0˚C) yields **3A-L** (Figure1). The next transformation is carried out into the **4A-L** by treating **3A-L** with chloroacetonitrile. Compound **3A-L**, treated with methyl bromoacetate in basic condition produce **5A-L.** Chemical transformation of **5A-L** by Knoevengel condensation was carried out by treating it with hydrazine hydrate to achieve **6A-L**. Thus hydrazide intermediate **6A-L** with the aforementioned cyclohexyl thiophene would be viable surrogate for the synthesis of **7A-L** by Schotten Baumann reaction, Schiff base **8A-L** formation with benzaldehyde. The NMR spectra confirmed formation of schiff bases from the hydrazides by the presence of the singlet peak of N=CH at 8.1 δ.

Our efforts to develop small molecular ATP-competitive cdk inhibitors as Alzheimer therapeutics have resulted in the discovery of novel series 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**. Kinase inhibition was measured by the use of scintillation proximate assays (SPA). The results of the assays are reported in **Table (1-3).** During the preliminary screening compound **2A** has emerged as hit cdk5/p25 (IC_{50} = 043 ± 02 nM) with good potency and more opportunities for chemical transformation for the optimization. Testing of **2A** against cdk2 revealed that **2A** was essentially equipotent at inhibiting cdk2/cyclin E (IC_{50} = 52 ± 5 nM), a cancer target. The final concentration used was the same in both the cdk5/p25 and cdk2/cyclin E assay.

The discovery of related series of cdk5/p25 inhibitors, **(2A)** obtained through structure based analogue synthesis and optimization. Certain chemical modification have been performed, to achieve an objective of improvement in cdk5 potency with minimize cdk2 activity.



SCHEME 1

Figure 1: Reaction protocol for the synthesis of clubbed triazolyl thiophene derivatives (2A-D) Cyclin-dependent kinase 5/p25 inhibiting activity

Table 1: cdk5 (IC_{50}) values of the compound 1A-7L.

Sr. No.	R1	R2	R3	[a]Cdk5 IC_{50} (nM)	Sr. No.	R1	R2	R3	[a]Cdk5 IC_{50} (nM)
1A	$NHCOCH_2Cl$	-	-	247 ± 37	**5H**	$NHCH_2CH_2COOH$	2,4,6-$Cl_3C_6H_2$	-	441 ± 109
1B	$NHCOCH_3$	-	-	346 ± 41	**5I**	$NHCOCH_2Cl$	2-ClC_6H_4	-	395 ± 112
1C	$NHCOC_6H_5$	-	-	373 ± 39	**5J**	$NHCOCH_3$	2-ClC_6H_4	-	389 ± 97
1D	$NHCH_2CH_2COOH$	-	-	397 ± 35	**5K**	$NHCOC_6H_5$	2-ClC_6H_4	-	467 ± 103
2A	$NHCOCH_2Cl$	-	-	**043 ± 02**	**5L**	$NHCH_2CH_2COOH$	2-ClC_6H_4	-	733 ± 137
2B	$NHCOCH_3$	-	-	386 ± 85	**6A**	$NHCOCH_2Cl$	CH_2CH_2Cl	-	439 ± 82
2C	$NHCOC_6H_5$	-	-	621 ± 23	**6B**	$NHCOCH_3$	CH_2CH_2Cl	-	964 ± 52
2D	$NHCH_2CH_2COOH$	-	-	268 ± 31	**6C**	$NHCOC_6H_5$	CH_2CH_2Cl	-	336 ± 34
3A	$NHCOCH_2Cl$	CH_2CH_2Cl	-	572 ± 63	**6D**	$NHCH_2CH_2COOH$	CH_2CH_2Cl	-	201 ± 31
3B	$NHCOCH_3$	CH_2CH_2Cl	-	460 ± 71	**6E**	$NHCOCH_2Cl$	2,4,6-$Cl_3C_6H_2$	-	**037 ± 01**
3C	$NHCOC_6H_5$	CH_2CH_2Cl	-	891 ± 112	**6F**	$NHCOCH_3$	2,4,6-$Cl_3C_6H_2$	-	**059 ± 08**
3D	$NHCH_2CH_2COOH$	CH_2CH_2Cl	-	457 ± 43	**6G**	$NHCOC_6H_5$	2,4,6-$Cl_3C_6H_2$	-	164 ± 72
3E	$NHCOCH_2Cl$	2,4,6-$Cl_3C_6H_2$	-	**041 ± 08**	**6H**	$NHCH_2CH_2COOH$	2,4,6-$Cl_3C_6H_2$	-	255 ± 79
3F	$NHCOCH_3$	2,4,6-$Cl_3C_6H_2$	-	183 ± 11	**6I**	$NHCOCH_2Cl$	2-ClC_6H_4	-	**061± 01**
3G	$NHCOC_6H_5$	2,4,6-$Cl_3C_6H_2$	-	343 ± 12	**6J**	$NHCOCH_3$	2-ClC_6H_4	-	249 ± 36
3H	$NHCH_2CH_2COOH$	2,4,6-$Cl_3C_6H_2$	-	674 ± 67	**6K**	$NHCOC_6H_5$	2-ClC_6H_4	-	180 ± 27
3I	$NHCOCH_2Cl$	2-ClC_6H_4	-	**064± 02**	**6L**	$NHCH_2CH_2COOH$	2-ClC_6H_4	-	531 ± 24
3J	$NHCOCH_3$	2-ClC_6H_4	-	384 ± 47	**7A**	$NHCOCH_2Cl$	CH_2CH_2Cl	CH_3	468 ± 64
3K	$NHCOC_6H_5$	2-ClC_6H_4	-	418 ± 46	**7AA**	$NHCOC_6H_5$	2-ClC_6H_4	CH_3	639 ± 67
3L	$NHCH_2CH_2COOH$	2-ClC_6H_4	-	633 ± 24	**7AB**	$NHCH_2CH_2COOH$	2-ClC_6H_4	CH_3	866 ± 48
4A	$NHCOCH_2Cl$	CH_2CH_2Cl	-	485 ± 68	**7AC**	$NHCOCH_2Cl$	2-ClC_6H_4	C_6H_5	773 ± 34
4B	$NHCOCH_3$	CH_2CH_2Cl	-	715 ± 75	**7AD**	$NHCOCH_3$	2-ClC_6H_4	C_6H_5	936 ± 46
4C	$NHCOC_6H_5$	CH_2CH_2Cl	-	651 ± 72	**7AE**	$NHCOC_6H_5$	2-ClC_6H_4	C_6H_5	911 ± 83
4D	$NHCH_2CH_2COOH$	CH_2CH_2Cl	-	2822 ± 92	**7AF**	$NHCH_2CH_2COOH$	2-ClC_6H_4	C_6H_5	676 ± 68
4E	$NHCOCH_2Cl$	2,4,6-$Cl_3C_6H_2$	-	**035 ± 04**	**7AG**	$NHCOCH_2Cl$	2-ClC_6H_4	CH_2Cl	652 ± 67
4F	$NHCOCH_3$	2,4,6-$Cl_3C_6H_2$	-	193 ± 61	**7AH**	$NHCOCH_3$	2-ClC_6H_4	CH_2Cl	674 ± 74
4G	$NHCOC_6H_5$	2,4,6-$Cl_3C_6H_2$	-	386 ± 78	**7AI**	$NHCOC_6H_5$	2-ClC_6H_4	CH_2Cl	758 ± 72
4H	$NHCH_2CH_2COOH$	2,4,6-$Cl_3C_6H_2$	-	874 ± 86	**7AJ**	$NHCH_2CH_2COOH$	2-ClC_6H_4	CH_2Cl	865 ± 38
4I	$NHCOCH_2Cl$	2-ClC_6H_4	-	**058 ± 06**	**7B**	$NHCOCH_3$	CH_2CH_2Cl	CH_3	641 ± 49

4J	$NHCOCH_3$	2-ClC_6H_4	-	358 ± 84	**7C**	$NHCOC_6H_5$	CH_2CH_2Cl	CH_3	518 ± 30
4K	$NHCOC_6H_5$	2-ClC_6H_4	-	664 ± 64	**7D**	$NHCH_2CH_2COOH$	CH_2CH_2Cl	CH_3	552 ± 28
4L	$NHCH_2CH_2COOH$	2-ClC_6H_4	-	441 ± 74	**7E**	$NHCOCH_2Cl$	CH_2CH_2Cl	C_6H_5	617 ± 23
5A	$NHCOCH_2Cl$	CH_2CH_2Cl	-	943 ± 106	**7F**	$NHCOCH_3$	CH_2CH_2Cl	C_6H_5	525 ± 47
5B	$NHCOCH_3$	CH_2CH_2Cl	-	789 ± 35	**7G**	$NHCOC_6H_5$	CH_2CH_2Cl	C_6H_5	683 ± 41
5C	$NHCOC_6H_5$	CH_2CH_2Cl	-	593 ± 38	**7H**	$NHCH_2CH_2COOH$	CH_2CH_2Cl	C_6H_5	771 ± 17
5D	$NHCH_2CH_2COOH$	CH_2CH_2Cl	-	841 ± 18	**7I**	$NHCOCH_2Cl$	CH_2CH_2Cl	CH_2Cl	429 ± 67
5E	$NHCOCH_2Cl$	2,4,6-$Cl_3C_6H_2$	-	281 ± 47	**7J**	$NHCOCH_3$	CH_2CH_2Cl	CH_2Cl	360 ± 38
5F	$NHCOCH_3$	2,4,6-$Cl_3C_6H_2$	-	278 ± 74	**7K**	$NHCOC_6H_5$	CH_2CH_2Cl	CH_2Cl	440 ± 22
5G	$NHCOC_6H_5$	2,4,6-$Cl_3C_6H_2$	-	353 ± 71	**7L**	$NHCH_2CH_2COOH$	CH_2CH_2Cl	CH_2Cl	585 ± 39

a: Inhibitory concentration

Table 2. cdk5 (IC_{50}) values of the compound 7M-8L

Sr. No.	**R1**	**R2**	**R3**	**[a]Cdk5 IC_{50} (nM)**	**Sr. No.**	**R1**	**R2**	**R3**	**[a]Cdk5 IC_{50} (nM)**
7M	$NHCOCH_2Cl$	2,4,6-$Cl_3C_6H_2$	CH_3	150 ± 61	**7Z**	$NHCOCH_3$	2-ClC_6H_4	CH_3	427 ± 48
7N	$NHCOCH_3$	2,4,6-$Cl_3C_6H_2$	CH_3	148 ± 46	**8A**	$NHCOCH_2Cl$	CH_2CH_2Cl	-	435 ± 21
7O	$NHCOC_6H_5$	2,4,6-$Cl_3C_6H_2$	CH_3	364 ± 77	**8B**	$NHCOCH_3$	CH_2CH_2Cl	-	513 ± 76
7P	$NHCH_2CH_2COOH$	2,4,6-$Cl_3C_6H_2$	CH_3	167 ± 48	**8C**	$NHCOC_6H_5$	CH_2CH_2Cl	-	617 ± 23
7Q	$NHCOCH_2Cl$	2,4,6-$Cl_3C_6H_2$	C_6H_5	268 ± 92	**8D**	$NHCH_2CH_2COOH$	CH_2CH_2Cl	-	758 ± 37
7R	$NHCOCH_3$	2,4,6-$Cl_3C_6H_2$	C_6H_5	213 ± 45	**8E**	$NHCOCH_2Cl$	2,4,6-$Cl_3C_6H_2$	-	**032 ± 01**
7S	$NHCOC_6H_5$	2,4,6-$Cl_3C_6H_2$	C_6H_5	266 ± 73	**8F**	$NHCOCH_3$	2,4,6-$Cl_3C_6H_2$	-	125 ± 28
7T	$NHCH_2CH_2COOH$	2,4,6-$Cl_3C_6H_2$	C_6H_5	234 ± 80	**8G**	$NHCOC_6H_5$	2,4,6-$Cl_3C_6H_2$	-	187 ± 52
7U	$NHCOCH_2Cl$	2,4,6-$Cl_3C_6H_2$	CH_2Cl	**065 ± 12**	**8H**	$NHCH_2CH_2COOH$	2,4,6-$Cl_3C_6H_2$	-	267 ± 52
7V	$NHCOCH_3$	2,4,6-$Cl_3C_6H_2$	CH_2Cl	243 ± 74	**8I**	$NHCOCH_2Cl$	2-ClC_6H_4	-	**032 ± 09**
7W	$NHCOC_6H_5$	2,4,6-$Cl_3C_6H_2$	CH_2Cl	347 ± 74	**8J**	$NHCOCH_3$	2-ClC_6H_4	-	353 ± 75
7X	$NHCH_2CH_2COOH$	2,4,6-$Cl_3C_6H_2$	CH_2Cl	362 ± 35	**8K**	$NHCOC_6H_5$	2-ClC_6H_4	-	367 ± 44
7Y	$NHCOCH_2Cl$	2-ClC_6H_4	CH_3	354 ± 49	**8L**	$NHCH_2CH_2COOH$	2-ClC_6H_4	-	435 ± 52

a: Inhibitory concentration

Table 3. Selectivity ratio of most active compounds

Compound	[a]Cdk5 IC_{50} (nm)	[a]Cdk2 IC_{50} (nm)	Select k2/k5
2A	43 ± 02	52 ± 5	1.2
3E	41 ± 08	879 ± 28	21.4
3I	64 ± 02	784 ± 48	12.3
4E	35 ± 04	1319 ± 89	21.6
4I	58 ± 06	1296 ± 154	22.3
6E	37 ± 01	748 ± 58	20.2
6F	59 ± 08	487 ± 68	8.3
6I	61 ± 01	4866 ± 132	79.8
7U	65 ± 12	936 ± 104	14.4
8E	32 ± 01	565 ± 21	17.7
8I	32 ± 09	48 ± 7	1.5

a: Inhibitory concentration

Variation of the amide side chain of **2A** with high speed and traditional chemistries allowed us to rapidly explore the first arm of the pharmacophore. Relative to the parent alkyl halide substitution over amide chain of thiophene demonstrated improved activity, however methyl, phenyl, ethanoic acids substantially decreases its activity. Amino group over the triazole was protected corresponding compound **3A-L** was furnish, all of these modification resulted in a increase activity. We observed little to no change in the selectivity of these analogs versus cdk2. The next modification made was s-alkylation with acetonitrile provides the first analogs **4E** and **4I** that demonstrated excellent activity, 21.6 and 22.3 fold selectivity versus cdk2 respectively, while others exhibited poor activity. Attention was then turned to optimization at sulfhydryl group, compounds **5A-L** were synthesized and investigated, which revealed loss of activity. A further modification of compounds **5A-L** produced compound **6A-L**. The results of the cdk5/p25 inhibitory activity was interesting because five **6E-I** compounds shown impressive percentage of inhibition. Compound **6A-L** was selected for further studies as they have a free amino group, which opened an area for further modification at this point. Compound **7A-7AJ** was obtained by Schotten Baumann reaction, which ultimately decreased potency except **7U**. Furthermore, compounds **6A-L** was converted to Schiff bases **8A-L** with benzaldehyde, and on investigation **8E-I** shows promising activity, while other remains inactive. On comparing the cdk5/p25 (IC_{50}) activity of **8E** afforded improved that is >17.7 fold selectivity versus cdk2. The compound **8I** was equally selective versus cdk2 and had slightly improved cdk5 (IC_{50}) as compare to other chemical modification. A possible change in binding conformation may occur and this will be the object for future computational studies.

CONCLUSION:

Screening of the in vitro cdk5/p25 inhibitory activity of a novel series of clubbed triazole and its derivatives 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 of triazole core and subsequently by the introduction of appropriate substitutes on both the heterocyclic rings leading to the most promising compound **8E** and **8I**. Therefore novel series has evidenced that derivatives with highly electronegative part at amino group and sulfhydryl group have emerged as new compounds endowed with cdk5/p25 inhibition activity. Specifically compounds **8E-I** i.e schiff bases probably due to their ability to increase the penetration in cell wall. Due to the better activity against the cdk5/p25, compounds **8E-I** has ample scope for further study.

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