CHAPTER 3

Design, Synthesis and Evaluation of N-Benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide Derivatives as Cholinesterase Inhibitors
3.1 Introduction

Thiazole is a five-membered heterocyclic molecule possessing two hetero atoms: nitrogen (N) and sulphur (S) with molecular formula, C$_3$H$_3$NS (Figure 3.1). Thiazole was first described by A. Hantzsch and J. H. Waber in 1887. This molecule possesses both an electron accepting (C=N) and electron donating group (-S-). The lone pair of electrons present on S atom makes the molecule 6π electron system that fulfills the criteria for aromaticity. The numbering in thiazole starts from the sulphur atom (Figure 3.1). When the thiazole ring fused at the 4, 5 positions with 6-membered benzene ring, the resulting molecules are known as benzothiazoles which are useful bicyclic heterocyclic molecules (Figure 3.1).

Thiazole and its derivatives is the important scaffold in the field of medicinal chemistry and display a wide range of biological activities. This ring is present in many natural and synthetic products with a broad range of biological applications such as antioxidant, antibacterial, antifungal, anti-tubercular, diurectic, anti-inflammatory and anti-cancerous. Vitamin B$_1$ (thiamine) contains thiazole moiety which helps in the normal functioning of the nervous system by its role in the synthesis of acetylcholine. Bacitracin and penicillin antibiotics also contains this moiety in their structures. Some of the synthetic drugs belonging to this family includes acinitrazole and sulfathiazole (antimicrobial agents), pramipexole (antidepressant), Bleomycin and Tiazofurin (antineoplastic agents), Ritonavir (anti-HIV drug), cinalukast (antiasthmatic drug) and Nizatidine (antiulcer agent) (Figure 3.2). Additionally, extensively thiazole derivatives have been successfully used in the past as potential neuroprotective agents. Tetrahydrobenzothiazoles, phenolic thiazoles and benzothiazoles are well known for their neuroprotective nature. Benzothiazole derivatives developed by Hofmann Le Roche is a potent adenosine receptor (A$_2$AR) antagonist and have been used for the treatment of Parkinson disease. The other therapeutic applications of benzothiazoles derivatives includes neurodegenerative disorder treatment, local brain ischemia, central muscle relaxants and cancer. Literature shows that thiazole–triazole linked derivative have shown potent anti-alzheimeric activity. Siddiqui et al in 2009 and Mishra et al in 2015 reviewed the diverse biological activities of thiazoles towards CNS activities like dopamine receptor ligands, nNOS inhibitors, adenosine receptor ligands, GABA receptor...
ligands, glutamate receptor ligands, 5-HT receptor ligands, cannabinoid receptor ligands, opioid receptor ligands, acetylcholine receptor ligands, neuroprotective and anticonvulsant agents.

![Thiazole and Benzothiazole structures](image)

**Figure 3.1**: Structures of thiazole and benzothiazole

![Thiazole moiety containing drugs](image)

**Figure 3.2**: Thiazole moiety containing drugs
In the 1950s, a number of 2-aminobenzothiazoles were intensively studied. The 2-aminobenzothiazole scaffold is one of the privileged structure in medicinal chemistry and reported cytotoxic on cancer cells.\(^{184}\) Several literatures highlighted that the combination of 2-aminobenzothiazole with other heterocyclic molecule lead to new drug molecule which allow to achieve new pharmacological action profile towards target with lower toxicity.

The benzothiazoles derivatives are used in neurodegenerative disorder treatment, local brain ischemia, central muscle relaxants and cancer.\(^{181}\) Literature also reveals that thiazole–triazole linked derivatives have shown potent anti-alzheimeric activity. Also, Vitamin B\(_1\) (thiamine) contains thiazole moiety that helps in the normal functioning of the nervous system by synthesizing acetylcholine. So, this chapter deals with the design, synthesis and evaluation of benzothiazole-triazole conjugates as anti-alzheimeric agents (Figure 3.3). The preliminary docking studies of fragments had indicated that benzothiazole and triazole interacted favourably with the active site of AChE and BuChE. Therefore, the compounds with these moieties were designed, synthesized and evaluated.

### 3.2 Experimental

#### 3.2.1 Preliminary *in silico* interaction studies of benzothiazole and triazole moieties with AChE and BuChE

Preliminary docking studies of the benzothiazole and triazole moieties with cholinesterase enzymes such as AChE (1EVE) and BuChE (4TPK) was performed using Glide module of Schrodinger Suite (Small-Molecule Drug Discovery Suite 2017-3, Schrödinger, LLC, New York, NY, 2017). Favourable interactions of these fragments with these enzymes (AChE and BuChE) were identified. These fragments were interacted in different parts of the active site. On the basis of these results, we designed and synthesized N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives. Further, the synthesized novel compounds were validated by doing *in silico* and *in vitro* experimental studies.
3.2.2 Synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino) acetamide derivatives (16a-i)

All commercially available solvents and reagents were purchased from reputed company and were used without further purification. Melting points were determined on a laboratory capillary
melting apparatus and are uncorrected. FTIR spectra were recorded on a Perkin Elmer Spectrum Version 10.5.3 FTIR spectrophotometer. The $v_{\text{max}}$ are expressed in cm$^{-1}$, and the chemical shifts are expressed in ppm. $^1$H and $^{13}$C NMR were recorded on a Bruker spectrophotometer and Jeol spectrophotometer (400/100MHz) using TMS as internal standard. The abbreviations s, d, t, q, m and bs stand for singlet, doublet, triplet, quartet, multiplet and broad singlet respectively. The elemental analysis was measured by PerkinElmer 2400. Thin-layer chromatography was performed on aluminium-coated silica plates purchased from Merck.

Synthesis of compounds 16a-i has been achieved by following three schemes 3.1-3.3. Scheme 3.1 deals with the synthesis of 2-aminobenzothiazole derivatives whereas scheme 3.2 shows the synthetic pathway of substituted triazoles. The covalent linking of triazole and benzothiazole is given in scheme 3.3.

**Synthesis of 2-aminobenzothiazole (10)**

A solution of aniline (9, 4.5 mL, 50 mmol) in glacial acetic acid (50 mL) was taken in a round bottom flask (250 mL), and then potassium thiocynate (KSCN) (4.8 g, 50 mmol) was added to the solution. The reaction mixture was kept at freezing mixture of ice and salt and was mechanically stirred till dissolution. Then Br$_2$ (2.5 mL in 4 mL glacial acetic acid, 50 mmol) was added from the dropping funnel at such rate the temperature does not raise beyond 5° C. After all bromine was added, the solution was stirred for 4 hours at room temperature. The progress of reaction was monitored by thin layer chromatography (TLC) (hexane: ethyl acetate, 7:3, v/v). After completion of reaction, the resulting crude solid product was filtered and washed with glacial acetic acid. The solid was dried and dissolved in hot water, and neutralized with aqueous ammonia solution (25%). The resulting precipitate was dried and purified by recrystallisation with ethanol to get a white solid as pure product.

Yield: 78%; Mp.: 126-128°C (Lit. Mp.$^{185}$ 129°C); FTIR (KBr): 3395, 3269, 3055, 2727, 1917, 1628, 1522, 1338, 1104, 887, 739 685 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$: 7.00 (2H, s, NH), 7.20-7.65 (4H, m, ArH); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$: 118, 121, 125, 131, 153, 166, 169.

**Synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11)**

A solution of 2-aminobenzothiazole (10, 5 g, 33.3 mmol) in 15 mL of tetrahydrofuran (THF) was taken in a round bottom flask (100 mL). Further, 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (1 mL, 6.6 mmol) was added to the solution. The reaction mixture was kept at 0°C. A
dropping funnel was fitted to the flask, and a solution of chloro acetylchloride (3.2 mL, 40 mmol, in 2 mL THF) was taken in a dropping funnel and added drop wise to the reaction mixture. The reaction mixture was allowed to come at room temperature and stirred for 6 hours. The progress of the reaction was monitored by TLC (hexane: ethyl acetate, 7:3, v/v). After completion of the reaction, crude solid product was obtained which was filtered and washed with water. The formed product was further re-crystallized using absolute ethanol.

Yield: 83%; Mp.: 142-145°C (Lit. Mp. 186°C); FTIR (KBr): 3372, 3255, 3164, 2986, 2852, 2735, 1692, 1596, 1442, 1396, 1269, 1176, 1015, 983, 865, 772, 677 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 4.47 (2H, s, CH₂), 7.32-8.01 (4H, m, ArH), 12.75 (1H, bs, NH); Anal. calc. for C₈H₇N₂OSCl: C, 47.69; H, 3.11; N, 12.36; S, 14.15; found C, 47.62; H, 3.08; N, 12.29; S, 14.09.

This reaction was performed with different aryl amines to check the versatility of the method (Table 3.3)

2-Chloro-N-(6-chlorobenzothiazole-2-yl)acetamide (11a)

Yield: 76%; Mp.: 210-213°C; FTIR (KBr): 3248, 2945, 2743, 1692, 1645, 1595, 1554, 1378, 1275, 781 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 4.46 (2H, s, CH₂), 7.35-8.05 (3H, m, ArH), 12.78 (1H, bs, NH); Anal. calc. for C₉H₆N₂OSCl: C, 41.40; H, 2.32; N, 10.73; S, 12.28; found C, 41.33; H, 2.29; N, 10.68; S, 12.23.

2-Chloro-N-(4-phenylthiazol-2-yl)acetamide (11b)

Yield: 86%; Mp.: 180-181°C; FTIR (KBr): 3354, 2967, 2765, 1678, 1572, 1442, 1327, 1264, 1140, 1025, 849, 722, 686, 575 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ: 4.23 (2H s, CH₂), 6.75(1H, s, CH of thiazole), 7.28-7.81 (5H, m, ArH), 10.20 (1H, bs NH); Anal. calc. for C₁₁H₉N₂OSCl: C, 52.28; H, 3.59; N, 11.08; S, 12.69; found C, 52.22; H, 3.54; N, 11.02; S, 12.60.

2-Chloro-N-[4-(4-fluorophenyl)thiazol-2-yl]acetamide (11c)

Yield: 85%; Mp.: 134-136°C; FTIR (KBr): 3362, 2998, 2742, 1680, 1576, 1488, 1266, 1161, 1067, 831, 707, 519 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ: 4.25 (2H s, CH₂), 6.67 (1H, s, CH of thiazole), 7.09 (2H, d, J = 8.8 Hz, ArH), 7.76 (2H, d, J = 8.8 Hz, ArH), 9.34 (1H, bs, NH); Anal.
calc. for C₁₁H₈N₂OSFCl: C, 48.80; H, 2.98; N, 10.35; S, 11.84; found C, 48.76; H, 2.91; N, 10.32; S, 11.80.

2-Chloro-N-[4-(4-chlorophenyl)thiazol-2-yl]acetamide (11d)
Yield: 85%; Mp.: 188-191°C; FTIR (KBr) : 3373, 2985, 2864, 1692, 1543, 1478, 1312, 1291, 1177, 1084, 1012, 841, 761, 670, 593 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ: 4.27 (2H, s, CH₂), 6.73 (1H, s, CH of thiazole), 7.36 (2H, d, J = 8.4 Hz, ArH), 7.73 (2H, d, J = 8.4 Hz, ArH), 9.75 (1H, bs, NH); Anal. calc. for C₁₁H₈N₂OSCl₂: C, 45.87; H, 2.80; N, 9.72; S, 11.09.

2-Chloro-N-[4-(4-bromo phenyl)thiazol-2-yl]acetamide (11e)
Yield: 86%; Mp.: 206-208°C; FTIR (KBr): 3472, 2973, 2885, 1678, 1586, 1515, 1465, 1326, 1268, 1072, 843, 712, 652, 521 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ: 4.12 (2H, s, CH₂), 6.69 (1H, s, CH of thiazole), 7.20 (2H, d, J = 7.6 Hz, ArH), 7.68 (2H, d, J = 7.6 Hz, ArH), 9.04 (1H, bs, NH); Anal. calc. for C₁₁H₈N₂OSBrCl: C, 39.78; H, 2.38; N, 8.41; S, 9.62.

2-Chloro-N-[4-(4-p-tolylthiazol-2-yl)acetamide (11f)
Yield: 90%; Mp.: 148-150°C; FTIR (KBr): 3340, 2967, 2872, 2740, 1699, 1567, 1426, 1328, 1268, 1140, 1070, 974, 820, 700, 652, 508 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ: 2.40 (3H, s, CH₃), 4.20 (2H, s, CH₂), 7.15 (1H, s, CH of thiazole), 7.25 (2H, d, J = 6.8 Hz, ArH), 7.72 (2H, d, J = 6.8 Hz, ArH), 10.23 (1H, bs, NH); Anal. calc. for C₁₂H₁₁N₂OSCl: C, 54.03; H, 2.43; N, 8.45; S, 9.67; found C, 53.98; H, 4.16; N, 10.50; S, 12.02; found C, 53.98; H, 4.10; N, 10.41; S, 11.96.

2-Chloro-N-[4-(4-methoxyphenyl)thiazol-2-yl]acetamide (11g)
Yield: 95%; Mp.: 232-234°C; FTIR (KBr): 3332, 2980, 2764, 1694, 1538, 1429, 1330, 1255, 1171, 1029, 972, 843, 735, 612, 534 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ: 3.87 (3H s, OCH₃), 4.27 (2H, s, CH₂), 6.97 (2H, d, J = 8.4 Hz, ArH), 7.08 (1H, s, CH of thiazole), 7.77 (2H, d, J = 8.4 Hz, ArH), 9.93 (1H, bs, NH); Anal. calc. for C₁₂H₁₁N₂O₂SCl: C, 50.97; H, 3.92; N, 9.91; S, 11.34; found C, 50.91; H, 3.87; N, 9.89; S, 11.29.

2-Chloro-N-[4-(4-nitrophenyl)thiazol-2-yl]acetamide (11h)
Yield: 75%; Mp.: 295-297°C; FTIR (KBr): 3315, 2992, 2874, 1688, 1560, 1542, 1436, 1345, 1265, 1180, 1038, 972, 854, 738, 642, 532 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 4.27 (2H, s, CH₂), 7.41 (1H, s, CH of thiazole), 8.04 (2H, d, J = 8.8 Hz, ArH), 8.23 (2H, J = 8.8 Hz, ArH), 9.75 (1H, bs, NH); Anal. calc. for C₁₁H₈N₂OSCl: C, 48.80; H, 2.98; N, 10.35; S, 11.84; found C, 48.76; H, 2.91; N, 10.32; S, 11.80.
9.87 (1H, bs, NH); Anal. calc. for C₁₁H₈N₃O₂SCl: C, 44.38; H, 2.71; N, 14.11; S, 10.17; found C, 44.32; H, 2.67; N, 14.06; S, 10.11.

2-Chloro-N-[4-(4-cyanophenyl)thiazol-2-yl]acetamide (11i)

Yield: 76%; Mp.: 276-278°C; FTIR (KBr): 3325, 2972, 2864, 2732, 1678, 1560, 1542, 1435, 1345, 1265, 1180, 1038, 854, 738, 642, 532 cm⁻¹; ¹H NMR (DMSO-­d₆, 400 MHz) δ: 4.24 (2H, s, CH₂), 6.98 (1H, s, CH of thiazole), 7.45 (2H, d, J = 8.4 Hz, ArH), 7.92 (2H, d, J = 8.4 Hz, ArH), 9.84 (1H, bs, NH); Anal. calc. for C₁₂H₈N₃OSCl: C, 61.34; H, 4.58; N, 15.90; S, 18.19; found C, 61.29; H, 4.55; N, 15.83; S, 18.11.

2-Chloro-N-[4-(3,4-dichlorophenyl)thiazol-2-yl]acetamide (11j)

Yield: 79% Mp.: 228-230°C; FTIR (KBr): 3349, 2968, 2854, 1684, 1528, 1415, 1355, 1246, 1132, 1060, 832, 746, 672, 538 cm⁻¹; ¹H NMR (DMSO-­d₆, 400 MHz) δ: 4.23 (2H, s, CH₂), 7.23 (1H, s, CH of thiazole) 7.61-8.02 (3H, m, ArH), 9.76 (1H, bs, NH); Anal. calc. for C₉H₈N₂S: C, 41.08; H, 2.19; N, 8.71; S, 9.97; found C, 40.98; H, 2.12; N, 8.67; S, 9.93.

General procedure for the synthesis of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol derivatives (15)

The derivatives were prepared according to the reported method in literature¹⁸⁷,¹⁸⁸ and given in scheme 3.2.

Synthesis of benzohydrazide derivatives (13)

The ester of substituted aromatic acid (12, 26 mmol) was dissolved in 30 mL ethanol, and hydrazine hydrate (0.1 mmol) was then added drop-wise to the mixture with stirring. The resulting mixture was allowed to reflux for 6 hours. The completion of the reaction was monitored by TLC (ethyl acetate: petroleum ether, 1:1, v/v). After completion of reaction, the excess ethanol was distilled out and the contents were allowed to cool. The crystals formed were filtered, washed thoroughly with water, and dried. This was used for further reactions without any purification.

Benzohydrazide (13a)

Yield: 64%; Mp.: 110-112°C; FTIR (KBr): 3298, 3196, 2978, 2874, 1672, 1578, 1476, 1368, 1256, 1178, 1056, 956, 840, 746, 651 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ: 4.54 (bs, 2H, NH₂),
7.26 (m, 1H, ArH), 7.41 (dd, 2H, J = 8.4 Hz, 3.2 Hz ArH), 7.79 (dd, 2H, J = 8.0 Hz, 2.8 Hz, ArH), 9.74 (bs, 1H, NH).

**4-Fluorobenzohydrazide (13b)**

Yield: 73%; Mp.: 160-162°C; FTIR (KBr): 3278, 3184, 2928, 2865, 1660, 1597, 1451, 1371, 1265, 1093, 990, 830, 729, 686 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 4.52 (bs, 2H, NH\(_2\)), 7.31 (d, 2H, J = 8.0 Hz, ArH), 7.97 (d, 2H, J = 8.0 Hz, ArH), 9.68 (bs, 1H, NH).

**4-Chlorobenzohydrazide (13c)**

Yield: 68%; Mp.: 166-168°C; FTIR (KBr): 3223, 3194, 2967, 2856, 1678, 1587, 1454, 1367, 1248, 1198, 1078, 967, 840, 735, 646 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 4.56 (bs, 2H, NH\(_2\)), 7.46-7.48 (d, 2H, J = 7.2 Hz, ArH), 7.79-7.80 (d, 2H, J = 7.6 Hz, ArH), 9.56 (bs, 1H, NH).

**4-Bromobenzohydrazide (13d)**

Yield: 62%; Mp.: 169-170°C; FTIR (KBr): 3270, 3156, 2971, 2884, 1685, 1578, 1436, 1340, 1286, 1194, 1067, 998, 856, 742, 650 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 4.50 (bs, 2H, NH\(_2\)), 7.43 (d, 2H, J = 6.8 Hz, ArH), 7.80 (d, 2H, J = 7.2 Hz, ArH), 9.59 (bs, 1H, NH).

**3-Bromobenzohydrazide (13e)**

Yield: 63%; Mp.: 154-158°C; FTIR (KBr): 3224, 3146, 2998, 2884, 1690, 1546, 1456, 1360, 1275, 1187, 1076, 995, 887, 640 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 4.53 (bs, 2H, NH\(_2\)), 7.48 (m, 1H, ArH), 7.69 (dd, 1H, J = 8.00 Hz, 2.4 Hz, ArH), 7.79 (dd, 1H, J = 8.00 Hz, 2.4 Hz, ArH), 9.64 (bs, 1H, NH).

**4-Methylbenzohydrazide (13f)**

Yield: 66%; Mp.: 114-116°C; FTIR (KBr): 3234, 3123, 2987, 2574, 1678, 1598, 1456, 1378, 1276, 1180, 1068, 992, 846, 768, 667 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 2.46 (s, 3H, CH\(_3\)), 4.56 (bs, 2H, NH\(_2\)), 7.30 (d, 2H, J = 7.7 Hz, ArH), 7.79 (d, 2H, J = 8.00 Hz, ArH), 9.67 (bs, 1H, NH).

**4-Methoxybenzohydrazide (13g)**

Yield: 64%; Mp.: 134-138°C; FTIR (KBr): 3224, 3176, 2996, 2874, 1685, 1575, 1468, 1340, 1166, 1078, 976, 867, 778, 698 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\): 3.83 (s, 3H, OCH\(_3\)), 4.51 (bs, 2H, NH\(_2\)), 7.02 (d, 2H, J = 8.00 Hz, ArH), 7.85 (d, 2H, J = 8.00 Hz, ArH), 9.74 (bs, 1H, NH).
4-Nitrobenzohydrazide (13h)

Yield: 67%; Mp.: 214-216°C; FTIR (KBr): 3238, 3179, 2989, 2854, 1682, 1538, 1470, 1352, 1274, 1168, 1098, 993, 897, 756, 648 cm⁻¹; ¹H NMR (DMSO-d$_6$, 400 MHz) δ: 6.92 (bs, 2H, NH$_2$), 7.99 (d, 2H, J = 7.60 Hz, ArH), 8.27 (d, 2H, J = 7.20 Hz, ArH), 9.68 (bs, 1H, NH).

3,4,5-Trimethoxybenzohydrazide (13i)

Yield: 61%; Mp.: 156-158°C; FTIR (KBr): 3246, 3136, 2978, 2869, 1686, 1575, 1498, 1368, 1265, 1189, 1076, 934, 871, 779, 653 cm⁻¹; ¹H NMR (DMSO-d$_6$, 400 MHz) δ: 3.69 (s, 3H, OCH$_3$), 3.86 (s, 6H, OCH$_3$), 7.01 (bs, 2H, NH$_2$), 7.21 (d, 1H, J = 3.20 Hz, ArH), 9.78 (bs, 1H, NH).

Synthesis of potassium 2-benzoylhydrazine-1-carbodithioate derivatives (14)

KOH (4.2 g, 75 mmol) was dissolved in absolute ethanol (200 ml). To the above solution, aryl acid hydrazide, (13, 50 mmol) was added and the solution was cooled on ice. To this, carbon disulfide (75 mmol) was added in small portions with constant stirring. The reaction mixture was agitated continuously for a period of 15 hours. It was then diluted with anhydrous ether. The precipitated potassium dithiocarbazinate was collected by filtration. The precipitate was further washed with anhydrous ether (100 mL) and evaporated under vacuum. The potassium salt thus obtained was in quantitative yield, and was used in the next step without further purifications.

Synthesis of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol derivatives (15)

A suspension of potassium dithiocarbazinate, (14, 100 mmol) in water (5 ml) and hydrazine hydrate (15 ml, 300 mmol) was refluxed for 30 min with occasional shaking. The colour of the reaction mixture changed to green with the evolution of hydrogen sulfide gas (lead acetate paper test and odour). A homogeneous reaction mixture was obtained during the reaction process. The completion of the reaction was monitored with TLC (ethyl acetate: petroleum ether, 1:1, v/v). The reaction mixture was cooled to room temperature, and was diluted with water (100 mL). On acidification with concentrated hydrochloric acid, the required triazole (15) was precipitated out. It was filtered, washed thoroughly with cold water, and then recrystallized from ethanol.
4-Amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (15a)

Yield: 60%; Mp.: 196-198°C; (Lit. Mp.: 198-200°C); FTIR (KBr): 3412, 3070, 2667, 1640, 1546, 1467, 1366, 1284, 1170, 1069, 966, 840, 732, 640 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 3.35 (1H, s, SH), 7.53-8.03 (m, 7H, ArH); Anal. calc. for C₈H₈N₄S: C, 49.98; H, 4.19; N, 29.14; S, 16.68; found: C, 49.90; H, 4.16; N, 29.13; S, 16.60.

4-Amino-5-(4-fluorophenyl)-4H-[1,2,4]triazole-3-thiol (15b)

Yield: 72%; Mp.: 204-206°C; (Lit. Mp.: 208°C); FTIR (KBr): 3423, 3091, 2943, 2656, 1615, 1508, 1476, 1350, 1298, 1187, 1073, 970, 846, 728, 691 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 5.68 (bs, 2H, NH₂), 7.28-7.33 (m, 2H, ArH), 7.99-8.11 (2H, m, ArH); Anal. calc. for C₈H₇N₄SF: C, 45.71; H, 3.36; N, 26.65; S, 15.25; found: C, 45.67; H, 3.28; N, 26.61; S, 15.20.

4-Amino-5-(4-chlorophenyl)-4H-[1,2,4]triazole-3-thiol (15c)

Yield: 68%; Mp.: 208-210°C; (Lit. Mp.: 210-212°C); FTIR (KBr): 3429, 3056, 2972, 2687, 1635, 1528, 1451, 1348, 1287, 1192, 1089, 998, 856, 740, 647 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 5.71 (bs, 2H, NH₂), 7.54-7.61 (m, 2H, ArH), 8.01-8.05 (m, 2H, ArH). Anal. calc. for C₈H₇N₄Cl: C, 42.39; H, 3.11; N, 24.72; S, 14.14; found: C, 42.30; H, 3.09; N, 24.68; S, 14.10.

4-Amino-5-(4-bromophenyl)-4H-[1,2,4]triazole-3-thiol (15d)

Yield: 67%; Mp.: 202-204°C; (Lit. Mp.: 205-206°C); FTIR (KBr): 3410, 3056, 2978, 2647, 1644, 1587, 1434, 1378, 1243, 1176, 1067, 989, 856, 747, 682 cm⁻¹; ¹H NMR [(CD₃)₂CO, 400 MHz] δ: 5.49 (bs, 2H, NH₂), 7.69-7.75 (m, 2H, ArH), 8.10-8.14 (m, 2H, ArH), 12.82 (bs, 1H, SH); Anal. calc. for C₈H₇N₄SBr: C, 35.44; H, 2.60; N, 20.66; S, 11.82; found: C, 35.41; H, 2.58; N, 20.62; S, 11.76.

4-Amino-5-(3-bromophenyl)-4H-[1,2,4]triazole-3-thiol (15e)

Yield: 63%; Mp.: 208-210°C; (Lit. Mp.: 212°C); FTIR (KBr): 3433, 3073, 2987, 2667, 1647, 1578, 1467, 1389, 1254, 1162, 1058, 948, 823, 749, 692 cm⁻¹; ¹H NMR [(CD₃)₂CO, 400 MHz] δ: 5.51 (bs, 2H, NH₂), 7.72-7.76 (m, 2H, ArH), 8.17 (m, 1H, ArH), 8.39 (m, 1H, ArH), 12.89 (bs, 1H, SH); Anal. calc. for C₈H₇N₄SBr: C, 35.44; H, 2.60; N, 20.66; S, 11.82; found: C, 35.43; H, 2.54; N, 20.61; S, 11.79.

4-Amino-5-(4-methylphenyl)-4H-[1,2,4]triazole-3-thiol (15f)

Yield: 71%; Mp.: 195-198°C; (Lit. Mp.: 201°C); FTIR (KBr): 3423, 3062, 2939, 2637, 1636, 1540, 1429, 1338, 1280, 1162, 1072, 938, 891, 749, 662 cm⁻¹; ¹H NMR [(CD₃)₂CO, 400 MHz] δ:
2.43 (s, 3H, CH₃), 5.51 (bs, 2H, NH₂), 7.82 (d, 2H, J = 8.00 Hz, ArH), 8.02 (d, 2H, J = 8.40 Hz, ArH), 12.79 (bs, 1H, SH); Anal. calc. for C₉H₁₀N₄S: C, 52.41; H, 4.89; N, 27.16; S, 15.54; found: C, 52.39; H, 4.84; N, 27.10; S, 15.49.

4-Amino-5-(4-methoxyphenyl)-4H-[1,2,4]triazole-3-thiol (15g)

Yield: 69%; Mp.: 210-212°C; (Lit.¹⁹⁰ Mp.: 215°C); FTIR (KBr): 3434, 3059, 2981, 2649, 1648, 1563, 1498, 1372, 1259, 1183, 1071, 917, 893, 749, 628 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 3.80 (s, 3H, OCH₃), 7.24 (d, 2H, J = 8.00 Hz, ArH), 7.83 (d, 2H, J = 8.40 Hz, ArH), 12.69 (bs, 1H, SH). Anal. calc. for C₉H₁₀N₄OS: C, 48.63; H, 4.54; N, 25.21; S, 14.42; found: C, 48.58; H, 4.51; N, 25.17; S, 14.40.

4-Amino-5-(4-nitrophenyl)-4H-[1,2,4]triazole-3-thiol (15h)

Yield: 76%; Mp.: 178-182°C; FTIR (KBr): 3430, 3109, 2998, 2689, 1641, 1546, 1476, 1353, 1292, 1139, 1074, 948, 879, 738, 661 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 5.98 (bs, 2H, NH₂), 8.00 (d, 2H, J = 7.60 Hz, ArH), 8.24 (d, 2H, J = 7.20 Hz, Ar-H), 12.89 (bs, 1H, SH). Anal. calc. for C₈H₇N₃O₂S: C, 40.50; H, 2.97; N, 29.52; S, 13.51; found: C, 40.47; H, 2.92; N, 29.49; S, 13.48.

4-Amino-5-(3,4,5-trimethoxyphenyl)-4H-[1,2,4]triazole-3-thiol (15i)

Yield: 63%; Mp.: 215-220°C; (Lit.¹⁹¹ Mp.: 221°C); FTIR (KBr): 3428, 3102, 2976, 2667, 1638, 1556, 1482, 1348, 1249, 1173, 1068, 928, 840, 728, 652 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 3.67 (s, 3H, OCH₃), 3.86 (s, 6H, OCH₃), 6.02 (bs, 2H, NH₂), 7.39 (2H, d, J = 2.80 Hz Ar-H), 12.03 (bs, 1H, SH); Anal. calc. for C₁₁H₁₄N₄O₅S: C, 46.80; H, 5.00; N, 19.85; S, 11.36; found: C, 46.75; H, 4.99; 19.78; S, 11.32.

**General procedure for the synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (Scheme 3.3, 16a-i)**

A solution of substituted triazoles (15, 2.6 mmol) in 6 mL dry acetone or acetonitrile and triethyl amine (0.4 mL, 2 mmol) were taken in a round bottom flask (50 mL). To this N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11, 2.6 mmol) was added. The reaction mixture was stirred at 50°C for 4 hours. The progress of reaction was monitored by TLC (CH₂Cl₂: methanol, 9:1, v/v). After completion of reaction, the solvent was removed under reduced pressure and reaction mixture was extracted with ethyl acetate (3×40 mL). The organic layer was dried over
anhydrous sodium sulphate, and the excess of solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (60-120 mesh) using chloroform: methanol 3:1 v/v as eluent.

**N-Benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide (16a)**

Yield: 75%; M.p.: 221-224°C; FTIR (KBr): 3361, 3064, 2995, 2064, 1682, 1597, 1553, 1485, 1318, 1256, 1082, 756 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz,) δ: 2.67 (s, 2H, CH₂), 3.50 (s, 1H, SH), 7.31-8.00 (m, 9H, ArH), 12.82 (bs, 1H, NH); ¹³C NMR (DMSO-d₆, 100 MHz) δ: 36, 121, 122, 123, 124, 126(3C of Ar), 129 (2C of Ar), 132 (2C of Ar), 163, 165, 167 (C=O); Anal. calc. for C₁₁H₁₅N₆O₄S: C, 53.39; H, 3.69; N, 21.97; S, 16.37; Yield: 16.71.

**N-Benzothiazol-2-yl-2-[3-(4-fluorophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16b)**

Yield: 68%; M.p.: 242-246°C; IR (KBr): 3230, 3164, 2958, 2012, 1679, 1610, 1548, 1480, 1324, 1276, 1086, 750 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz,) δ: 2.87 (s, 2H, CH₂), 3.38 (s, 1H, SH), 7.28-8.21 (m, 8H, ArH), 12.75 (bs, 1H, NH); ¹³C NMR (DMSO-d₆, 100 MHz) δ: 38, 114, 117, 120, 122, 124, 125, 127, 131, 148, 155, 162, 164, 169, 173; Anal. calc. for C₁₁H₁₃N₆O₄S₂F: C, 50.99; H, 3.27; N, 20.99; S, 16.01; found C, 50.93; H, 3.13; N, 20.96; S, 15.99.

**N-Benzothiazol-2-yl-2-[3-(4-chlorophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16c)**

Yield: 62%; M.p.: 258-260°C; IR (KBr): 3332, 3176, 3062, 2938, 1928, 1673, 1620, 1555, 1476, 1439, 1417, 1234, 1093, 829, 746, 718, 677 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 2.76 (s, 2H, CH₂), 3.36 (s, 1H, SH), 7.26-8.19 (m, 8H, ArH), 12.65 (bs, 1H, NH); ¹³C NMR (DMSO-d₆, 100 MHz) δ: 36, 116, 122, 124, 126, 127, 129, 131, 133, 149, 152, 166, 169, 172; Anal. calc. for C₁₁H₁₃N₆O₂Cl: C, 48.98; H, 3.14; N, 20.16; S, 15.38; found C, 49.94; 3.08; N, 20.10; S, 15.32.

**N-Benzothiazol-2-yl-2-[3-(4-bromophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16d)**

Yield: 58%, M.p.: 278-281°C; IR (KBr): 3321, 3165, 3042, 2942, 1936, 1686, 1612, 1548, 1472, 1430, 1232, 1086, 832, 735, 694 cm⁻¹; ¹H NMR (DMSO-d₆, 400 MHz) δ: 2.82 (s, 2H, CH₂), 3.42 (s, 1H, SH), 7.21-8.02 (m, 8H, ArH), 12.63 (bs, 1H, NH); ¹³C NMR (DMSO-d₆, 100 MHz) δ: 39,
117, 120, 122, 124, 126, 131, 132, 133, 150, 165, 169, 175; Anal. calc. for C$_{17}$H$_{13}$N$_6$OS$_2$Br: C, 44.26; H, 2.84; N, 18.22; S, 13.90; found C, 44.21; H, 2.80; N, 18.19; S, 13.86.

N-Benzothiazol-2-yl-2-[3-(3-bromophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16e)

Yield: 53%, M.p.: 241-245°C; IR (KBr): 3329, 3156, 3048, 2947, 1948, 1678, 1618, 1568, 1478, 1236, 1094, 746, 675 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 400 MHz) δ: 2.67 (s, 2H, CH$_2$), 3.56 (s, 1H, SH), 7.19-8.06 (m, 8H, ArH), 12.78 (bs, 1H, NH); $^{13}$C NMR (DMSO-d$_6$, 100 MHz) δ: 38, 118, 120, 122, 123, 125, 126, 127, 129, 131, 132, 136, 152, 154, 166, 169, 173; Anal. calc. for C$_{17}$H$_{13}$N$_6$OS$_2$Br: C, 44.26; H, 2.84; N, 18.22; S, 13.90; found C, 44.23; H, 2.80; N, 18.21; S, 13.83.

N-Benzothiazol-2-yl-2-[3-(4-methylphenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16f)

Yield: 62%, M.p.: 232-236°C; IR (KBr): 3346, 3168, 3036, 2946, 1928, 1688, 1626, 1540, 1465, 1245, 1087, 766, 640 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 400 MHz) δ: 2.32 (s, 3H, CH$_3$), 2.86 (s, 2H, CH$_2$), 3.46 (s, 1H, SH), 7.34-7.99 (m, 8H, ArH), 12.78 (bs, 1H, NH); $^{13}$C NMR (DMSO-d$_6$, 100 MHz) δ: 20, 36, 119, 122, 125, 126, 128, 130, 131, 132, 151, 154, 167, 168, 172; Anal. calc. for C$_{18}$H$_{16}$N$_6$OS$_2$: C, 54.53; H, 4.07; N, 21.20; S, 16.17; found C, 54.48; H, 3.98; N, 21.19; S, 16.15.

N-Benzothiazol-2-yl-2-[3-(4-methoxyphenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16g)

Yield: 57%, M.p.: 256-258°C; IR (KBr): 3336, 3146, 3028, 2968, 1967, 1679, 1614, 1529, 1457, 1240, 1076, 748, 656 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 400 MHz) δ: 2.72 (s, 2H, CH$_2$), 3.67 (s, 1H, SH), 3.83 (s, 3H, OCH$_3$), 7.36-8.12 (m, 8H, ArH), 12.72 (bs, 1H, NH); $^{13}$C NMR (DMSO-d$_6$, 100 MHz) δ: 38, 52, 115, 119, 122, 123, 125, 126, 129, 131, 150, 159, 168, 173; Anal. calc. for C$_{18}$H$_{16}$N$_6$O$_2$S$_2$: C, 52.41; H, 3.91; N, 20.37; S, 15.54; found C 52.38; H, 3.87; N 20.32; S, 15.48.

N-Benzothiazol-2-yl-2-[3-(4-nitrophenyl)-5-mercapto-[1,2,4]triazol-4-ylamino]acetamide (16h)

Yield: 48%, M.p.: 296-298°C; IR (KBr): 3340, 3160, 2958, 1950, 1685, 1618, 1542, 1473, 1352, 1248, 1056, 773, 642 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 400 MHz) δ: 2.85 (s, 2H, CH$_2$), 3.67 (s, 1H, SH), 7.34-814 (m, 8H, ArH), 12.89 (bs, 1H, NH); $^{13}$C NMR (DMSO-d$_6$, 100 MHz) δ: 39,
120, 123, 126, 127, 129, 132, 138, 149, 153, 155, 169, 170, 176; Anal. calc. for C$_7$H$_{13}$N$_7$O$_3$S$_2$: C, 47.77; H, 3.07; N, 22.94; S, 15.00; found C, 47.72; H, 3.04; N, 22.90; S, 14.96.

N-Benzothiazol-2-yl-2-[3-(3,4,5-trimethoxyphenyl)-5-mercapto-[1,2,4]triazol-4-ylamino] acetamide (16i)

Yield: 44%, M.p.: 241-245°C; FTIR (KBr): 3397, 3156, 2942, 1939, 1681, 1606, 1560, 1448, 1241, 1046, 765, 637 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) δ: 2.88 (s, 2H, CH$_2$), 3.66 (s, 1H, SH), 3.72 (s, 3H, OCH$_3$), 3.84 (s, 6H, OCH$_3$), 7.31-8.14 (m, 6H, ArH), 12.92 (bs, 1H, NH); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) δ: 36, 59, 115, 118, 120, 123, 124, 125, 126, 129, 148, 152, 164, 166, 171; Anal. calc. for C$_{20}$H$_{20}$N$_6$O$_4$S$_2$: C, 50.84; H, 4.27; N, 17.79; S, 13.57; found C, 50.79; H, 4.21; N, 17.71; S, 13.55.

3.2.3 In-silico (Docking) studies

Geometries of the compounds 10, 15a-i, and 16a-i were optimized at the B3LYP/6-31G* level using Gaussian 09 quantum chemistry software (http://gaussian.com/). The global minima of the structures were verified using vibrational frequencies. Crystal structure of the protein AChE (PDB Id: 1EVE) was downloaded from protein data bank (PDB: www.rcsb.org). Though many structures of AChE are available, but the above protein structure from Tetronarce californica organism was opted as assay used for in vitro experiment was also carried on enzyme from the same organism. Similarly for BuChE structure PDB Id (4TPK) was used.

Before docking, the ligand molecules and enzymes were prepared by Glide ‘ligprep’ and ‘Protein preparation’ modules respectively. The ligand was refined in torsional space using the force field OPLS3 (Glide XP) with a distance-dependent dielectric model. Finally, a small number of poses are minimized within the field of the receptor with full ligand flexibility. The Glide module of Schrodinger uses high throughput virtual screening (HTVS), standard Precision (SP) and Xtra precision (XP) docking methodologies. As the last one provided more appropriate results, the current study provided XP docking score for all the ligands (Table 3.4).
3.2.4  *In-vitro* experimental studies

*Inhibition of acetylcholinesterase (AChE) and butrylcholinesterase (BuChE) activity assay*

The synthesized molecules were tested for AChE and BuChE inhibitory activities according to the method described by Najafi et al, 2017\(^{145}\) with some modifications. Enzyme inhibition assay was performed in a 96-well plate by using Ellman’s reagent 5,5’-dithio-bis-[2-nitrobenzoic acid] (DTNB) method. Briefly, 25 µL AChE/BuChE (25 mU in 100 µM PBS) was incubated with 75 µL DTNB (100 µM PBS containing 600 µM NaHCO\(_3\)) for 5 min at room temperature. To this, 25 µL of test compounds (1 – 1000 µM), and 50 µL PBS (pH 7.4) were added. The reaction mixture was then incubated for 15 min at room temperature. Reaction was initiated by adding 25 µL of acetylthiocholine iodide and butylthiocholine (75 mM in PBS) for AChE and BuChE inhibitory assay respectively. Change in absorbance was recorded spectrophotometrically during the experimental duration of 4 min at 412 nm by using UV-spectrophotometer. A blank reaction was run simultaneously, which was having 25 µL solvent (1% DMSO) in place of drugs. Percent inhibition of AChE activity was calculated by using following equation. Similar method was also used to determine the inhibition of BuChE activity.

\[
\%AChE/BuChE\text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}}
\]

3.3  Results and discussion

3.3.1  Synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives

The synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (16a-i) were achieved by using schemes 3.1, 3.2 and 3.3. The synthesized compounds were characterized by FTIR, \(^1\)H NMR, \(^{13}\)C NMR and elemental analysis.
Scheme 3.1: Synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11)

Scheme 3.2: Synthesis of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol derivatives (15a-i)

Scheme 3.3: Synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives (16a-i)
**Synthesis of 2-aminobenzothiazole (10)**

2-Aminobenzothiazole (10) are important starting materials for many useful and biologically active heterocycles.\(^{192-195}\) There have been several reports for their synthesis.\(^{196}\) Kim, et.al reported its synthesis this molecule using oxidising agent potassium ferricyanide in aqueous sodium hydroxide \([\text{NaCN/K}_3\text{Fe(CN)}_6]\) (Scheme 3.4).\(^{192}\) The compound 17 was also cyclized using sodium hydride (NaH) in the presence of N-methylpyrrolidinone (NMP) at 140°C.\(^{193}\) The 2-aminobenzothiazoles were successfully synthesized by Qiuping et al using 2-iodobenzenamine and isothiocyanate as starting material.\(^{194}\) This reaction was carried out by using CuI as catalyst in presence of 1,4-diazobicyclo(2,2,2)-octane (DABCO) in toluene at 50°C (Scheme 3.5).

![Scheme 3.4: Synthesis of 2-alkylbenzothiazole by using NaOH/K$_3$Fe(CN)$_6$](image)

**Scheme 3.4:** Synthesis of 2-alkylbenzothiazole by using NaOH/K$_3$Fe(CN)$_6$

![Scheme 3.5: Synthesis of 2-N-alkylbenzothiazole by using CuI/DABCO](image)

**Scheme 3.5:** Synthesis of 2-N-alkylbenzothiazole by using CuI/DABCO

Tweit, et al. reported the synthesis of substituted 2-aminobenzothiazole by refluxing alkyl isothiocyanate and 2-aminothiol in alcohol as solvent (Scheme 3.6).\(^{195}\)

![Scheme 3.6: Synthesis of 2-(N-alkyl)benzothiazole](image)

**Scheme 3.6:** Synthesis of 2-(N-alkyl)benzothiazole
We synthesized the compound 10 from aniline and potassium thiocynate. The potassium thiocynate was added to the solution of aniline in glacial acetic acid. Then bromine (Br₂) in glacial acetic acid was added with the help of dropping funnel. During addition of Br₂, the temperature of reaction mixture was maintained below 5°C. Br₂ in this reaction are acting as an oxidizing agent and is used for cyclization. After addition of all bromine, the reaction mixture was allowed to come at room temperature and stirred for 4 hours. The solid mass was separated and filtered, and then was washed with glacial acetic acid to remove the unreacted Br₂. After washing the solid residue was dried at vacuum under reduced pressure and subsequently dissolved in hot water. The resulting solution was neutralized by adding 25% ammonia solution. The white precipitate of 2-aminobenzothiazole was obtained which was characterized by spectroscopic techniques. The absorption at 3395 and 1522 cm⁻¹ in the IR spectrum of 2-aminothiazole have been assigned for N-H stretching of NH₂ and C=N stretching of thiazole respectively (Figure 3.4). In the ¹H NMR spectrum, the aromatic protons of benzothiazole appeared in the region 7.00-7.65 ppm (Figure 3.5). The peaks at 118, 121, 125, 131, 153, 166 and 169 in ¹³C NMR spectrum further confirms the formation of 2-aminobenzothiazole (Figure 3.6).

Figure 3.4: FTIR spectrum of 2-aminobenzothiazole (10)
Figure 3.5: $^1$H NMR spectrum of 2-aminobenzothiazole (10)

Figure 3.6: $^{13}$C NMR spectrum of 2-aminobenzothiazole (10)
Synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11)

The synthesis of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11) was achieved by reacting 2-aminobenzothiazole with chloro acetylchloride in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU). Amide bond formation is the most common reaction and plays a vital role in organic synthesis. A large number of synthetic and natural molecules are known which possess this functional group. The synthetic chemists are always looking for better and easier methods for the formation of amide bond. The condensation of an amine or aniline with carboxylic acid or its derivatives is commonly employed method for amide bond formation. For the synthesis of compound 11, getting the quantitative yield using the reported methods is a major challenge. Some of the important reported methods for the synthesis of 2-chloroacetamide in solution phase using a various solvents with different bases include triethylamine (TEA) in DMF, TEA in DCM, toluene, K₂CO₃ in benzene, TEA in THF, TEA in dioxane and so on. In spite of their potential utility, many of these reported methods suffer from drawbacks such as harsh reaction conditions, long reaction times, unsatisfactory yields, tedious product isolation procedures and needs purification by column chromatography. As a part of our ongoing effort towards the synthesis of biologically active compounds, we herein developed an efficient high yielding synthetic protocol for the one-pot synthesis of amides from aryl amines and chloro acetychloride using DBU as non-nucleophilic base in THF solvent (Table 3.1). This method gave 75 to 95% yields in 3-6 hours at room temperature (rt) for the synthesis of amides like N-phenylacetamides from substituted aryl amines (9-9h), N-benzothiazol-2-ylacetamide (11) and N-(4-phenylthiazol-2-yl)acetamides from substituted 4-phenylthiazole-2-amines (7a-7l) by DBU. The reactions have also been performed in TEA and DABCO using different solvent systems. The combination of DBU and THF gave best result (Table 3.1). This method ensures the wide substrate scope with excellent yields. The products were isolated and purified by recrystallization. DBU is commercially available and cheap homogenous catalyst. It is a sterically hindered bicyclic amidine base and especially useful where side reactions due to nucleophilicity of basic nitrogen are a problem. It is one of the strongest organic neutral base (pKa = 12) in which the +M effect of the adjacent nitrogen stabilizes the protonated species. It has been used in many organic reactions including amide bond formations in recent years. In a typical reaction, aniline (6 mmol) was dissolved in THF (5 ml) and then DBU (1.2 mmol) was added (Scheme 3.7). The reaction mixture was placed on the freezing mixture of ice and salt, and
mechanically stirred for 15 min. After that the chloroacetyl chloride (6.1 mmol) was added from dropping funnel at such rate so that the temperature does not rise beyond 5°C. The reaction mixture was further stirred at room temperature for 3 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into cold water. The compound was precipitated out which was filtered and washed with water. The precipitate was dried and recrystallized using ethanol. The product, N-phenylacetamide was obtained as a solid powder with 86% yield (Table 3.1 and 3.2). The same procedure was also repeated for the substrates 2-aminobenzothiazole and 2-amino-4-phenylthiazole to check the versatility of the process. The optimization of catalysts DBU, DABCO (1,4-diazobicyclo(2,2,2)-octane) and TEA was also performed for aniline in the different solvent systems, and the same ratio applied to all other substrates under optimized reaction conditions (Table 3.1). The reactions in the bases like TEA (triethylamine) and DABCO remained non-completed even after performing the reaction for the longer time.

The reactant, aryl amines in both the cases was not consumed completely even after stirring for 10 hours at room temperature as observed in TLC. Hence, the products were separated by using column chromatography leading to low yield in comparison to DBU. The summary of comparative studies for different bases in the different solvent is given in table 3.1. The comparison of % isolated yield in case of DABCO and DBU for 6 mmol of aniline is given in figure 3.7 According to the proposed mechanisms, DBU provides significant acceleration compared to other amine bases. This suggests that DBU is not only acting as a base rather playing another role also. There are different mechanisms postulated to explain the role of DBU in these types of reactions. According to our observation, the most suitable catalytic mechanism is the displacement of chloride ion by DBU and hence activates the carbonyl for attack by the lone pair of nitrogen present on aryl amines (Figure 3.8). The synthesized compounds were characterized by spectroscopic technique and melting point for known compounds. The absorption at 3228 and 1689 cm$^{-1}$ in the IR spectrum of compound 11 have been assigned for N-H stretching for NH and C=O stretching for amide bond respectively (Figure 3.9). In the $^1$H NMR spectrum, a singlet at 4.47 ppm is for the CH$_2$ proton, which is present in between C=O and Cl atom. A multiplet in the 7.31-8.01 ppm region is due to four aromatic protons and a broad singlet at 12.72 ppm is due to NH proton (Figure 3.10). The peaks at 43, 121, 122, 124, 126, 131, 148, 158 and 166 in $^{13}$C NMR spectrum for aromatic carbon and
carbonyl carbon atoms respectively (Figure 3.11), further confirm the formation of compound 11.

Table 3.1: Optimization of catalysts and solvents for compounds 9, 10 & 7a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compd. No.</th>
<th>Solvent</th>
<th>% yield by using different catalysts</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DBU</td>
</tr>
<tr>
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<td>9</td>
<td>THF</td>
<td>86</td>
</tr>
<tr>
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<td>10</td>
<td>THF</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>7a</td>
<td>THF</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>1,4-dioxane</td>
<td>75</td>
</tr>
<tr>
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<td>10</td>
<td>1,4-dioxane</td>
<td>71</td>
</tr>
<tr>
<td>6</td>
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<tr>
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<tr>
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<tr>
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</tr>
<tr>
<td>15</td>
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<td>DMF</td>
<td>73</td>
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</table>
Figure 3.7. Comparative % yield optimization with DBU & DABCO catalysts for aniline

Scheme 3.7: Reaction of aryl amine with chloroacetyl chloride in the presence of DBU

Synthesis of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol derivatives (15a-i)

4-Amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (15a-i) compounds were synthesized using literature methods\(^\text{187}\) starting from esters of benzoic acid and their derivatives (Scheme 3.2). The reactions between benzoates (12) and hydrazine hydrate gave the corresponding hydrazide derivatives (13). The formed hydrazide derivatives (13) were further treated with carbon disulfide under basic conditions and stirred at room temperature for 15 hours to form the corresponding disulfide salts (14), which were used for subsequent reaction without purification. The formed salts were then reacted with hydrazine hydrate in water. The mixture was refluxed for 24 hours to form the corresponding triazole derivatives (15).
Table 3.2: Amidation of chloroacetyl chloride with different aryl amines 7a-h, 7l, 9, 9a-i, 10, and 10a

<table>
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<tr>
<th>R</th>
<th>Time (hrs)</th>
<th>% Yield of products</th>
<th>Mp (°C)</th>
<th>Lit mp ref</th>
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<tr>
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<tr>
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<td>5</td>
<td>85 (11c)</td>
<td>134-136</td>
<td>-</td>
</tr>
<tr>
<td><img src="image" alt="Structure 7c" /></td>
<td>5</td>
<td>85 (11d)</td>
<td>188-181</td>
<td>-</td>
</tr>
<tr>
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<td>86 (11e)</td>
<td>206</td>
<td>-</td>
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<tr>
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<td>90 (11f)</td>
<td>149</td>
<td>-</td>
</tr>
<tr>
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<td>95 (11g)</td>
<td>234</td>
<td>-</td>
</tr>
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<td>-----</td>
</tr>
<tr>
<td><img src="image" alt="7g" /></td>
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<td>75 (11h)</td>
<td>295-297</td>
<td></td>
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<tr>
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<td>76 (11i)</td>
<td>276-278</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="7l" /></td>
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<td>79 (11j)</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="9" /></td>
<td>3</td>
<td>86</td>
<td>136</td>
<td>134&lt;sup&gt;214&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="image" alt="9a" /></td>
<td>3</td>
<td>82</td>
<td>69-70</td>
<td>73&lt;sup&gt;214&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="image" alt="9b" /></td>
<td>3</td>
<td>80</td>
<td>100</td>
<td>98-100&lt;sup&gt;215&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="image" alt="9c" /></td>
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<td>85</td>
<td>176</td>
<td>178&lt;sup&gt;215&lt;/sup&gt;</td>
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<tr>
<td><img src="image" alt="9d" /></td>
<td>3</td>
<td>85</td>
<td>182</td>
<td>180-184&lt;sup&gt;215&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="image" alt="9e" /></td>
<td>5</td>
<td>76</td>
<td>98</td>
<td>96-98&lt;sup&gt;216&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Figure 3.8: Proposed catalytic cycle for amidation of chloroacetyl chloride with different aryl amines using DBU

The formation of triazole derivatives (15a-i) were confirmed by spectroscopic techniques. The absorptions at 3412 and 1546 cm\(^{-1}\) in the IR spectrum of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol were assigned for NH\(_2\) and C=N stretching for triazole respectively. In the \(^1\)H NMR spectrum, the broad singlet at 3.35 ppm is for SH proton, while aromatic protons appeared in the region 7.53-8.03 ppm (Figure 3.12).
Figure 3.9: FTIR spectrum of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11)

Figure 3.10: $^1$H NMR spectrum of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11)
Figure 3.11: $^{13}$C NMR spectrum of N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (11)

Figure 3.12: $^1$H NMR spectrum of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (15a)
Synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives (16a-i)

The synthesis of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide derivatives was achieved by following scheme 3.3. The products were formed by nucleophilic substitution reaction of NH$_2$ of triazoles (15) to chloroacetamide derivative of 2-aminobenzothiazole (11). For this reaction, the triazole was dissolved in dry acetone or CH$_3$CN, and then added weak base triethyl amine. The reaction mixture was stirred at 50°C. After dissolution of triazole, the compound 11 was added and stirred the reaction mixture for 4 hours. After completion of reaction, the product was purified by column chromatography and characterized by FTIR, NMR spectroscopy and elemental analysis. The absorptions at 3361, 1682 and 756 cm$^{-1}$ in IR spectrum are assigned for NH stretching, C=O stretching of amide and C-S stretching respectively (Figure 3.13). In the $^1$H NMR spectrum, a singlet at 2.67 ppm is for CH$_2$ proton; another singlet at 3.50 ppm is for SH proton attached to triazole ring; multiplet in the region 7.31-8.00 ppm is for 9 aromatic protons; and a broad singlet at 12.62 ppm is for NH proton adjacent to carbonyl group (Figure 3.14). These proton NMR peaks confirms the formation of products. The $^{13}$C NMR spectrum peaks at 36, 121-132, and 163-167 ppm further confirms the formation of product (Figure 3.15).

**Figure 3.13:** FTIR spectrum of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)-acetamide (16a)
**Figure 3.14:** $^1$H NMR spectrum of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide (16a)

**Figure 3.15:** $^{13}$C NMR spectrum of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide (16a)
3.3.2 *In-silico* interaction analysis

Potential binding profile of the novel synthesized compounds (16a-i) and compounds (10, 15a-i) into AChE and BuChE enzymes was studied by performing docking studies. In spite of diverse series of compounds, the docking scores of these molecules are quite high for almost all molecules. This may be an indication of inaccurate score calculation. *In-vitro* results have also indicated that none of the molecule is active against AChE (Table 3.3). However, a range of docking scores indicating favorable to unfavorable interactions are obtained from docking of diverse compounds against BuChE. Further, the docking results of BuChE are correlating well with the *in vitro* experimental studies (Table 3.3). Analysis of the docked structure revealed that the Trp 231 and Phe 329 makes an π-π interaction with benzene ring of benzothiazole moiety. Phe 329 also makes π-π interaction with the thiazole ring, the nitrogen atom of thiazole and oxygen atom of carbonyl makes a H-bond with Ser 198. His 438 makes an π-π interaction with phenyl ring present at 5-position of triazole and SH present at 2-position makes H-bond with Thr 120 in 16a (Figure 3.16). In many compounds, we also observed hydrophobic and aromatic interactions among the compounds and enzyme indicating compounds good binding affinity with the BuChE. The docking results are in consistent with the *in-vitro* results (Table 3.3).

![Figure 3.16: Interactions of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide with active site of BuChE (16a)](image)

83
3.3.3 *In-vitro* inhibition studies of AChE and BuChE

The inhibiton activity of N-benzothiazol-2-yl-2-(3-mercapto-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives against AChE & BuChE are given in table 3.3. Based on the IC$_{50}$ value, the synthesized compounds showed poor to no activity towards AChE, but a remarkably high activity towards BuChE. The better inhibition is being displayed by 16a, 16b and 16f with IC$_{50}$ value of 25.18, 95.52 and 83.25 μM respectively. The synthesized compounds are composed of two fragments: 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol and 2-aminobenzothiazole joined *via* acetamide linkers. The IC$_{50}$ value of 2-aminobenzothiazole (10) was found to be 691.26 μM and of 4-amino-5-phenyl-4H-[1,2,4]triazole-3-thiol (15a) 604.25 μM. On coupling both the moieties, the activity increases apparently. The data reveals that unsubstituted or no substitution on phenyl ring of triazole, and 4-fluoro or 4-methyl substitution on phenyl ring of triazole have increased the anti BuChE activity remarkably. Substitution of 4-Cl, 4-Br, 4-NO$_2$, 3-Br and 3,4,5-trimethoxy at phenyl ring in triazole showed moderate anti BuChE activity. On comparing 16d and 16e, 4-Br is found to be more active than 3-Br. It is useful to note that the substitution at 4-position on phenyl ring of triazole results in higher activity against BuChE than 3-substituted counterparts. It has also been also found that the compound 10 and substituted triazole (with exception 15b) alone are inactive against both the enzyme.
Table 3.3: The IC$_{50}$ value and docking score of synthesized compounds 10, 15a-i and 16a-i against AChE and BuChE

<table>
<thead>
<tr>
<th></th>
<th>IC$_{50}$ (μM)</th>
<th>Docking Score</th>
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<tbody>
<tr>
<td></td>
<td>AChE</td>
<td>BuChE</td>
<td>AChE</td>
<td>BuChE</td>
</tr>
<tr>
<td>10</td>
<td>2903.66 ± 234.97</td>
<td>691.26 ± 215.36</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>15a</td>
<td>1320.4 ± 140.09</td>
<td>604.25 ± 110.08</td>
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<tr>
<td>15b</td>
<td>974.86 ± 130.99</td>
<td>168.47 ± 56.24</td>
<td>-6.14</td>
<td>-6.43</td>
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<tr>
<td>15c</td>
<td>643.56 ± 9.47</td>
<td>1548.52 ± 68.89</td>
<td>-5.83</td>
<td>-5.97</td>
</tr>
<tr>
<td>15d</td>
<td>216.06 ± 7.84</td>
<td>1200.91 ± 103.98</td>
<td>-6.34</td>
<td>-5.98</td>
</tr>
<tr>
<td>15e</td>
<td>671.28 ± 71.53</td>
<td>1023.59 ± 23.28</td>
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<td>-5.67</td>
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<tr>
<td>15f</td>
<td>501.41 ± 0.82</td>
<td>1525.55 ± 366.47</td>
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<td>-5.96</td>
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<tr>
<td>15g</td>
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<td>1441.27 ± 218.14</td>
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<td>-5.68</td>
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<tr>
<td>15h</td>
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<td>1382.56 ± 5.69</td>
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<tr>
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</tr>
<tr>
<td>16d</td>
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<tr>
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</tr>
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<tr>
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<td>4.66 ± 0.503</td>
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**Figure 3.17:** IC\textsubscript{50} value in μM of N-benzothiazol-2-yl-2-(3-mercaptop-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives against AchE enzyme. IC\textsubscript{50} value less than 100 μM concentration is considered significant inhibition against AchE enzyme.

**Figure 3.18:** IC\textsubscript{50} value in μM of N-benzothiazol-2-yl-2-(3-mercaptop-5-phenyl-[1,2,4]triazol-4-ylamino)acetamide derivatives against BuChE enzyme. IC\textsubscript{50} value less than 100 μM concentration is considered significant inhibition against BuChE enzyme.