2-Mercaptobenzimidazole Derivatives as Novel Butyrylcholinesterase Inhibitors: Biology-Oriented Drug Synthesis (BIODS), *In-Vitro* and *In-Silico* Evaluation

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Summary: Schiff bases gaining remarkable importance day by day in the current situation Schiff bases are found to be a valuable pharmacophore for the synthesis and development of various biologically active heterocyclic compounds. In recent past, we have reported various classes of compounds as enzyme inhibitors, in continuation; A series of 2-Mercaptobenzimidazole hydrazone derivatives **(9-42)** were synthesized through multistep reactions in high yields and evaluated for butyrylcholinesterase inhibition. In present study, 2-ethylthio benzimidazole was formed by the reaction of 2-Mercaptobenzimidazole with bromoethane. In the second step (2-(2-(ethylthio)benzimidazolyl)acetate) obtained by the reaction of 2-ethylthio benzimidazole with ethyl chloroacetate. In the third step, 2-(2-(ethylthio)benzimidazolyl)acetate was refluxed in methanol with hydrazine hydrate and get 2-((ethylthio)benzimidazolyl)acetohyrazide. In the last step, 2-((ethylthio)benzimidazolyl)acetohyrazide was reacted with different aldehydes in the presence of glacial acetic acid (catalyst) to get a series of 2-Mercaptobenzimidazole hydrazone derivatives. Product was characterized by ^{1}H NMR and ^{13}C NMR.

These newly synthesized compounds showed varying degree of butyrylcholinesterase inhibition. Compound no. **15** with (IC_{50} = 25.10 ± 0.90 μM) was found to be most active in the whole series. Similarly, compounds **42**, **12**, **40**, **17**, **22**, **28** and **09** exhibited excellent activity with IC_{50} values are (IC_{50} = 25.36 ± 0.57 μM, IC_{50} = 27.30 ± 0.52 μM, IC_{50} = 34.31 ± 0.59 μM, IC_{50} = 51.29 ± 0.64 μM, IC_{50} = 54.52 ± 0.95 μM, IC_{50} = 57.90 ± 0.45 μM and IC_{50} = 60.93 ±0.67 μM) respectively as compared to standard galantamine 18.13±0.20 μM. Molecular docking helped to find interactions between butyrylcholinesterase enzyme and test compounds.

This study results that Schiff bases have been discovered a new class of butyrylcholinesterase inhibitors which have not been discovered earlier.

Keywords: Benzimidazole, Benzimidazole-2-thiol, *In silico*, Butyryl Cholinesterase, Hydrazone Schiff's bases.

Introduction

Schiff bases are the compounds generally represented with a formula of $R_2R_3C=NR_1$. They are the condensation products of primary amines and carbonyl compounds, i.e., aldehydes and ketones. German Chemist Hugo Schiff first reported Schiff bases in 1864. Schiff bases contain azomethine structurally or imine functional group (–C=N–) gaining remarkable importance day by day in the current situation [1-3]. Schiff bases are found to be a valuable pharmacophore for the synthesis and development of various biologically active heterocyclic compounds. Various literatures reported that Schiff bases have many applications in different fields, including analytical, biological, and inorganic chemistry. They are used in dyes, pigments and intermediate in organic synthesis, polymers, and corrosion inhibitors [4]. Schiff bases have achieved a broad spectrum of biological activities such as antibacterial, antimalarial, antimicrobial, anti-inflammatory, antioxidant, antiviral, antiglycation and antidepressant activities [5-10].

The basic nitrogen atom of imine is also involved in hydrogen bond formation with the active sites of cell constituents responsible for normal cell function [11, 12]. Introduction of metal complexes into Schiff bases show decent biological activities [13].

Butyrylcholinesterase enzyme is also known as pseudocholinesterase or plasma cholinesterase enzyme. Its abbreviated form is (BChE). It is made mostly in blood plasma in humans and encoded by the butyrylcholinesterase gene [14]. Butyrylcholinesterase enzyme has distinctive enzymatic properties and is broadly dispersed in the nervous system due to the possible involvement in neural function. It is a non-specific cholinesterase enzyme that hydrolysis ester of choline including acetylcholine. Literature reported

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that inhibitors of butyrylcholinesterase enzyme are valuable approaches to treat neurological diseases including Alzheimer's disease [15] and carried out possible significant applications in the treatment of different diseases such as ataxia, dementia and Parkinson disease [16].

In the present study, thirty-four 2-Mercaptobenzimidazole based novel hydrazone derivatives (**09**-**42**) were screened for butyrylcholinesterase activity. The binding mode of the synthesized compounds was studied by molecular docking and found moderate results.

Experimental

Materials and Methods

During this experimental study, analytical grade solvents were used. The purity of products was monitored through alumina TLC plates, and the melting point was determined through melting point apparatus. Generally, n-hexane and ethyl acetate solvent medium were used for checking of reaction through TLC plates. Progress of the reaction was monitored by thin layer chromatography. Ultraviolet lamp was used as a visualizing agent.

The whole reactions were carried out in clean glassware with specific catalysts, basic or acidic conditions. All synthesized compounds were characterized by using different spectroscopic techniques such as ^{1}H NMR and ^{13}C NMR.

^{1}H NMR experiments were performed on Advance Bruker AM 300, 400 and 500 MHz. Thin Layer Chromatography (TLC) was performed on pre-coated silica gel aluminum plates with dimension 3x8 cm (Kieselgel 60, 254, E. Merck, Germany). Chromatogram was visualized with dual wave length by UV at 254 and 365 nm. Melting point was found out on Gallon kemp apparatus.

General procedure for the synthesis of novel hydrazone derivatives based on 2-Mercaptobenzimidazole **(09-42)**

2-Mercaptobenzimidazole based hydrazone derivatives were carried out through multistep reactions. First 2-Mercaptobenzimidazole was refluxed with bromoethane in basic condition (KOH) in ethanol with equimolar amounts for about 10 h. After completion of reaction, the reaction mixture was filtered. The filtrate so obtained was kept until whole ethanol was evaporated and gets shiny white needle like crystals of 2-ethylthio benzimidazole. In second step 2-ethylthio benzimidazole was taken in round bottom flask refluxed with ethyl chloroacetate (drop wise) using anhydrous potassium carbonate in DMF (solvent) for about 15h. After completion of reaction, the product **(**2-(2-(ethylthio)benzimidazolyl)acetate**)** obtained and get through separating funnel in semisolid form. In third step, 2-(2-(ethylthio)benzimidazolyl)acetate was refluxed in methanol with hydrazine hydrate for about 10h. The product, 2-((ethylthio)benzimidazolyl)acetohyrazide get was poured into ice cold water until precipitate was formed. Precipitate was filtered and then dried in open atmosphere. In fourth step, 2-((ethylthio)benzimidazolyl)acetohyrazide was dissolved in methanol with 2-3 drops of acetic acid (catalyst) on hotplate. After 10 minutes aldehyde was added and refluxed the whole mixture for about 5-6 hours. Progress of reaction was monitored by TLC. After completion of reaction, the mixture was poured into ice cold water until precipitate was formed. Precipitate was collected by filtration, washed with water and then dried in open atmosphere.

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-hydroxy benzylidene) acetohydrazide **(09)**

Yield: 0.78g (90%), melting point: 165-170 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.14 (s, 1H), 9.96 (s, 1H), 7.67 (s, 1H), 7.59 – 7.48 (m, 4H), 7.20 – 7.24 (m, 2H), 6.85 – 6.78 (m, 2H), 5.39 (s, 2H), 3.38 (q, *J* = 7.5 Hz, 2H), 1.42 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.09, 155.21, 153.26, 147.84, 143.66, 138.19, 134.19, 127.06, 123.57, 121.33, 116.31, 113.22, 110.43, 47.43, 29.91, 16.38

Synthesis of N'-(4-chlorobenzylidene)-2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl) acetohydrazide **(10)**

Yield: 0.75g (86%), melting point: 155-160 ˚C, ^{1}H NMR (400 MHz, DMSO-*d6*): δ 11.92 (s, 1H), 8.13 (s, 1H), 7.84 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 8.6 Hz, 2H), 7.47 (d, J = 7.0 Hz, 1H), 7.40 (d, J = 7.0 Hz, 1H), 7.24 – 7.21 (m, 2H), 5.41 (s, 2H), 3.10 (q, J = 7.5 Hz, 2H), 1.35 (t, J = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.09, 158.19, 146.83, 142.68, 139.99, 138.15, 135.61, 127.97, 125.72, 122.94, 120.39, 117.31, 105.49, 42.30, 25.81, 12.39

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(3,4,5-trimethoxybenzylidene) acetohydrazide **(11)**

Yield: 0.80g (88%), melting point: 218-220 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.86 (s, 1H), 8.10 (s, 1H), 7.67 (d, *J* = 7.5 Hz, 1H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.24 (m, 2H), 7.05 (s, 2H), 5.35 (s, 2H), 3.87

(s, 6H), 3.76 (s, 3H), 3.05 (q, *J* = 7.2 Hz, 2H), 1.34 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.40, 158.08, 155.83, 149.61, 142.42, 140.71, 138.25, 131.71, 128.08, 124.30, 121.30, 115.75, 108.44, 67.89, 59.24, 49.41, 28.97, 18.38

Synthesis of N'-(2,4-dichlorobenzylidene)-2-(2-(ethylthio)-1H-benzo[d] imidazol-1-yl)acetohydrazide **(12)**

Yield: 0.79g (85%), melting point: 205-210 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (s, 1H), 8.73 (s, 1H), 7.97 (d, *J* = 2.0 Hz, 1H), 7.67 (d, *J* = 7.0 Hz, 1H), 7.52 (d, *J* = 7.0 Hz, 1H), 7.47 (d, *J* = 8.5, 1H), 7.40 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.24 – 7.20 (m, 2H), 5.11 (s, 2H), 3.09 (q, *J* = 7.2 Hz, 2H), 1.34 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.16, 153.18, 148.87, 143.69, 135.19, 132.27, 131.21, 127.81, 126.59, 125.26, 124.91, 121.94, 120.72, 116.39, 107.57, 41.37, 24.98, 12.76

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-formyl benzylidene) acetohydrazide **(13)**

Yield: 0.81g (89%), melting point: 195-199 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.97 (s, 1H), 9.92 (s, 1H), 8.18 (s, 1H), 7.80 (d, *J* = 8.5 Hz, 2H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 7.2 Hz, 1H), 7.53 (d, *J* = 7.2 Hz, 1H), 7.23 – 7.18 (m, 2H), 5.37 (s, 2H), 3.12 (q, *J* = 7.3 Hz, 2H), 1.35 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.95, 163.07, 155.14, 147.90, 145.67, 136.62, 135.11, 133.80, 128.85, 157.02, 122.84, 121.60, 117.90, 106.42, 40.84, 24.51, 13.50

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-nitro benzylidene) acetohydrazide **(14)**

Yield: 0.80g (84%), melting point: 162-167 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.12 (s, 1H), 8.29 (d, *J* = 8.5 Hz, 2H), 8.17 (s, 1H), 8.04 (d, *J* = 8.5 Hz, 2H), 7.64 – 7.45 (m, 2H), 7.26 – 7.08 (m, 2H), 5.46 (s, 2H), 3.30 (q, *J* = 7.3 Hz, 2H), 1.36 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.02, 151.89, 147.84, 142.96, 142.05, 140.16, 136.92, 128.01, 123.96, 121.57, 117.55, 109.71, 44.54, 26.47, 14.98

Synthesis of N'-(2,4-dimethoxybenzylidene)-2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl) acetohydrazide **(15)**

Yield: 0.830g (85%), melting point: 200-205 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.07 (s, 1H), 8.58 (s, 1H), 7.61 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.59 – 7.50 (m, 2H), 7.29 – 7.17 (m, 2H), 6.65 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.57 (d, *J* = 2.1 Hz, 1H), 5.09 (s, 2H), 3.88 (s, 3H), 3.83 (s, 3H), 3.08 (q, *J* = 7.2 Hz, 2H), 1.37 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.19, 152.17, 150.87, 147.65, 136.91, 135.27, 133.21, 130.85, 128.52, 125.26, 125.01, 122.94, 121.32, 117.30, 105.55, 48.36, 25.41, 18.77

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(3-hydroxy benzylidene) acetohydrazide **(16)**

Yield: 0.88g (86%), melting point: 150-157 C˚,^{1}H NMR (400 MHz, DMSO-d_6): δ 10.17 (s, 1H), 9.87 (s, 1H),7.90 (s, 1H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.34 (d, *J* = 7.2 Hz, 1H), 7.22 – 7.14 (m, 5H), 6.85 (d, *J* = 7.8 Hz, 1H), 5.33 (s, 2H), 2.99 (q, *J* = 7.3 Hz, 2H), 1.29 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.97, 159.35, 157.80, 149.42, 146.97, 138.82, 134.32, 128.25, 124.89, 123.90, 116.83, 115.99, 113.61, 110.91, 106.42, 48.90, 27.91, 17.24

Synthesis of N'-(4-(diethylamino)benzylidene)-2-(2-(ethylthio)-1H-benzo[d] imidazol-1-yl)acetohydrazide **(17)**

Yield: 0.82g (87%), melting point: 165-170 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.28 (s, 1H), 7.96 (s, 1H), 7.60 – 7.54 (m, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.29 – 7.17 (m, 2H), 6.62 (d, *J* = 8.4 Hz, 2H), 5.53 (s, 2H), 3.38 (q, *J* = 7.2 Hz, 4H), 3.13 (q, 7.3 Hz, 2H), 1.34 (t, *J* = 7.3 Hz, 3H), 1.18 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.05, 153.67, 151.98, 146.66, 146.69, 138.25, 126.98, 123.11, 122.08, 120.67, 115.32, 110.23, 104.59, 45.54, 43.76, 25.91, 18.45, 13.57

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-hydroxy-3-methoxybenzylidene) acetohydrazide **(18)**

Yield: 0.72g (91%), melting point: 177-180 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.09 (s, 1H), 10.09 (s, 1H), 8.47 (s, 1H), 7.58 – 7.52 (m, 2H), 7.28 – 7.16 (m, 3H), 7.05 (d, *J* = 8.5, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 5.33 (s, 2H), 3.77 (s, 3H), 3.15 (q, *J* = 7.3 Hz, 2H), 1.37 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.67, 156.15, 146.85, 145.38, 144.97, 143.65, 138.98, 128.69, 126.78, 123.92, 121.98, 118.30, 113.94, 107.57, 105.08, 55.15, 46.99, 29.51, 18.79

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(2-hydroxy-3-methoxybenzylidene) acetohydrazide **(19)**

Yield: 0.79g (90%), melting point: 150-162 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.05 (s, 1H), 10.12 (s, 1H), 8.78 (s, 1H), 7.58 – 7.51 (m, 2H), 7.25 –

7.20 (m, 2H), 7.01 – 6.96 (m, 2H), 6.88 (d, *J* = 7.8 Hz, 1H), 5.56 (s, 2H), 3.87 (s, 3H), 3.17 (q, *J* = 7.3 Hz, 2H), 1.34 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.11, 156.15, 155.30, 147.55, 145.01, 143.65, 135.11, 123.13, 121.47, 120.61, 118.75, 117.30, 115.79, 114.81, 108.57, 53.15, 45.36, 24.91, 13.07

Synthesis of N'-(2-chlorobenzylidene)-2-(2-(ethylthio)-1H-benzo[d] imidazol-1-yl) acetohydrazide **(20)**

Yield: 0.70g (90%), melting point: 198-206 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H), 8.21 (s, 1H), 7.99 (dd, *J* = 7.5, 1.9 Hz, 1H), 7.77 – 7.67 (m, 1H), 7.50 – 7.26 (m, 4H), 7.24 – 7.17 (m, 2H), 5.35 (s, 2H), 3.38 (q, *J* = 7.4 Hz, 2H), 1.44 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.78, 155.17, 148.45, 143.67, 135.82, 133.88, 132.45, 131.98, 127.36, 125.49, 124.18, 123.17, 121.84, 17.31, 110.42, 49.78, 27.91, 15.19

Synthesis of N'-(3,4-dimethoxybenzylidene)-2-(2-(ethylthio)-1H-benzo[d] imidazol-1-yl)acetohydrazide **(21)**

Yield: 0.91g (81%), melting point: 225-230 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.67 (s, 1H), 8.63 (s, 1H), 7.57 – 7.51 (m, 3H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.25 – 7.20 (m, 2H), 7.03 (d, *J* = 8.3 Hz, 1H), 5.39 (s, 2H), 3.88 (s, 6H), 3.21 (q, *J* = 7.3 Hz, 2H), 1.27 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.44, 155.18, 152.91, 150.78, 147.69, 145.65, 138.17, 129.23, 126.98, 122.13, 120.76, 119.31, 113.09, 108.54, 107.45, 58.89, 53.67, 48.41, 28.97, 13.34

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(naphthalen-1-ylmethylene) acetohydrazide **(22)**

Yield: 0.71g (83%), melting point: 222-225 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.06 (s, 1H), 8.46 (s, 1H), 8.02 (d, *J* = 7.7 Hz, 1H), 7.97 (d, *J* = 7.7 Hz, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.69 (d, *J* = 7.7 Hz, 1H) 7.64 – 7.50 (m, 5H), 7.26 – 7.19 (m, 2H), 5.52 (s, 2H), 3.12 (q, *J* = 7.0 Hz, 2H), 1.26 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.07, 154.15, 148.93, 144.65, 137.11, 133.18, 131.98, 130.15, 128.29, 128.22, 127.46, 126.25, 126.21, 126.13, 123.84, 123.76, 122.33, 119.31, 109.54, 44.43, 26.91, 14.19

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(2-methoxybenzylidene) acetohydrazide **(23)**

Yield: 0.78g (80%), melting point: 180-186 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.44 (s, 1H), 8.37 (s, 1H), 7.59 – 7.53 (m, 2H), 7.44 (d, *J* = 8.1 Hz, 1H), 7.40 (td, *J* = 8.0, 1.5 Hz, 1H), 7.25 – 7.20 (m, 2H), 7.03 – 6.94 (m, 2H), 5.47 (s, 2H), 3.86 (s, 3H), 3.19 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.11, 156.67, 154.17, 150.66, 144.67, 137.11, 130.15, 127.07, 125.63, 124.14, 122.70, 122.15, 119.30, 110.88, 109.42, 55.80, 44.39, 26.91, 14.18

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-methylbenzylidene)acetohydrazide **(24)**

Yield: 0.80g (82%), melting point: 167-170 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.72 (s, 1H), 8.05 (s, 1H), 7.69 – 7.62 (m, 3H), 7.52 (d, *J* = 7.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.24 – 7.15 (m, 2H), 5.39 (s, 2H), 3.24 (q, *J* = 7.2 Hz, 2H), 2.38 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.07, 154.20, 145.66, 144.67, 140.03, 136.82, 129.86, 129.19, 126.81, 124.00, 122.90, 119.31, 109.43, 44.40, 26.91, 21.33, 14.28

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(2-hydroxy benzylidene) acetohydrazide **(25)**

Molecular Formula: $C_{18}H_{18}N_4O_2S$, Molecular Weight: 354.43

Yield: 0.86g (77%), melting point: 175-180 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.55 (s, 1H), 9.70 (s, 1H), 8.57 (s, 1H), 8.56 (d, *J* = 1.0 Hz, 1H), 7.59 – 7.45 (m, 3H), 7.32 – 7.18 (m, 3H), 6.95 – 6.84 (m, 2H), 5.36 (s, 1H), 3.06 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.11, 158.80, 154.20, 152.50, 144.67, 136.82, 132.56, 131.47, 124.00, 122.90, 120.25, 119.31, 117.28, 114.95, 109.42, 44.42, 26.91, 14.29

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-octylidene acetohydrazide **(26)**

Yield: 0.82g (87%), melting point: 176-177 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.59 (s, 1H), 7.55 (dd, J =7.1, 1.6 Hz 1H), 7.45 (dd, J =7.1, 1.6 Hz), 7.28 – 7.16 (m, 2H), 7.02 (t, J = 4.5 Hz, 1H), 5.41 (s, 2H), 3.06 (q, J = 7.2 Hz, 2H), 2.23 (td, J = 6.6, 4.4 Hz, 2H), 1.56 – 1.44 (m, 2H), 1.43 – 1.32 (m, 5H), 1.32 – 1.22 (m, 6H), 0.95 – 0.83 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.31, 156.22, 148.41, 144.27, 136.35, 126.20, 123.36, 115.31, 111.51, 49.80, 34.99, 28.39, 28.13, 27.77, 26.81, 24.02, 22.61, 16.31, 14.78

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-((5-methylfuran-2-yl)methylene) acetohydrazide **(27)**

Yield: 0.77g (88%), melting point: 215-220 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.69 (s, 1H), 8.29 (s, 1H), 7.69 – 7.62 (m, 1H), 7.59 – 7.51 (m, 1H), 7.30 – 7.17 (m, 2H), 6.59 (d, J = 8.5 Hz, 1H), 6.23 (dq, J = 8.5, 0.7 Hz, 1H), 5.47 (s, 2H), 3.12 (q, J = 7.3 Hz, 2H), 2.38 (d, J = 0.6 Hz, 3H), 1.32 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.94, 155.82, 145.67, 144.92, 139.15, 135.14, 132.39, 129.23, 126.37, 124.00, 122.86, 118.31, 104.54, 49.30, 27.91, 18.97, 13.19

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(3-methoxybenzylidene)acetohydrazide **(28)**

Yield: 0.79g (83%), melting point: 134-140 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.87 (s, 1H), 8.08 (s, 1H), 8.23 (t, *J* = 1.0 Hz, 1H), 7.69 – 7.60 (m, 1H), 7.59 – 7.50 (m, 1H), 7.33 – 7.16 (m, 5H), 6.96 – 6.88 (m, 1H), 5.41 (s, 2H), 3.81 (s, 3H), 3.29 (q, *J* = 7.3 Hz, 2H), 1.36 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.74, 158.22, 153.14, 149.52, 145.67, 136.02, 134.72, 128.69, 124.05, 121.34, 120.32, 119.39, 116.75, 113.77, 109.94, 56.92, 47.40, 23.21, 15.71

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(thiophen-2-ylmethylene) acetohydrazide **(29)**

Yield: 0.86g (81%), melting point: 187-190 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.53 (s, 1H), 8.44 (s, 1H), 7.70 – 7.60 (m, 1H), 7.60 – 7.48 (m, 2H), 7.31 – 7.17 (m, 3H), 7.10 (dd, J = 7.8, 5.7 Hz, 1H), 5.40 (s, 2H), 3.13 (q, J = 7.0 Hz, 2H), 1.37 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.44, 153.27, 146.47, 138.45, 135.36, 133.24, 129.46, 128.16, 125.38, 122.37, 118.31, 108.16, 45.39, 27.41, 19.51

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-isopropylbenzylidene)acetohydrazide **(30)**

Yield: 0.76g (86%), melting point: 212-217 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.29 (s, 1H), 8.08 (s, 1H), 7.68 – 7.59 (m, 1H), 7.60 – 7.50 (m, 3H), 7.33 – 7.16 (m, 4H), 5.47 (s, 2H), 3.16 (q, J = 7.2 Hz, 2H), 2.96 (dtt, J = 13.0, 6.5, 1.0 Hz, 1H), 1.38 (t, J = 7.2 Hz, 3H), 1.25 (d, J = 6.5 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.34, 158.42, 148.40, 146.70, 144.24, 135.22, 131.23, 126.59, 125.01, 123.06, 122.34, 114.31, 109.50, 49.30, 35.93, 24.91, 22.93, 15.78.

Synthesis of N'-butylidene-2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl) acetohydrazide **(31)**

Yield: 0.88g (78%), melting point: 145-150 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.41 (s, 1H), 7.59 – 7.53 (m, 2H), 7.32 (t, J = 4.4 Hz, 1H), 7.29 – 7.17 (m, 2H), 5.29 (s, 2H), 3.11 (q, J = 7.1 Hz, 2H), 2.18 (td, J = 6.5, 4.4 Hz, 2H), 1.51 (qt, J = 7.5, 6.5 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H), 0.94 (t, J = 7.6 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6): δ 164.35, 158.31, 148.25, 143.61, 134.84, 125.11, 123.22, 117.21, 108.38, 48.29, 33.28, 27.21, 19.87, 17.31, 12.15.

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-fluorobenzylidene) acetohydrazide **(32)**

Yield: 0.95g (81%), melting point: 202-204 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 10.42 (s, 1H), 7.73 (dt, *J* = 7.3, 1.3 Hz, 1H), 7.69 (s, 1H), 7.61 (dd, *J* = 8.7, 5.5 Hz, 2H), 7.24 – 7.17 (m, 3H), 7.10 (t, *J* = 8.6 Hz, 2H), 5.34 (s, 2H), 3.38 (q, *J* = 7.4 Hz, 2H), 1.43 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.68, 152.63, 145.20, 143.64, 136.65, 129.51, 129.43, 122.40, 122.29, 118.66, 116.28, 116.07, 108.72, 44.92, 27.67, 15.02

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(furan-2-ylmethylene) acetohydrazide **(33)**

Yield: 0.87g (76%), melting point: 234-240 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 12.10 (s, 1H), 8.29 (s, 1H), 7.69 (dd, J = 7.8, 1.4 Hz, 1H), 7.62 – 7.50 (m, 2H), 7.30 – 7.18 (m, 2H), 6.82 (dd, J = 7.8, 1.4 Hz, 1H), 6.60 (dd, J = 7.9, 1.4 Hz, 1H), , 5.56 (s, 2H), 3.23 (q, J = 7.3 Hz, 2H), 1.33 (t, J = 7.3 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6): δ 162.35, 158.87, 147.87, 145.94, 144.19, 138.29, 137.96, 128.24, 124.97, 118.31, 113.13, 110.91, 108.26, 48.99, 24.91, 17.91

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-methoxy benzylidene) acetohydrazide **(34)**

Yield: 0.92g (79%), melting point: 187-190 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.97 (s, 1H), 8.18 (s, 1H), 7.65 –7.55 (m, 2H), 7.51 – 7.45 (m, 2H), 7.28 – 7.16 (m, 2H), 7.03 – 6.95 (m, 2H), 5.39 (s, 2H), 3.83 (s, 3H), 3.11 (q, J = 7.3 Hz, 2H), 1.43 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.94, 158.66, 152.74, 144.57, 141.97, 138.22, 129.52, 126.90, 125.95, 121.34, 118.31, 114.70, 107.94, 57.33, 48.40, 25.91, 17.71

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(3-nitro benzylidene) acetohydrazide **(35)**

Yield: 0.82g (83%), melting point: 172-178 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.78 (s, 1H), 8.57 (s, 1H), 8.13 (dd, J = 7.7, 1.2 Hz, 1H), 8.06 (s, 1H), 7.83 (dd, J = 7.7, 1.1 Hz, 1H), 7.68 (t, J = 7.7 Hz,

1H), 7.58 – 7.54 (dd, J = 6.9, 1.5 Hz, 1H), 7.49 (dd, J = 6.9, 1.5 Hz, 1H), 7.29 – 7.17 (m, 2H), 5.46 (s, 2H), 3.23 (q, J = 7.3 Hz, 2H), 1.32 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.94, 155.12, 149.45, 146.64, 143.00, 138.82, 134.42, 129.22, 128.45, 126.36, 123.34, 122.95, 121.02, 118.31, 108.52, 43.40, 28.21, 15.67.

Synthesis of N'-(3-bromobenzylidene)-2-(2-(ethylthio)-1H-benzo[d] imidazol-1-yl)acetohydrazide **(36)**

Yield: 0.88g (80%), melting point: 138-143 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.92 (s, 1H), 8.01 (s, 1H), 7.78 (s, 1H), 7.63 (dd, J = 7.8, 2.0 Hz, 1H), 7.60 – 7.52 (m, 3H), 7.41 (t, J = 7.9 Hz, 1H), 7.29 – 7.17 (m, 2H), 5.59 (s, 2H), 3.15 (q, J = 7.2 Hz, 2H), 1.41 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.65, 156.80, 146.28, 144.69, 138.15, 134.32, 130.56, 129.32, 128.76, 126.04, 123.31, 122.95, 121.30, 119.31, 111.59, 54.99, 28.31, 16.81

Synthesis of N'-(3-ethoxy-2-hydroxybenzylidene)-2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)acetohydrazide **(37)**

Yield: 0.85g (81%), melting point: 165-174 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.09 (s, 1H), 11.28 (s, 1H), 8.55 (s, 1H), 7.59 – 7.49 (m, 2H), 7.29 – 7.17 (m, 2H), 7.10 (dd, J = 7.6, 1.6 Hz, 1H), 6.98 – 6.85 (m, 2H), 5.51 (s, 2H), 4.17 (q, J = 6.9 Hz, 2H), 3.18 (q, J = 7.1 Hz, 2H), 1.45 (t, J = 6.9 Hz, 3H), 1.37 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.37, 159.92, 154.34, 147.99, 146.38, 141.23, 134.52, 125.96, 123.68, 122.57, 119.99, 118.32, 117.44, 116.62, 100.48, 68.74, 48.41, 29.31, 19.63, 14.76

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(4-fluoro-3-methoxybenzylidene) acetohydrazide **(38)**

Yield: 0.75g (77%), melting point: 185-188 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.69 (s, 1H), 8.23 (s, 1H), 7.57 – 7.47 (m, 2H), 7.31 (dd, J = 8.3, 1.7Hz, 1H), 7.29 – 7.17 (m, 3H), 6.96 (s, 1H), 5.59 (s, 2H), 3.86 (s, 3H), 3.21 (q, J = 7.3 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.67, 156.32, 154.82, 152.35, 149.04, 147.54, 145.37, 143.93, 136.22, 131.10, 129.37, 124.06, 123.74, 121.97, 121.29, 118.31, 116.98, 115.28, 111.26, 110.08, 109.50, 61.21, 56.31, 49.19, 29.91, 19.91

Synthesis of N'-(2,3-dihydroxybenzylidene)-2-(2-(ethylthio)-1H-benzo[d] imidazol-1-yl) acetohydrazide **(39)**

Yield: 0.85g (79%), melting point: 213-214 ˚C,^{1}H NMR (400 MHz, DMSO-d_6): δ 11.98 (s, 1H), 10.69 (s, 1H), 9.82 (s, 1H), 8.47 (s, 1H), 7.59 – 7.50 (m, 2H), 7.29 – 7.17 (m, 2H), 6.90 (dd, J = 7.1, 1.2 Hz, 1H), 6.75 – 6.63 (m, 2H), 5.48 (s, 2H), 3.27 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.97, 157.14, 152.88, 148.13, 145.65, 144.69, 138.22, 127.05, 125.34, 122.26, 120.45, 119.81, 118.58, 117.05, 107.44, 54.40, 23.91, 18.71

Synthesis of N'-(2,4-dihydroxybenzylidene)-2-(2-(ethylthio)-1H-benzo[d] imidazol-1-yl)acetohydrazide **(40)**

Yield: 0.81g (82%), melting point: 187-188 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.93 (s, 1H), 11.54 (s, 1H), 9.98 (s, 1H), 8.58 (s, 1H), 7.61 – 7.50 (m, 2H), 7.29 – 7.17 (m, 2H), 7.05 (dd, J = 8.2, 1.1 Hz, 1H), 6.35 – 6.26 (m, 2H), 5.53 (s, 2H), 3.16 (q, J = 7.3 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.56, 162.82, 156.38, 154.14, 151.68, 147.67, 136.22, 132.72, 126.05, 121.34, 119.67, 112.44, 107.93, 107.38, 103.96, 44.45, 26.91, 18.21

Synthesis of 2-(2-(ethylthio)-1H-benzo[d]imidazol-1-yl)-N'-(2-methyl benzylidene) acetohydrazide **(41)**

Yield: 0.87g (86%), melting point: 155-160 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (s, 1H), 8.22 (s, 1H), 7.63 – 7.51 (m, 3H), 7.37 (td, J = 7.5, 1.6 Hz, 1H), 7.33 – 7.19 (m, 5H), 5.44 (s, 2H), 3.23 (q, J = 7.3 Hz, 2H), 2.56 (s, 3H), 1.39 (t, J = 7.2 Hz, 3H). 13C NMR (100 MHz, DMSO-d_6): δ 165.54, 158.22, 153.74, 145.67, 139.95, 137.77, 132.84, 130.57, 128.86, 127.42, 126.51, 124.97, 121.36, 114.31, 106.93, 49.40, 28.61, 22.56, 16.87

Synthesis of N'-(anthracen-9-ylmethylene)-2-(2-(ethylthio)-1H-benzo[d] imidazol-1-yl)acetohydrazide **(42)**

Yield: 0.80g (82%), melting point: 221-230 ˚C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.09 (s, 1H), 8.63 (s, 2H), 8.24 – 8.16 (m, 2H), 8.08 – 7.98 (m, 2H), 7.62 – 7.50 (m, 6H), 7.30 – 7.18 (m, 2H), 5.59 (s, 2H), 3.14 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.67, 155.79, 147.92, 142.87, 138.58, 133.71, 131.37, 128.86, 128.46, 127.23, 126.98, 126.71, 126.21, 123.56, 121.39, 119.38, 114.05, 48.78, 29.91, 17.54.

Molecular Docking Approach

Molecular Operating Environment (MOE) software package was used to perform molecular

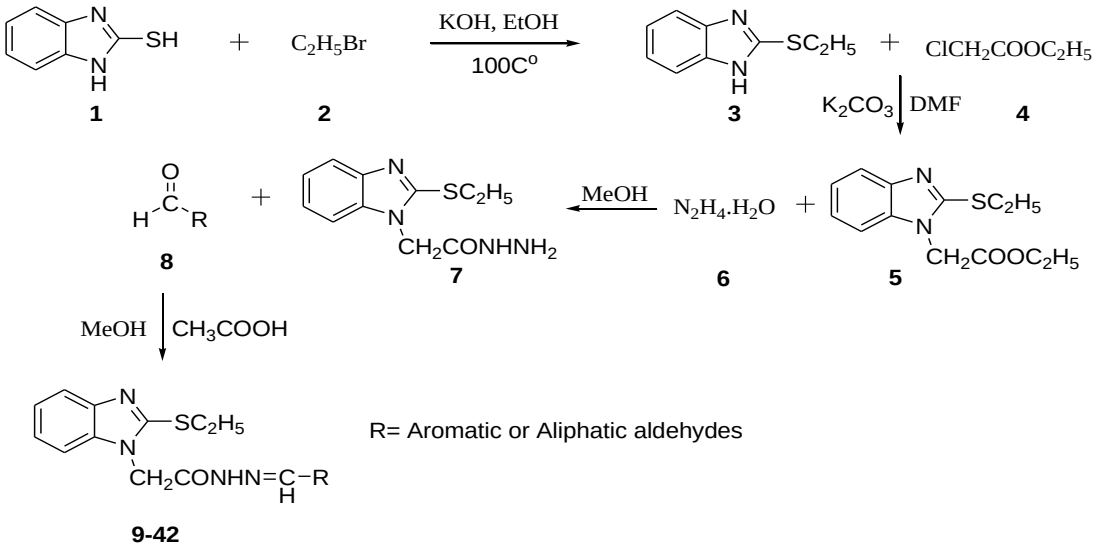
docking study of the synthesized derivatives against human butyryl-cholinesterase enzyme. The 3D structural coordinates for all derivatives generated by using the builder tool, then protonated and energy minimized using the default parameters of the MOE package. The crystallographic structure of the human butyryl-cholinesterase enzyme was retrieved (PDB code: **1P0P)** from online free server protein databank (www.rcsb.org). Next, the structure coordinate was subject for protonation and was energy minimized to get a stable conformation of the protein. The parameters were used to perform the molecular docking study, i.e., **Placement**: Triangle Matcher, **Rescoring 1**: London dG, **Refinement**: Forcefield, **Rescoring 2**: GBVI/WSA. For each compound, a total of ten conformations were allowed to be formed. Later, the top-ranked conformations based on docking score (S) were selected for protein-ligand interaction analysis.

Results and Discussion

Chemistry

2-Mercaptobenzimidazole derivatives **(09-42)** were carried out through multistep reactions. First, 2-Mercaptobenzimidazole was refluxed with bromoethane using necessary condition (KOH) in ethanol with equimolar amounts for about 10h. After completion of the reaction, the reaction mixture was filtered. The filtrate so obtained was kept until the whole ethanol was evaporated and got shiny white needle-like crystals of 2-ethylthio benzimidazole. In the second step 2-ethylthio benzimidazole was taken in round bottom flask refluxed with ethyl chloroacetate (dropwise) using anhydrous potassium carbonate in DMF (solvent) for about 15h. After completion of the reaction, the product **(**2-(2-(ethylthio)benzimidazolyl)acetate**)** obtained and got through separating funnel in semisolid form. In the third step, 2-(2-(ethylthio)benzimidazolyl)acetate was refluxed in methanol with hydrazine hydrate for about 10h. The product, 2-((ethylthio)benzimidazolyl)acetohyrazide get was poured into cold water until a precipitate was formed. The precipitate was filtered and then dried in an open atmosphere. In the fourth step, 2-((ethylthio)benzimidazolyl)acetohyrazide was dissolved in methanol with 2-3 drops of glacial acetic acid (catalyst) on the hotplate. After 10 minutes, aldehyde was added and refluxed the whole mixture for about 5-6 hours. TLC monitored the progress of the reaction. After completion of the reaction, the mixture was poured into cold water until a precipitate was formed. The precipitate was collected by filtration, washed with water and then dried in an open atmosphere.

Synthetic Procedure



Scheme-1: Synthesis of 2-Mercaptobenzimidazole hydrazone derivatives **09-42**.

Table-1: 2-Mercaptobenzimidazole hydrazone derivatives **9-42.**

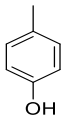
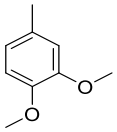
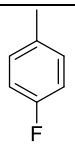
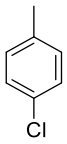
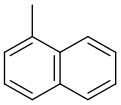
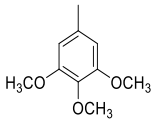
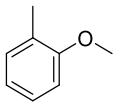
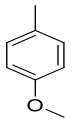
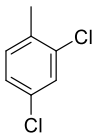
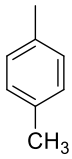
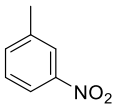
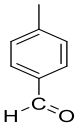
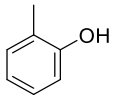
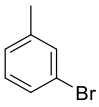
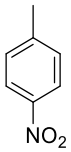
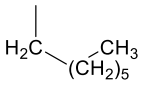
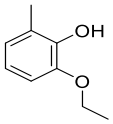
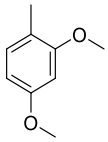
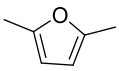
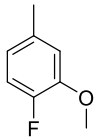
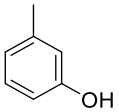
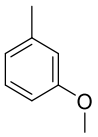
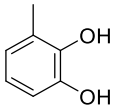
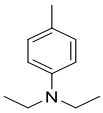
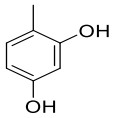
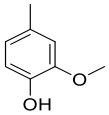
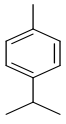
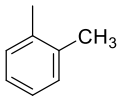
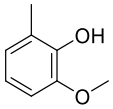
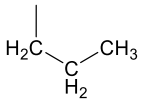
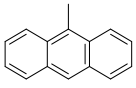
Comp. No.	R	Comp. No.	R	Comp. No.	R
09		**21**		**32**	
10		**22**		**33**	
11		**23**		**34**	
12		**24**		**35**	
13		**25**		**36**	
14		**26**		**37**	
15		**27**		**38**	
16		**28**		**39**	
17		**29**		**40**	
18		**30**		**41**	
19		**31**		**42**	
20		-	-	-	-

Table-2: IC_{50} values and docking scores of 2-MBI hydrazone derivatives against BChE **9-42.**

Compound No.	IC_{50} (μM)	Docking Score (S)	Compound No.	IC_{50} (μM)	Docking Score (S)
09	**60.93±0.67**	**-6.9573**	**27**	**144.66±0.91**	**-12.05581**
10	**165.65±0.91**	**-6.50024**	**28**	**57.90±0.45**	**-7.51345**
11	**75.56±1.73**	**-6.5578**	**29**	**316.50±0.56**	**-3.98745**
12	**27.30±0.52**	**-8.1353**	**30**	**202.49±0.60**	**-4.82034**
13	**75.90±0.48**	**-6.5954**	**31**	**351.47±0.15**	**-3.93240**
14	**148.29±0.64**	**-5.02569**	**32**	**614.07±0.35**	**-3.5987**
15	**25.10±0.90**	**-8.2095**	**33**	**524.08±0.47**	**-4.06475**
16	**181.23±1.83**	**-4.5897**	**34**	**89.23±0.22**	**-6.4325**
17	**51.29±0.64**	**-7.6401**	**35**	**56.34±1.92**	**-6.9459**
18	**530.42±0.43**	**-3.95147**	**36**	**77.58±0.63**	**-6.5210**
19	**134.78±0.45**	**-6.3896**	**37**	**142.67±0.94**	**-6.47854**
20	**151.11±0.06**	**-6.5364**	**38**	**166.26±1.27**	**-6.48752**
21	**62.49±0.060**	**-6.6125**	**39**	**67.19±0.73**	**-6.6075**
22	**54.52±0.95**	**-7.5573**	**40**	**34.31±0.59**	**-7.8083**
23	**205.33±0.29**	**-5.03214**	**41**	**134.43±0.79**	**-6.50102**
24	**348.40±0.44**	**-3.85698**	**42**	**25.36±0.57**	**-7.9546**
25	**617.42±0.43**	**-3.68974**			
26	**770.80±0.90**	**-4.5678**	**Galantamine**	**18.13±0.20**	

Molecular docking

Molecular docking study was carried out through Molecular Operating Environment (MOE) software package to illustrate the binding mode of all the synthesized compound enlisted in the current study against the butyrylcholinesterase enzyme to validate the experimental results. The primarily favorable docking conformations were ascertained within the active site with the proper orientation for all the compounds. The catalytic active site residues of the corresponding enzyme include acidic residue Glu197, hydrophobic side chain residue Tyr 332 and Trp 82. Generally, we have noticed that the most active compound (**15**) in the series adopt fit-well interaction pattern of binding in the active (**Fig 1A**).

The most active compound in the whole series against butyrylcholinesterase is **15** (2,4-Dimethoxy group) with IC_{50} value is 25.10 ± 0.90 μM, which is very close to the IC_{50} value of standard galantamine 18.13 ± 0.20 μM. The high activity of this compound may be due to the robust electron donating capacity of $–OCH_3$ group. Compound **15** contains two $–OCH_3$ group one is at position-2 and second is at position-4 on the benzene ring. It means that the methoxy group has more electron donating effect (+ I.E.) at ortho and para positions as compared to other places on benzene. The Second most active compound **42** with IC_{50} value is 25.36 ± 0.57 μM, which is precisely equal to the IC_{50} value of compound **15** was observed in pi-stacking interaction with active site residues (**Fig 1C**). A little bit decrease in IC_{50} value of compound **42** as compared to the IC_{50} value of compound **15** might be due to the delocalization of π-electrons in the anthracene ring, which is highly conjugated and show –M effect. The 3rd active compound **12** with IC_{50} value is (27.30 ± 0.52 μM) adopt favourable interaction with hydrophilic side chain residue Phe 329 and acidic side chain donor Glu197 (**Fig 1B**). The decrease in the IC_{50} value of compound **12** was observed and it might be due to the negative inductive effect (- I.E.) of two chloro groups at position-2 and second is at position-4 on the benzene ring. The fourth active compound **40** contains -OH groups at ortho and para position. Hydroxyl groups are powerfully activating at ortho and para position on benzene ring and mostly increased enzymatic activity, while some time decreased enzymatic activity due to the nature of the compound. Increase activity of hydroxyl groups may be due to the involvement of oxygen and hydrogen interactions with different residues of the protein.

Other more active compounds in the series are **17, 21** and **22** with IC_{50} values 51.29 ± 0.64, 62.49 ± 0.060, 54.52 ± 0.95 μM respectively, and additionally, these compounds showed similar binding mode as compared with the most potent compound in the series (**Fig 1D-F**). The enhanced activity of compounds **17** might be due to the robust electron donating groups such as N, N-Diethyl group on benzene ring due to + I.E. Compound **22** contains naphthalene moiety and showed low enzymatic activity as compared to compound **15** due to missing of one benzene ring in naphthalene as compared to anthracene in **15**. So, the reduction in activity was observed in **22,** due to less involvement of π-electrons in the naphthalene ring as compared to the anthracene ring due to the –M effect. The docking pose of almost all potent compounds computationally inhibited the catalytic activities of the enzyme by binding determinedly through strong hydrogen bonding, hydrophobic, pi-stacking and polar interactions with critical residues.

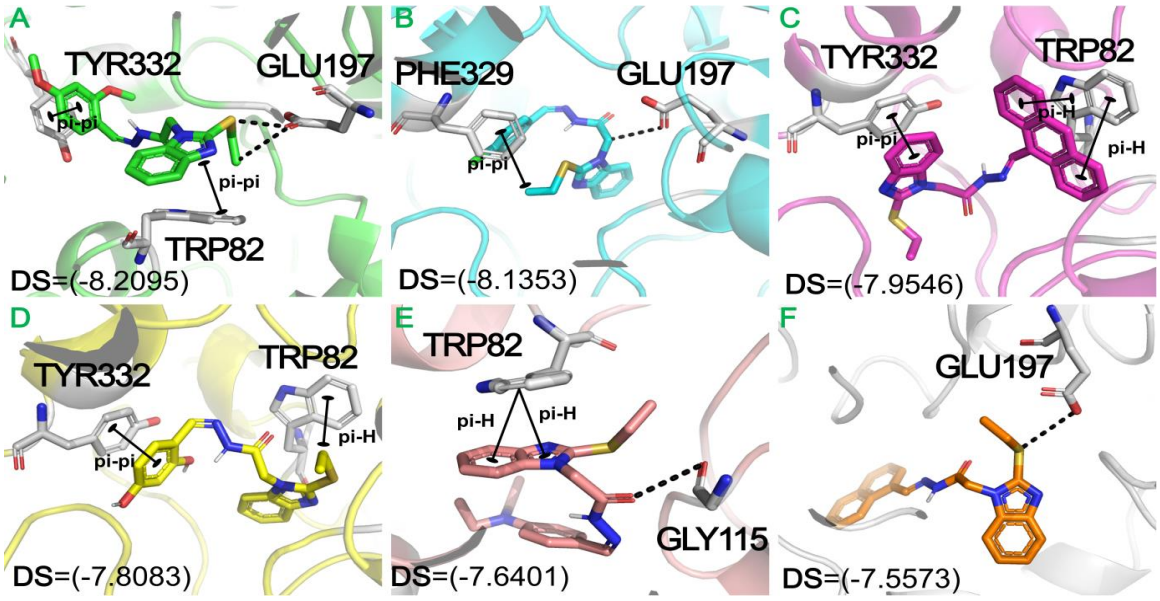


Fig. 1: The Docked conformations of most active compounds. (**A**) binding mode of compound **15**, (**B**) for compound **12**, (**C**) for compound **42** and (**D**) for compound **40,** (**E**) for compound **17** and **(F)** for compound **22** against the human butyrylcholinesterase enzyme (PDB code: **1P0P**).

Butyrylcholinesterase (BChE) Assay

Butyrylcholinesterase (BChE) from equine serum was used to expose the enzymes inhibitory power of drug by using Ellman's assay [17, 18]. The assay is mainly based on the hydrolysis of butyrylthiocholine iodide by the specific enzymes BChE and the formation of 5-thio-2-nitrobenzoate anion followed by complexation with DTNB to give yellow color compound which is detected with a spectrophotometer beside the reaction time.

Preparation of solutions

The drug was first dissolved in phosphate buffer (0.1 M) in concentrations ranging from 125-1000 µg/ml. For the preparation of 0.1 M and 8.0 ± 0.1 pH phosphate buffer solution, K_2HPO_4 (17.4 g/L) and KH_2PO_4 (13.6 g/L) were prepared and were mixed with the ratio of 94% and 6% respectively. Finally, KOH was used to adjust pH. BChE (7-16 U/mg) was diluted in freshly prepared buffer pH 8.0 until a final concentration of 0.01 U/ml was obtained. Solutions of DTNB (0.0002273 M), BTchI (0.0005 M) were prepared in distilled water and were kept in Eppendorf caps in the refrigerator. Galanthamine (Positive control) was dissolved in methanol, and the dilutions, as mentioned earlier, were prepared [19].

Spectroscopic analysis

For the preparation of each assay, an enzyme solution of 5 µl was added to the cuvette, proceeded by addition of drug solution (205 μl), and finally DTNB reagent (5 μl). The solution mixture was kept at 30 °C for 15 minutes using a water bath, and consequently, the substrate solution (5 μl) was added. A double beam spectrophotometer was used to measure the absorbance at 412 nm. Except for the drug, negative control contained all components, whereas positive control galanthamine (10 μg/mL) was used in the assay as a standard cholinesterase inhibitor. The absorbance, along with the reaction time, was taken for 4 minutes at 30 °C and was repeated in triplicate. Finally, the enzyme activity and enzyme inhibition by control and tested samples were calculated from the rate of absorption with a change in time ($V=\Delta Abs/\Delta t$) as follow;

% enzyme inhibition = 100 - percent enzyme activity

% enzyme activity = 100 × V/Vmax where (Vmax) is an enzyme activity in the absence of inhibitor drug.

Conclusion

A series of 2-mercaptobenzimidazole hydrazone derivatives **(9-42)** were synthesized and screened for butyrylcholinesterase studies. All compounds showed potential activity against butyrylcholinesterase enzymes, but compounds **15**, **12**, **42**, **40**, **17**, **22**, **28** and **09** showed excellent activity against butyrylcholinesterase enzymes. Docking studies confirmed the inhibition values of the synthesized compounds against butyrylcholinesterase enzymes.

Acknowledgements

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