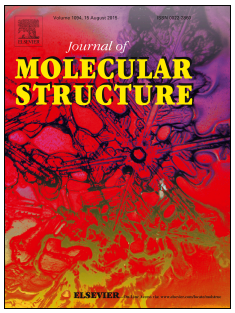

Synthesis, characterization, biological evaluation and molecular docking studies of *N*-functionalized derivatives of 2-aminobenzohydrazide

Naghmana Kausar, Shahzad Murtaza, Muhammad Nadeem Arshad, Robina Rashid, Abdullah M. Asiri, Noman Javid, Mulazim Hussain Asim, Zaman Ashraf

PII: S0022-2860(20)30367-7

DOI: https://doi.org/10.1016/j.molstruc.2020.128042

Reference: MOLSTR 128042

To appear in: *Journal of Molecular Structure*

Received Date: 27 January 2020

Revised Date: 6 March 2020

Accepted Date: 8 March 2020

Please cite this article as: N. Kausar, S. Murtaza, M.N. Arshad, R. Rashid, A.M. Asiri, N. Javid, M.H. Asim, Z. Ashraf, Synthesis, characterization, biological evaluation and molecular docking studies of *N*-functionalized derivatives of 2-aminobenzohydrazide, *Journal of Molecular Structure* (2020), doi: https://doi.org/10.1016/j.molstruc.2020.128042.

Credit Author Statement

Naghmana Kausar: Methodology, Data curation, Writing- Original draft preparation, Software. **Shahzad Murtaza.**: Supervision. Conceptualization, Writing- Reviewing and Editing, **Muhammad Nadeem Arshad**: X-ray crystallographic analysis ***Robina Rashid****:* Free radical activity**: Abdullah M. Asiri**: X-ray crystallographic analysis, Software, Validation: **Noman Javid:** Structural Analysis. **Mulazim Hussain Asim**: Biological evaluation. **Zaman Ashraf:** Biological evaluation.

Synthesis, characterization, biological evaluation and molecular docking studies of *N*-functionalized derivatives of 2-aminobenzohydrazide:

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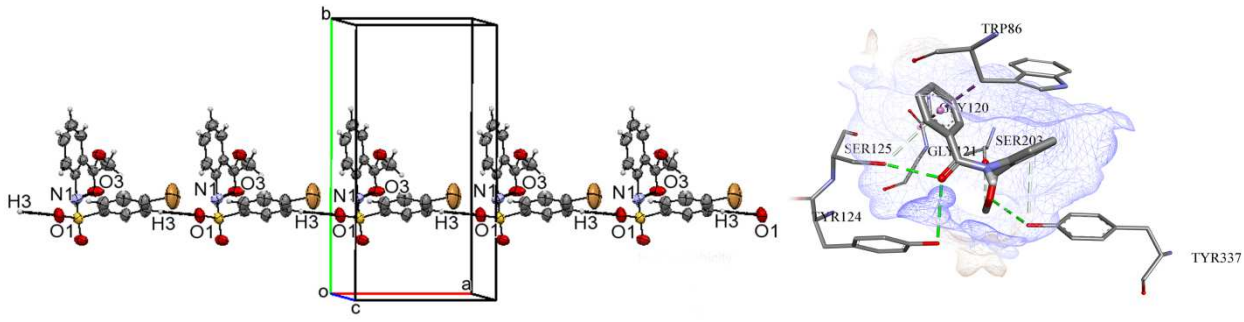
Abstract:

In this paper, *N*-functionalized derivatives of methyl anthranilate **(1-3)** were prepared by reacting it with various acid chlorides to give amide derivatives **(1-3)** which were further converted to benzohydrazide derivatives **(4-6)** by reacting them with hydrazine. These *N*-functionalized derivatives of 2-aminobenzohydrazide **(4-6)** were reacted with 4-triflouromethyl benzaldehyde to give schiff base derivatives **(7-9)**. All the synthesized compounds **(1-9)** were characterized by Mass spectrometry as well as NMR and FTIR spectroscopic techniques. Single Crystal X-ray diffraction analysis technique (XRD) was utilized to analyze crystalline compound **1**. AChE and BChE enzymes inhibition potential was checked for the synthesized compounds. Putative binding approaches of screened compounds were explored by molecular docking studies. Synthesized compounds were also checked for antioxidant activity. Enzyme inhibition analysis showed that compounds **2** and **9** exhibited good AChE inhibition showing 68% and 60% percentage inhibition, respectively, while very good BChE inhibition activities were shown by compounds **2** (70%) and **5** (76%). Enzyme inhibition results were also supported by molecular docking studies with lowest binding energy values of -9.79 Kcal mol^{-1} for BChE and -9.55 Kcal mol^{-1} for AChE for compound **9**. Hydrazide derivatives **(4-6)** showed very significant results for antioxidant activity with percentage scavenging greater than 90%.

Keywords: Benzohydrazide, heterocyclic, enzyme inhibition, crystal description, sulfonamide, DPPH

Graphical Abstract:



1. Introduction

The development of new biologically important molecules is decisive to cater with diseases and for improved health conditions. The incorporation of pharmacophoric moiety on food-

grade molecule could be a promising approach to get valuable molecules. Methyl 2-aminobenzoate (methyl anthranilate) is used as a flavouring agent with fruity grape smell and chemically consist of two important functional groups (amino and ester). In this research work, these functional groups were derivatized to get amides, sulfonamides, benzohydrazides and schiff bases. Benzohydrazides are the aromatic derivatives of hydrazine and benzoic acid derivatives. Now a day, benzohydazide derivatives have become very important due to their various pharmacological actions. They are found to act as analgesics [1] and anti-inflammatory agents [2]. Benzohydrazides derivatives have also shown promising antimicrobial activities [3] along with their importance as anti-cancerous drugs [4]. Significant anti-tubercular [5] and anti HIV activities [6] are being possessed by benzohydrazide derivatives. They have also been found to possess significant AChE and BChE inhibition activities [7-8] where flouro substituted derivative of benzohydrazides have shown considerable AChE inhibition activities [9-10]. Because of possessing a large range of biological activities, benzohydrazide moiety can be considered as an important pharmacophore.

Sulfonamides have also attracted the attention of the researchers because of their diverse pharmaceutical importance due to possessing anti-cancerous [11], HIV protease inhibition [12], antibacterial [13] and anti-Alzheimer properties [14]. Schiff base derivatives possessing versatile therapeutic activities such as anti-HIV and anti-cancerous activity [15], antitumor activities [16] and antifungal activities [17] are also getting eminence. Amides have their own biological significance due to showing various important biological activities such as *N*-acylethanolamine acid amidase (NAAA) inhibitors [18], anticancer [19] and antimicrobial agents [20].

Enormous biological importance of schiff bases, amides, sulfonamides and benzohydrazide derivatives has urged us to design this research work to synthesize schiff base derivatives of 2-aminobenzohydrazide with functionalization of amino group with substituents of various types including sulfonamide along with amides, of both aromatic as well as heterocyclic nature and further investigated these compounds for their prospective of enzyme inhibition against BChE as well as AChE enzymes and antioxidant potential.

2. Materials and Methods

All the chemicals including methyl anthranilate, hydrazine hydrate, 4-bromobezenesulfonyl chloride, benzoyl chloride, thiophene-2-carbonyl chloride and aldehyde were bought from Sigma-Aldrich (USA) and Merck (Germany) and were utilized as received. Electrothermal melting point apparatus was utilized for measuring melting points and these melting points are uncorrected. FTIR spectra were recorded on a Bio-red spectrophotometer using KBr discs. NMR spectra were taken using JEOL- 300 MHz DELTA2_NMR Spectrometer (^{1}H, 300 MHz and ^{13}C, 75 MHz) utilizing DMSO-d_6 as solvent. Mass spectra were taken by Tandem Mass Spectrometric analysis (LTQ XL Linear Ion Trap Mass Spectrophotometer, Thermo Scientific, USA), using Electrospray (ESI) ionization probe.

*2.1. Synthesis of N-functionalized derivatives of methyl anthranilate **(1-3)***

Weighed quantity of methyl anthranilate (1.29 mL, 10.0 mmol) was taken and its solution was prepared in pyridine (10 mL). Solutions of acid chlorides (15mmol) in distilled methanol were added drop wise to the methyl anthranilate solution followed by stirring of reaction mixture. pH of the solution was kept up to 7 by constantly adding sodium carbonate. The products precipitated out from the reaction mixture, which were washed with cold, distilled methanol. Obtained products were recrystallized using methanol to get pure products in decent yield. (Scheme 1)

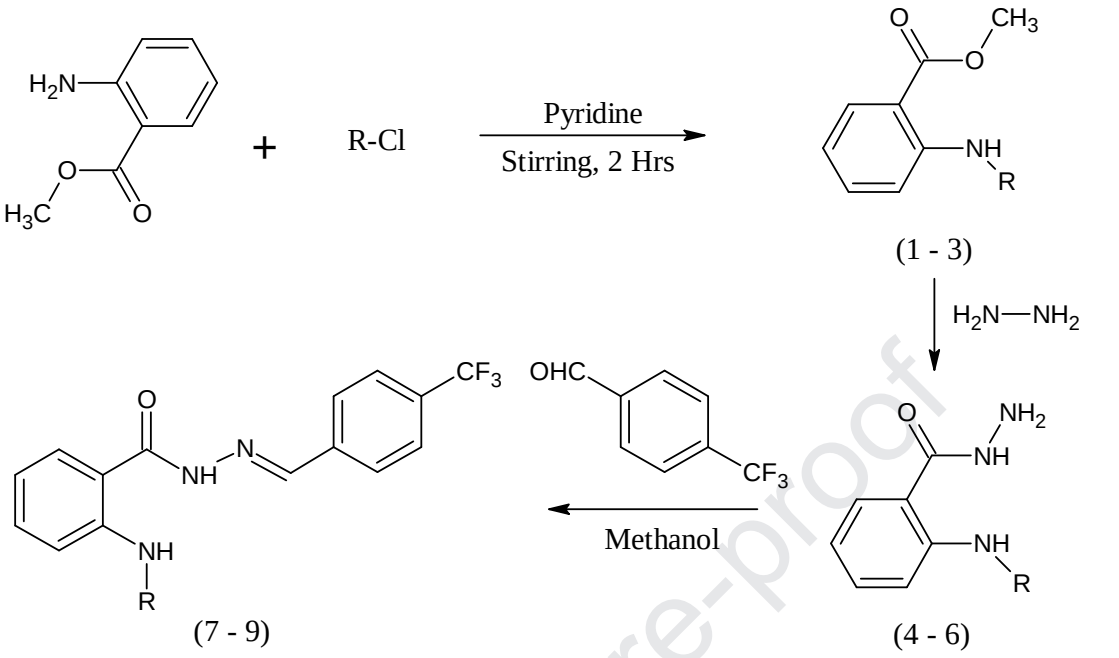
*2.2. Synthesis of hydrazides of N-functionalized derivatives of methyl anthranilate **(4-6)***

Weighed amount of *N*-functionalized derivatives of methyl anthranilate **(1-3)** were dissolved in acetonitrile. Excess hydrazine was added to the reaction mixture and was subjected to refluxing for two hours. Excess solvents were removed under vacuum. Ethyl acetate /water extraction was performed to remove excess hydrazine. Ethyl acetate was vaporized under reduced pressure to get crude products. Recrystallization of the products was done using ethanol to get pure products. (Scheme 1)

*2.3. Synthesis of Schiff bases of hydrazides of N-functionalized derivatives of methyl anthranilate **(6-9)***

Stoichiometric amounts of synthesized hydrazides **(4-6)** and 4-triflouromethyl benzaldehyde were dissolved in methanol (10 mL). The reaction mixture was refluxed for two hours. Solvents were vaporized under vacuum. Washing and recrystallization of the product was

done using cold ethanol and methanol, respectively to get products in decent yield (about 90%). (Scheme 1)



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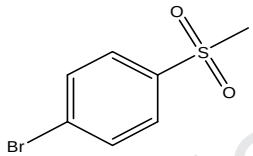
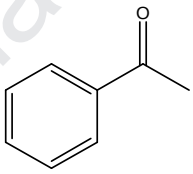
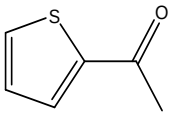
R =  (**1, 4, 7**), R =  (**2, 5, 8),** R =  (**3, 6, 9)**

Scheme 1.

2.4. Characterization

The physico-chemical data for the synthesized compounds is given below

Methyl 2-{[(4-bromophenyl)sulfonyl]amino}benzoate **(1)**

M.F: $C_{14}H_{12}BrNO_4S$; Yield: 64%; m.p. 110±1°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 10.43 (1H, br, s, NH-SO_2), 7.18-7.84 (8H, m, Aromatic CH), 3.81 (3H, s, CH_3O). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 167.7 (C=O), 138.5, 138.3, 134.7, 132.9 (2), 131.4, 129.3 (2), 127.8, 124.9, 121.3, 119.6 (2 benzene rings), 53.0 (CH_3O). FTIR (cm^{-1}): 3188 (NH, stretch), 1686 (ester C=O, stretch), 1342 (S=O, stretch), 1261 (C - O, stretch). MS (ESI): m/z (%), (369 (25), $[M]^{-\cdot}$), 311 (10), 220 (27), 214 (28).

Methyl 2-benzamidobenzoate **(2)**

M.F: $C_{15}H_{13}NO_3$; Yield: 75%; m.p. 104±1°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.17 (1H, br, s, NHCO), 7.35-8.38 (9H, m, Aromatic CH), 3.47 (3H, s, CH_3O). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 167.6 (COO), 165.9 (CONH), 142.6, 138.4, 135.7 (2), 131.5 (2), 129.3, 126.8 (2), 124.7, 121.6, 120.5 (benzene rings), 52.6 (CH_3O). FTIR (cm^{-1}): 3263 (NH, stretch), 3023 (C-H Stretch), 1691 (ester C=O, stretch), 1667 (C=O, stretch), 1267 (C-O, stretch). MS (ESI): m/z (%), (254 (30), $[M]^{-\cdot}$), 224(12), 196 (24), 178 (27).

Methyl 2-(thiophene-2-carboxamido)benzoate **(3)**

M.F: $C_{13}H_{11}NO_3S$; Yield: 78%; m.p. 131±1°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.37 (1H, br, s, NHCO), 7.32-8.21 (7H, m, Aromatic CH), 3.81 (3H, s, CH_3O). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.9 (COO), 160.8 (CONH), 143.2, 140.1, 135.2, 134.7, 131.6 (2), 129.8, 124.9, 121.2, 117.1 (aromatic rings), 54.1 (CH_3O). FTIR (cm^{-1}): 3262 (NH, stretch), 3087 (C-H Stretch), 1686 (ester C=O, stretch), 1665 (C=O, stretch), 1250 (C-O, stretch). MS (ESI): m/z (%), 260 (20, $[M]^{-\cdot}$), 230(12), 202 (24), 178(26)

4-Bromo-*N*-(2-(hydrazinecarbonyl)phenyl)benzene sulfonamide **(4)**

M.F: $C_{13}H_{12}BrN_3O_3S$; Yield: 62%; m.p. 165±1°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.62 (1H, br, NH-NH_2), 10.21 (1H, br, s, NH-SO_2), 7.10-7.75 (8H, m, Aromatic CH), 4.84 (2H, br, NH_2, amine). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 163.7 (NHCO), 147.2, 144.7, 138.3, 135.6 (2), 132.7, 130.5 (2), 128.9, 125.6, 122.1, 119.2 (benzene rings). FTIR (cm^{-1}): 3559 (NH_2 anti-sym. stretch), 3385 (NH_2 sym. stretch), 3331 (CON-H, Stretch), 3273 (SO_2N-H, stretch), 1630 (C=O, stretch), 1336 (S=O, stretch). MS (ESI): m/z (%), (370 (100), $[M]^{-\cdot}$), 354 (16), 310 (15), 221 (21), 200 (10), 171 (10).

N-(2-(hydrazinecarbonyl)phenyl)benzamide **(5)**

M.F: $C_{14}H_{13}N_3O_2$; Yield: 71%; m.p. 192±1°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 12.57 (1H, s, NH-CO), 10.21 (1H, br, NH-NH_2), 7.17-8.68 (8H, m, Aromatic CH), 4.70 (2H, br, NH_2, amine). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 167.9 ($CONHNH_2$), 164.6 (NHCO), 139.7, 134.9, 132.5 (2), 129.4 (2), 128.1, 127.4 (2), 123.3, 120.6, 119.5 (benzene rings). FTIR (cm^{-1}): 3316 (NH_2 stretch), 3204 (CON-H, Stretch), 3054 (C-H, stretch), 1646 (C=O, stretch). MS (ESI): m/z (%), 254 (40, $[M]^{-\cdot}$), 240 (35), 236 (11), 196(10).

N-(2-(hydrazinecarbonyl)phenyl)thiophene-2-carboxamide **(6)**

M.F: $C_{12}H_{11}N_3O_2S$; Yield: 80%; m.p. 217±1°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 12.57 (1H, br, s, NH-CO), 10.22 (1H, br, NH-NH_2), 7.14-8.54 (7H, m, Aromatic CH), 4.70 (2H, br, NH_2, amine). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 167.9 ($CONHNH_2$), 159.5 (NHCO), 140.2, 139.3, 132.8, 132.6, 128.7 (2), 128.1, 123.3, 120.5, 119.2 (aromatic rings). FTIR (cm^{-1}): 3375 (NH_2 anti-sym. stretch), 3314 (NH_2 sym. stretch), 3213 (CON-H, Stretch), 3083 (C-H, stretch), 1630 (C=O, stretch). MS (ESI): m/z (%), (260 (40), $[M]^{-\cdot}$), 246 (60), 229 (10), 202 (20), 176 (40), 137 (10).

(*E*)-4-bromo-*N*-(2-(2-(4-(trifluoromethyl)benzylidene)hydrazine-1-carbonyl)phenyl)benzene-sulfonamide **(7)**

M.F: $C_{21}H_{15}BrF_3N_3O_3S$; Yield: 91%; m.p. 195±1°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 12.03 (1H, br, s, NH-N), 10.59 (1H, br, s, NH-SO_2), 8.42 (1H, s, N=CH), 7.26 – 7.97 (12H, m, Aromatic CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 164.6 (NHCO), 147.4 (CH=N), 138.4, 137.2, 133.0, 132.8 (3), 129.3 (3), 128.3 (2), 127.7, 126.2 (3), 125.1, 122.7 (2) (benzene rings), 124.2 (CF_3). FTIR (cm^{-1}): 3326 (HN, stretch), 3089 (C-H, stretch), 1644 (C=O, stretch), 1598 (N=CH, Stretch), 1322 (S=O, stretch). MS (ESI): m/z (%), (524 (20), $[M]^{-\cdot}$), 496 (70), 353 (55), 336 (05), 310 (20), 272 (100), 219 (05), 182(10).

(*E*) -*N*- (2-(2- (4-(trifluoromethyl)benzylidene)hydrazinecarbonyl)phenyl)benzamide **(8)**

M.F: $C_{22}H_{16}F_3N_3O_2$; Yield: 91%; m.p. 229±1°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 12.12 (1H, br, s, NH-CO), 11.78 (1H, br, s, NH-N), 8.50(1H, s, N=CH), 7.38-8.59 (13H, m, Aromatic CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.2 (=N-NHCO), 164.9 (NHCO), 147.6 (CH=N), 138.7, 134.9, 134.2, 133.5, 132.7, 131.6, 130.2 (6), 129.7, 128.1 (2), 122.1, 121.6, 120.9 (benzene rings), 122.8 (CF_3). FTIR (cm^{-1}): 3192 (HN, stretch), 3067 (C-H, stretch), 1657 (C=O, stretch), 1604 (N=CH, Stretch). MS (ESI): m/z (%), 410 (10, $[M]^{-\cdot}$), 239 (25), 221 (11), 187 (100), 159 (15).

(*E*)-*N*-(2-(2-(4-(trifluoromethyl)benzylidene)hydrazinecarbonyl)phenyl)thiophene-2-carbox-amide **(9)**

M.F: $C_{20}H_{14}F_3N_3O_2S$; Yield: 82%; m.p. 248±1°C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 11.97 (1H, br, s, NH-CO), 11.91 (1H, br, s, NH-N), 8.51 (1H,s, N=CH), 7.21 – 8.45 (11H, m, Aromatic CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.6 (=N-NHCO), 163.2 (NHCO), 146.7 (CH=N), 141.3, 138.9, 138.1, 133.8, 132.5, 132.1, 130.9, 129.2 (2), 128.4, 127.3, 125.7

(2), 124.1, 122.9, 122.5 (aromatic rings), 126.4 (CF_3). FTIR (cm^{-1}): 3323 (HN, stretch), 3056 (C-H, stretch), 1640 (C=O, stretch), 1587 (N=CH, Stretch). MS (ESI): m/z (%), (416 (15), $[M]^{-\cdot}$), 388 (11), 332(12), 245 (06), 227 (10), 187 (100), 161 (15).

2.5. Crystallography

The selected sample of prismatic crystal was fixed, using glue, over the glass tip. This assemblage was attached on the Agilent SuperNova (Dual source) Agilent Technologies Diffractometer, furnished with microfocus Cu/Mo Kα radiation for the collection of data. CrysAlisPro, software was utilized for the collection of data [21] maintaining temperature of the system at 296 K taking Mo Kα radiation as source of light radiation. Direct method was employed to solve structures utilizing SHELXS–97 [22] and refinement of the results was performed by employing full–matrix least–squares methods on F2 utilizing SHELXL–97 [22]. Full–matrix least squares methods were utilized for the refinement of all non–hydrogen atoms anisotropically [22]. PLATON [23] and ORTEP software packages were used for the generation of figures [24].

There are three different types of hydrogen atoms available in the molecules i.e. aromatic, methyl and amino hydrogen atoms. All types of hydrogen atoms were positioned geometrically with the *d*C-H = 0.93 Å for hydrogen atoms of aromatic ring, *d*C-H =0.96Å for carbon atom of methyl group and *d*N-H = 0.86Å. The Uiso(H) for nitrogen and aromatic atoms (X = C & N) was set to 1.2 Ueq(X). For the methyl carbon atom, the Uiso(H) was fixed to 1.5 Ueq(C). The crystal data was submitted to Cambridge Crystallographic Data Centre, as CCDC No. 1976272, which can be obtained free of charges.

2.6. Biological Evaluation

2.6.1. Enzyme Inhibition Essay

Enzyme inhibition studies were performed by following well-known Ryan and Elman technique [25] with little adjustment. Enzyme solutions (AChE/BChE) were mixed with solutions of the synthesized compounds (1-9) followed by the addition of substrate solution i.e., acetylthiocholine iodide for AChE and butyrylthiocholine chloride for BChE together with DTNB solution and phosphate buffer of pH=8 and mixed properly. The mixture was incubated for 30 minutes at 37 °C. Substrate hydrolysis was estimated by spectrophotometer by recording absorbance at λ=400 nm for AChE and λ=412 nm for BChE. Enzyme inhibition rate was measured by the following equation.

$$\%age\ Inhibition = \left(\frac{Y - X}{Y}\right) \times 100$$

Where X = Value of Absorption for enzyme including test sample

Y = Value of Absorption for enzyme devoid of test sample

Every test was performed thrice and mean was calculated. Donepzil was taken as reference.

2.6.2. Antioxidant analysis

DPPH method [26] was used for determining the antioxidant potential of the compounds (1-9). Ethanolic solution of DPPH was mixed with the solution of the test compounds followed by the incubation of the mixture at 37 ^{o}C for 20 minutes. Extent of change in colour was determined by spectrophotometry by recording the absorbance at λ=517 nm. For positive control ascorbic acid was used. The equation operated to compute free radical scavenging (%age) for each of test compound is given below

$$\%age\ Scavenging = \left(\frac{Ad - As}{Ad}\right) \times 100$$

Where

Ad = Absorbance of the DPPH solution, As = Absorbance of the sample solution

2.7. Molecular Docking

For exploration of binding approaches of synthesized compounds with the enzymes, BChE and AChE, molecular docking examination was executed. The proteins were taken from Protein Data Bank. For the sketching of the molecular structures and 3D optimization, ACD/ChemSketch and Chem 3D Pro 12.0, soft ware packages were utilized. The structures were saved as SYBYL mol2 document form. The software used to carry out docking procedure was AutoDock Tool v1.5.6. In this docking procedure, hundred diverse conformations were obtained and was chosen superior amongst all the conformations in the wake of imagining each posture by utilizing Discovery Studio Visualizer v4.0.23 [27].

3. Results and Discussion

3.1. Chemistry

N-functionalized derivatives of methyl anthranilate were synthesized according to Scheme 1 as reported in literature [28]. Methyl anthranilate has two active sites i.e., an amino group and an ester linkage on which transformations can be done. Firstly its amino group was functionalized by carrying out its reaction with different acid chlorides using pyridine to obtain compounds **(1-3)**. Then the ester linkage of these *N*-functionalized derivatives was converted to their hydrazide derivatives **(4-6)** by reacting them with hydrazine hydrate. These synthesized benzohydrazide derivatives **(4-6)** were further transformed to their schiff base analogues by reacting them with 4-triflouromethylbenzaldehyde to synthesize compounds **(7-9)**. ^{1}H-NMR, ^{13}C NMR, FTIR and X-ray-Crystallographic techniques were employed to carry out characterization of the synthesized compounds.

3.1.1. FT-IR spectral analysis

Different functional groups present in all the synthesized compounds were determined by FTIR spectral analysis. Shifting of the signal of (C=O) group of ester linkage in compounds **(1-3)** from $\bar{\upsilon}$ = 1691 cm^{-1} – 1686 cm^{-1} to lower values of $\bar{\upsilon}$ = 1646 cm^{-1} – 1630 cm^{-1} in compounds **(4-6)** and also the appearance of signals for NH_2 at $\bar{\upsilon}$ = 3559 cm^{-1} – 3314 cm^{-1} confirmed the formation of hydrazide linkage. Similarly formation of imine linkage in compounds **(7-9)** was authenticated with the appearance of at $\bar{\upsilon}$ = 1604 cm^{-1} – 1588 cm^{-1} (N= CH).

3.1.2. ^{1}H-NMR spectral analysis

^{1}H-NMR spectral data also confirmed the structures of all the synthesized compounds. Signals of three protons of -OCH_3 group of ester linkage in compounds **(1-3)** at δ = 3.47 ppm – 3.81 ppm disappeared accompanying with the appearance of peaks at δ = 4.70 ppm – 4.84 ppm relating to –NH_2 group in compounds **(4-6)** [28]. Singlet peaks were also observed for one proton of NH-NH_2 in the range of δ = 10.21 ppm – 11.62 ppm in these compounds **(4-6)**. Appearance of characteristic singlets between δ = 8.42 ppm – 8.51 ppm relating to the imine linkage (N=CH) also gave confirmation of the conversion of compounds **(4-6)** to their schiff base derivatives. Signals appeared in the range of 7.10 ppm – 8.68 ppm were due to aromatic protons. ^{1}H-NMR spectral data supported the structures of all the synthesized compounds strongly.

3.1.3. ^{13}C NMR spectral analysis

^{13}C NMR spectral data also confirmed the structures of the synthesized compounds. Signals appearing at of δ = 52.6 ppm – 54.1 ppm for –OCH_3 carbon of ester linkage in compounds **(1-3)** has disappeared in compounds **(4-6)** due to conversion of ester linkage into hydrazide in these compounds. The signal for –HC=N- carbon atom of benzylidene group was appeared at δ = 146.7 ppm – 147.6 ppm in compounds **(7-9).**

3.1.4. Mass spectroscopic analysis

Electrospray Ionization technique (ESI) was employed for the establishment of the structures of the synthesized compounds. Characteristic peaks corresponding to [M-H] ions in -ve mode appeared in mass spectrum of all compounds. In compound **5**, cyclization leading to the formation of quinazolinone nucleus gave peak at m/z = 236 [29].

3.1.5. Crystal Description

The title compound 2-(4-bromo-benzenesulfonylamino)-benzoic acid methyl ester **(1)** belongs to the sulfonamide branch of organic chemistry. The molecule contains sulfonamide and ester functional group in it as shown in Fig. 1 (a). The crystal data is being presented in Table 1. The compound adopted *V*-shape where the dihedral angle between the two aromatic rings (C1-C6) & (C7-C12) is 83.84(5)°. In the central sulfonamide functional group, the geometry around the sulphur group is disordered tetrahedral geometry where the angles are ranging from 105.47(2)° to 119.42(2)Å around it. The methyl acetate group (O3/C13/O4/C14) is twisted by 1.77(3)° with reference to parent aromatic ring (C7-C12). The molecules afford inter and intramolecular hydrogen bonding in their unit cell. A strong intramolecular interaction like N1—H1N…O3 produce six membered ring motif with the root mean square deviation value of about 0.1121 Å. This six membered ring motif is leaning at dihedral angle of 77.39(2)° and 6.47(2) with reference to the aromatic rings (C1-C6) & (C7-C12) respectively. A non-classical hydrogen bonding interaction where C3 is acting as donor atom via H3 atom to the O1 oxygen atom by following the symmetry operation x+1, y, z is depicted in Fig. 2(b). The hydrogen bond interactions are presented in Table 2. This hydrogen bond association joins the molecules along a-axis to make long chain as presented in Fig. 2 and supramolecular nature of compound. The bond angles and bond lengths are shown in Table 3.

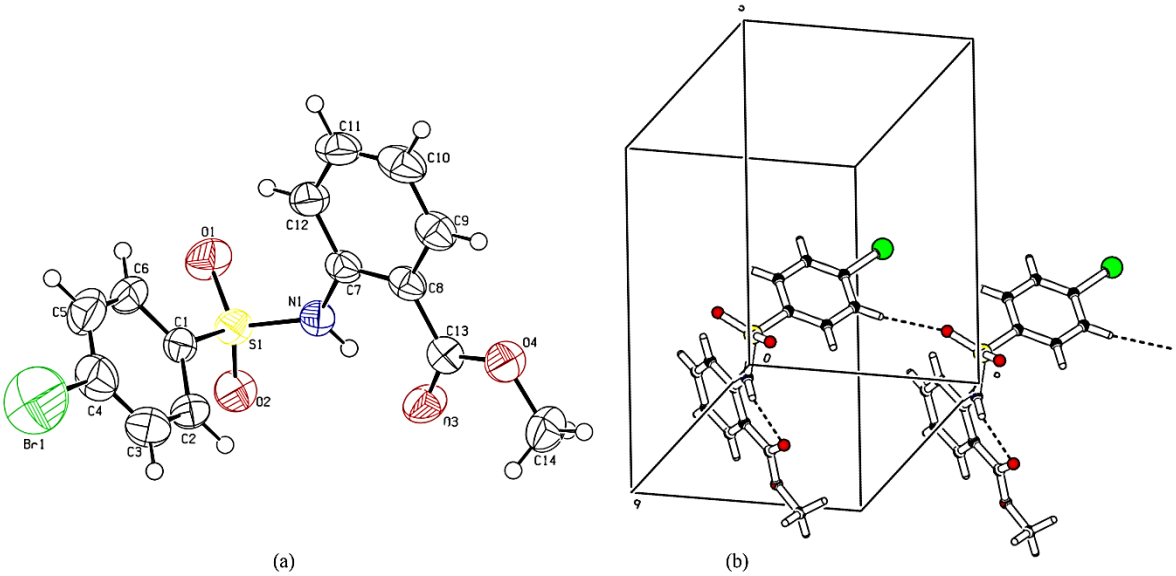


Fig. 1. a) Compound **1** *ORTEP* diagram, thermal ellipsoids sketched at 50% probability level. b) Hydrogen bonding associations of both inter and intra-molecular type in a unit cell in compound 1.

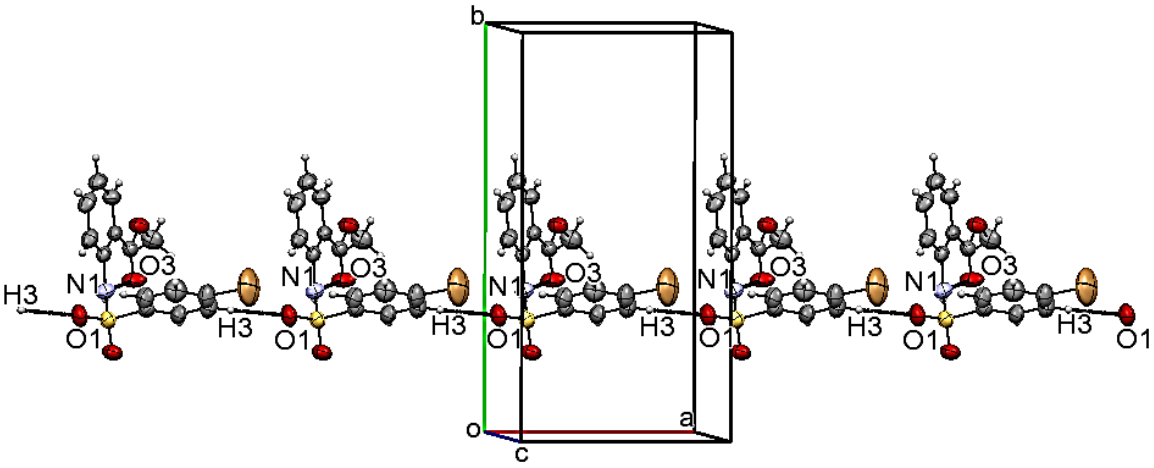


Fig. 2. Long chain formation along a-axis via C3-H3…O1 interaction

Table 1

Crystal data and structure refinement for compound **1**

Crystal data and structure refinement for compound 1

CCDC number	1976272
Formula weight	370.22
Empirical formula	$C_{14}H_{12}BrNO_4S$
Crystal system	monoclinic

Temperature/K	296(2)
Space group	$P2_1/c$
c/Å	12.2255(12)
b/Å	15.6090(13)
a/Å	8.0748(5)
γ/°	90
β/°	91.191(7)
α/°	90
Z	4
μ/mm^{-1}	2.817
Volume/$Å^3$	1540.6(2)
$\rho_{calc}g/cm^3$	1.596
Crystal size/mm^3	0.43 × 0.28 × 0.15
F(000)	744.0
2Θ range for data collection/°	5.682 to 58.462
Radiation	MoKα (λ = 0.7107)
Index ranges	$-11 \le h \le 10$, $-16 \le k \le 21$, $-15 \le l \le 15$
Reflections collected	8864
Independent reflections	3662 [R_{int} = 0.0339, R_{sigma} = 0.0446]
Goodness-of-fit on F^2	1.044
Data/restraints/parameters	3662/0/191
Final R indexes [all data]	R_1 = 0.1105, wR_2 = 0.1835
Final R indexes [I>=2σ (I)]	R_1 = 0.0659, wR_2 = 0.1554
Largest diff. peak/hole / e $Å^{-3}$	0.92/-1.01

Table 2

Hydrogen bond associations in structure of compound **1.**

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C3	H3	O1[1]	0.93	2.36	3.278(5)	171.0
N1	H1N	O3	0.86	2.02	2.611(5)	124.9

[1]1+X,+Y,+Z

Table 3

Selected Bond Angles and Bond lengths for Compound **1**.

Bond Angles				Bond Lengths		
Atom	**Atom**	**Atom**	**Angle/˚**	**Atom**	**Atom**	**Length/Å**
C6	C1	S1	120.5(3)	C1	S1	1.763(4)

C2	C1	S1	118.5(3)	C4	C5	1.364(7)
C3	C4	C5	122.2(5)	C4	Br1	1.895(5)
C3	C4	Br1	119.4(4)	C5	C6	1.381(7)
C5	C4	Br1	118.4(4)	C7	C12	1.390(6)
C12	C7	N1	121.6(4)	C7	N1	1.411(5)
C8	C7	N1	118.8(3)	C13	O3	1.207(5)
O3	C13	O4	122.0(4)	C13	O4	1.318(5)
O3	C13	C8	124.6(4)	C14	O4	1.445(5)
O4	C13	C8	113.4(3)	N1	S1	1.620(3)
C7	N1	S1	127.2(3)	O1	S1	1.422(3)
C13	O4	C14	115.6(3)	O2	S1	1.428(3)
O1	S1	O2	119.42(18)			
O1	S1	N1	109.95(19)			
O2	S1	N1	104.52(18)			
O1	S1	C1	107.2(2)			
O2	S1	C1	109.42(18)			
N1	S1	C1	105.47(17)			

3.2. Antioxidant activity

DPPH free radical scavenging method was used to find the antioxidant potential of the synthesized compounds. Literature has discovered that the compounds having reducing properties converted DPPH to 1, 1-diphenyl-2-picrylhydrazine [30]. DPPH having deep violet color absorbed light at λ= 517 nm due to the delocalization of free electrons [31]. The compounds owning antioxidant potential do pair up the single electron of DPPH, cause reduction in the absorption at the λ_{max} of DPPH. Ascorbic acid as an antioxidant was chosen as reference. *N*-functionalized derivatives of methyl anthranilate **(1-3)** exhibited moderate antioxidant activity whereas, hydrazide derivatives of *N*-functionalized methyl anthranilate **(4-6)** showed very good antioxidant activity. Higher antioxidant activity could be due to the presence of $-NH_2$ group. The relatively easier availability of the hydrogen atoms of NH_2 could make them as an efficient reducing agent [32, 33]. Schiff base derivatives **(7-9)** have shown reduction in antioxidant activity. However by proper selection of the substituent on the schiff bases can lead to their improved antioxidant potential. The results are described as %age scavenging activity and are displayed in Table 4.

Table 4

Scavenging potential (%) of compounds **1-9** at 1μg/μl concentration

Compounds	*Absorbance*	*Percentage scavenging*
1	1.14	43.87
2	1.27	37.46
3	1.33	34.51
4	0.083	93.91
5	0.080	95.06
6	0.081	92.01
7	1.46	28.11
8	1.43	29.59
9	1.47	27.62
+ve Control (Ascorbic acid)	0.08	96.06
DPPH	2.031	

3.3. Enzyme inhibition studies

Screening of the compounds **(1–9)** in vitro was done against acetyl cholinesterase and butyryl cholinesterase enzymes for their inhibition by utilizing spectrophotometric technique taking Donepzil as positive control. Results are described in terms of %age inhibition of enzyme activities as presented in Table 5. Results clearly indicated that the compounds **2, 3** and **9** showed good inhibition potential against AChE whereas, **2, 4** and **5** against BChE. It is significant that the methyl 2-benzamidobenzoate **(2)** was established to be the two fold inhibitor of AChE as well as BChE which directed that it could be utilized as dual inhibitor for both the enzymes and for treatment of over expression of both the enzymes. However, compound methyl 2-(thiophene-2-carboxamido)benzoate **(3)** was also found to have considerable activity against both BChE and AChE enzyme. In general, hydrazide derivatives of *N*-functionalized methyl anthranilate **(4-6)** showed good inhibition against BChE as compared to AChE. The conversion of the hydrazide derivatives **(4-6)** into schiff bases **(7-9)** had overall reduced the inhibition potential against both the enzymes. In case of *N*-derivatization of methyl anthranilate with thiophenyl carbonyl group caused the overall increase in the inhibition activity against both the enzymes as depicted by inhibition activities of compound **3, 6** and **9**. These results of enzyme inhibition were further reinforced by molecular docking studies. By following the results of our recent study, we planned to carry on the synthesis of new *N*-functionalized derivatives of 2-aminobenzohydrazide accompanying with little modifications on compound **9** by altering substituents to synthesize a series of compounds which could act as an effective class of BChE and AChE inhibitors.

Table 5

AChE and BChE inhibition (%) of compounds **1-9** at concentration of 50μM.

Compound #	*Percentage Inhibition against AChE*	*Percentage Inhibition against BChE*
1	24	32
2	68	70
3	54	59
4	30	61
5	39	76
6	52	51
7	34	56
8	18	24
9	60	42
Donepzil	95	92

3.4. Molecular Docking Studies

Molecular docking studies were executed to discover probable binding interactions of inhibitor compounds with amino acids of active site of the enzymes taken and also to certify the experimental findings. Furthermore, to establish SAR studies, these expected interpretations were utilized to discover interactions between protein and ligand at molecular level. Templates taken were human AChE (PDB ID: 4BDT) and BChE (PDB ID: 4BDS) X-ray structures to carry the process of molecular docking. The lowest bonding energies of the compounds **(1–9)** found after docking investigation are presented in Table 6.

Table 6

Lowest binding energies related to selected conformations against human AChE and BChE

Compound #	*Lowest Binding Energy ΔG (kJ mol^{-1}) h AChE*	*Lowest Binding Energy ΔG (kJ mol^{-1}) h BChE*
1	-9.27	-7.51
2	-7.55	-7.05
3	-7.51	-7.33
4	-9.07	-8.72
5	-7.43	-7.08
6	-7.96	-6.98
7	-10.46	-9.64
8	-9.53	-9.50
9	-9.55	-9.79
Standard	−10 (HUW)	−6.83 (THA)

The molecular docking studies revealed that a wide variety of interactions have been established by most stable conformations of all the inhibitors with the amino acid residues of

the active sites of BChE and AChE and most of them were well accommodated there. The most active compound **2**, when visualized in the active site of human AChE established a lot of significant binding interactions as represented in Fig. 3. This inhibitor utilized oxygen atom of its ester linkage to make H-bonding interactions with AChE, catalytic triad amino acid residue Ser-203 with distance 3.03 Å. Similarly, compound **2** made H-bonding interaction using ester oxygen and another π-donor hydrogen bond association with electron deficient hydrogen atom of –OH group attached to amino acid residue Tyr-337 of AChE (3.18 Å). In the same way, carbonyl oxygen of amide linkage of compound **2** was involved in H-bonding interactions with –OH group of TYR-124 (3.22 Å) and SER-125 (2.90 Å). Trp-86 established π-sigma binding with aromatic ring of the benzamide linkage with distance of 3.68 Å). This compound also utilized its aromatic ring in forming amide π-stacked bonding with Gly-121 and Gly-122, amino acid residues of AChE (4.32 Å).

In the same way, many important interactions were revealed upon visualization of compound **2** in active site of BChE enzyme. It established two H-bonding interactions, one by utilizing its NH hydrogen atom with Ser-198 (2.26 Å), amino acid residue from BChE catalytic triad and second with amino acid residue of oxyanion hole, Ala-199 through its carbonyl oxygen atom (2.55 Å). Compound **2** also established carbon hydrogen bond with Glu-197, amino acid residue of BChE with distance of 3.78 Å. Trp-82, an amino acid residue from choline binding site established π-sigma interactions with methyl group of ester linkage (3.85 Å). Leu-286, amino acid from hydrophobic pocket of BChE made π-π stacked bonding with the under observed compound with distance of 5.04 Å. The expected binding interactions of compound **2** with BChE can be seen in Fig. 4.

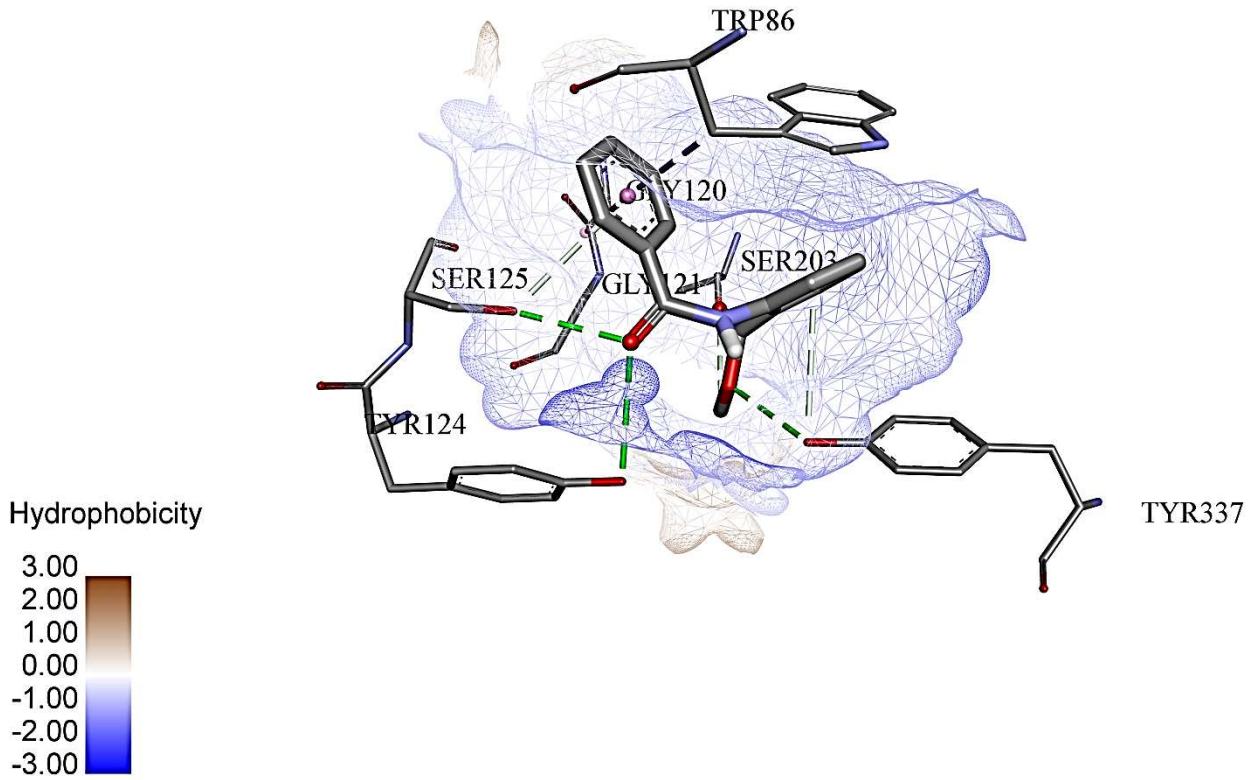


Fig. 3. Putative binding approaches of compound **2** inside AChE enzyme.

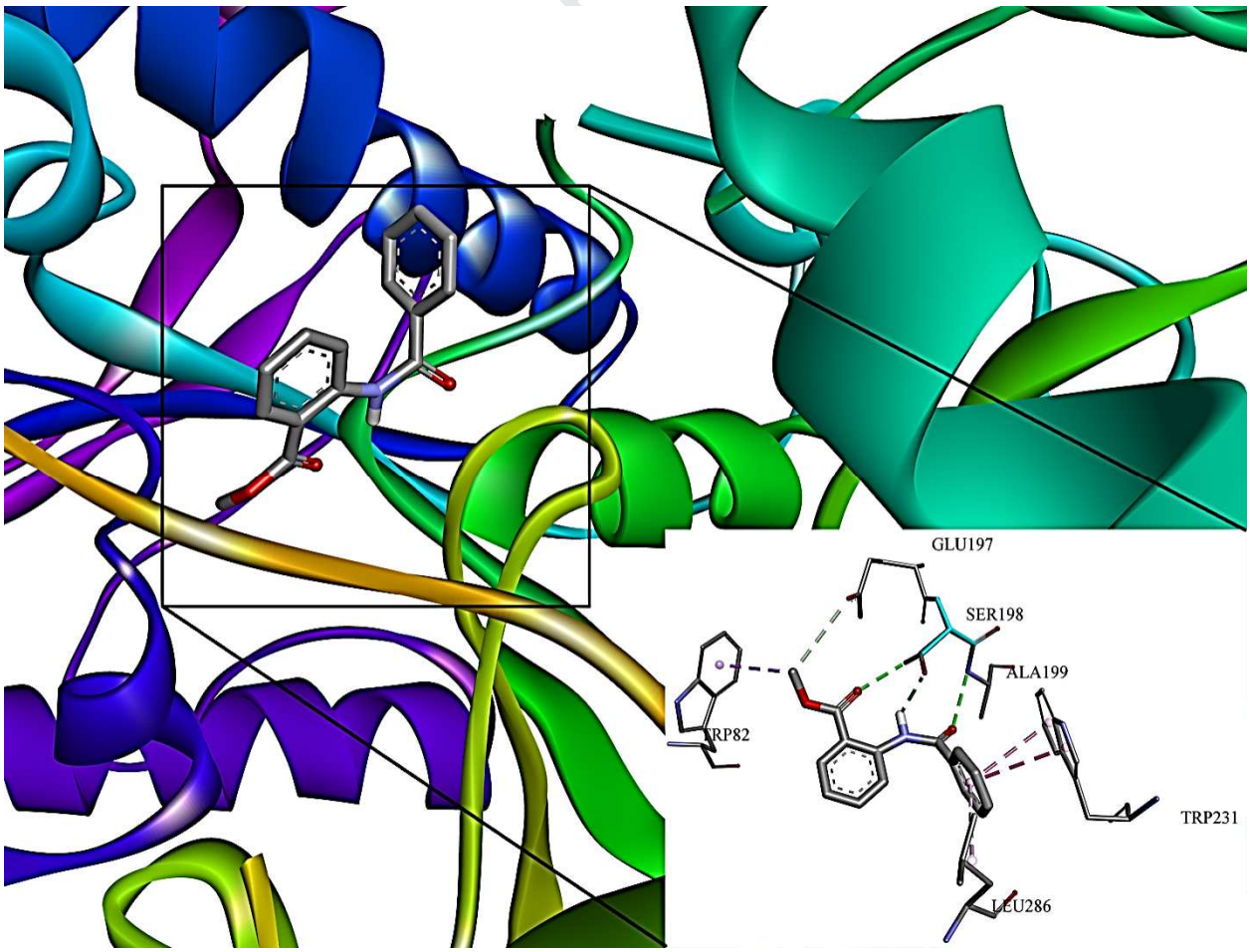


Fig. 4. Compound **2** interactions with BChE at 3D space; Interactions of the compound displayed in the box are with specific amino acid residues of the enzyme. Enzyme is exemplified by 3D ribbon; stick model is the lowest energy conformation of the compound 2.

3.5. Conclusion

Schiff bases, amides and sulfonamides of benzohydrazide were synthesized in good yield. All the synthesised molecules were characterised by spectroscopic techniques. Their potential as antioxidant and as anticholinesterases was also evaluated. The benzoyl, thiophenecarboxamide and sulfonamide benzohydrazides showed excellent antioxidant activities. The amide benzohydrazides were emerged as the potential candidate as anticholinesterases since having good inhibition potential against both BChE and AChE. This study may be further extended with different amide bezohydrazide derivatives to find out potential molecules against Alzheimer disease.

Acknowledgement

The authors are highly thankful to Dr. Mazhar Iqbal, National Institute for Biotechnology & Genetic Engineering (NIBGE), Faisalabad, Pakistan for providing assistance in LC-MS analysis of the compounds and Higher Education Commission (HEC), Pakistan, for financial support for the said analysis.

References

1. A. Deep, S. Jain, P.C. Sharma, P. Phogat, M. Malhotra, Synthesis of 2-(aryl)-5-(arylidene)-4-thiazolidinone derivatives with potential analgesic and anti-inflammatory activity. Med. Chem. Res. 21(8) (2012) 1652-1659.
2. R. Jarapula, K. Gangarapu, S. Manda, S. Rekulapally, Synthesis, in vivo anti-inflammatory activity, and molecular docking studies of new isatin derivatives. Int. J. Med. Chem. (2016).
3. R. Manivannan, R. Shopna, Anti-microbial and Anti-inflammatory Activity of New 4-methoxy-3-(methoxymethyl) Phenol and (E)-N′-(5-bromo-2-methoxybenzylidene)-4-methoxy Benzohydrazide Isolated from Calotropis gigantean white. Nat. Prod. Sci. 23(1) (2017) 69-74.
4. A. Katiyar, M. Hegde, S. Kumar, V. Gopalakrishnan, K.D. Bhatelia, K. Ananthaswamy, S.C. Raghavan, Synthesis and evaluation of the biological activity of N′-[2-oxo-1, 2 dihydro-3 H-indol-3-ylidene] benzohydrazides as potential anticancer agents. RSC Adv. 5(56) (2015) 45492-45501.

5. V.B. Tatipamula, G.S. Vedula, Antimicrobial and anti-tubercular activities of isolates and semi synthetic derivatives of lichen Ramalina leiodea (Nyl.) Nyl. J. Serb. Chem. Soc. 84(6) (2019) 555-562.
6. A. Balachandran, R. Wong, P. Stoilov, S. Pan, B. Blencowe, P. Cheung, A. Cochrane, Identification of small molecule modulators of HIV-1 Tat and Rev protein accumulation. Retrovirology, 14(1) (2017) 7.
7. N. Karaman, Y. Sıcak, T. Taşkın-Tok, M. Öztürk, A. Karaküçük-İyidoğan, M. Dikmen, E.E. Oruç-Emre, New piperidine-hydrazone derivatives: Synthesis, biological evaluations and molecular docking studies as AChE and BChE inhibitors. Eur. J. Med. Chem. 124 (2016) 270-283.
8. M.U. Nisa, M.A. Munawar, A. Iqbal, A. Ahmed, M. Ashraf, A.G. Qurra-tul-Ann, M.A. Khan, Synthesis of novel 5-(aroylhydrazinocarbonyl) escitalopram as cholinesterase inhibitors. Eur. J. Med. Chem. 138 (2017) 396-406.
9. A.B. Özçelik, M. Gökçe, I. Orhan, M.F. Sahin, Synthesis and Acetylcholinesterase/Butyrylcholinesterase Inhibitory Activities of (Substituted/Nonsubstituted Benzal)Hydrazone Derivatives of 3-(6-Substituted-3(2*H*)-Pyridazinon-2-yl)propionohydrazides. FABAD J. Pharm. Sci. 35(3) (2010) 153-161.
10. B. Kaya, Y. Özkay, H.E. Temel, Z.A. Kaplancıklı, Synthesis and biological evaluation of novel piperazine containing hydrazone derivatives. J. Chem. (2016) DOI:10.1155/2016/5878410.
11. J. Mun, A.A. Jabbar, N.S. Devi, S. Yin, Y. Wang, C. Tan, D. Culver, J.P. Snyder, E.G. Van Meir, M.M. Goodman, Design and in-vitro activities of N-alkyl-N-[(8-R-2,2-dimethyl-2H-chromen-6-yl)methyl]heteroarylsulfonamides, novel, small-molecule hypoxia inducible factor-1 pathway inhibitors and anticancer agents, J. Med. Chem. 15 (2012) 6738-6750.
12. E.D. Clercq, New developments in anti-HIV chemotherapy, Curr. Med. Chem. 8 (2001) 1543-1572.
13. B. Ahmed, S.A. Khan, T. Alam, Synthesis and antihepatotoxic activity of some heterocyclic compounds containing the 1, 4-dioxane ring system, Pharmazie. 58 (2003) 173-176.
14. M. Markowicz-Piasecka, K.M. Huttunen, J. Sikora, Metformin and its sulphonamide derivative simultaneously potentiateanti-cholinesterase activity of donepezil and inhibit beta-amyloid aggregation, J. Enzyme Inhib. Med. Chem. 33 (1) (2018) 1309-1322.
15. D. Marton, U. Russo, D. Stivanello, G. Tagliavini, P. Ganis, G.C. Valle, Preparation of Benzylstannanes by Zinc-Mediated Coupling of Benzyl Bromides with Organotin Derivatives. Physicochemical Characterization and Crystal Structures, Organometallics. 15 (6) (1996) 1645–1650.
16. V.S.V. Satyanarayana, P. Sreevani, A.S. Kumar, V. Vijayakukar, Synthesis and antimicrobial activity of new Schiff bases containing coumarin moiety and their spectral characterization, ARKIVOC. 17 (2008) 221-233.
17. M. Yildiz, A. KÍraz, B. Dülger, Synthesis and antimicrobial activity of new crown ethers of Schiff base type, J. Serb. Chem. Soc. 72(3) (2007) 215-224.
18. P. Zhou, L. Xiang, D. Zhao, J. Ren, Y. Qiu, Y. Li, Synthesis, biological evaluation, and structure activity relationship (SAR) study of pyrrolidine amide derivatives as N-acylethanolamine acid amidase (NAAA) inhibitors. MedChemComm, 10(2) (2019) 252-262.
19. V. Ramesh, G.P.C. Rao, D. Ramachandran, A.K. Chakravarthy, Synthesis and Biological Evaluation of Amide Derivatives of Imidazopyridine as Anticancer Agents. Russ. J. Gen. Chem. 89(7) (2019) 1491-1495.

20. J.N. Soni, S. S. Soman, Synthesis and antimicrobial evaluation of amide derivatives of benzodifuran-2-carboxylic acid. Eur. J. Med. Chem. 75 (2014) 77-81.
21. Agilent CrysAlis PRO Agilent Technologies, Yarnton, England (2012).
22. G.M. Sheldrick, A short history of SHELX, Acta Cryst. A. 64 (1) (2008) 112-122.
23. A.L. Spek PLATON-a Multipurpose Crystallographic Tool Utrecht University, Utrecht, (2005).
24. L.J. Farrugia, WinGX and ORTEP for Windows: an update, J. Appl. Cryst. 45 (4) (2012) 849-854.
25. G.L. Ellman, K.D. Courtney, J.V. Andres R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7(2) (1961) 88-95.
26. S. Murtaza, S. Shamim, N. Kousar, M.N. Tahir, M. Sirajuddin, U.A. Rana, Synthesis, biological investigation, calf thymus DNA binding and docking studies of the sulfonyl hydrazides and their derivatives. . J. Mol. Struct. 1107 (2016) 99-108.
27. K. Iftikhar, S. Murtaza, N. Kousar, A. Abbas, M.N. Tahir, Aminobenzoic acid derivatives as antioxidants and cholinesterase inhibitors; synthesis, biological evaluation and molecular docking studies, Acta. Pol. Pharm. 75(2) (2018) 385-396.
28. N. Kausar, S. Muratza, M.A. Raza, H. Rafique, M.N. Arshad, A.A. Altaf, S. Shafqat, Sulfonamide hybrid schiff bases of anthranilic acid: Synthesis, characterization and their biological potential. J. Mol. Struct.1185 (2019) 8-20.
29. W.S. El-serwy, N.A. Mohamed, E.M. Kassem, K. Mahmoud, M.M. Mounier, Synthesis, biological evaluation and docking analysis of some novel quinazoline derivatives as antitumor agents. Iran. J. Pharm. Res. 15(1) (2016) 179.
30. Z. Akar, M. Küçük, H. Doğan, A new colorimetric DPPH• scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. J. Enzyme inhib. Med. Chem. 32(1) (2017) 640-647.
31. S.B. Kedare, R.P. Singh, Genesis and development of DPPH method of antioxidant assay. J. Food Sci. technol. 48(4) (2011) 412-422.
32. D. Amic, D. Davidovic-Amic, D. Beslo, V. Rastija, B. Lucic, N. Trinajstic, SAR and QSAR of the antioxidant activity of flavonoids, Curr. Med. Chem. 14 (7) (2007) 827-845.
33. E. Khan, Z. Gul, A. Shahzad, M.S. Jan, F. Ullah, M.N. Tahir, A. Noor, Coordination compounds of 4, 5, 6, 7-tetrahydro-1 H-indazole with Cu (II), Co (II) and Ag (I): structural, antimicrobial, antioxidant and enzyme inhibition studies. J. Coord. Chem. 70(24) (2017) 4054-4069.

- *N*-functionalized derivatives of 2-aminobenzohydrazide were synthesized.
- The benzohydrazides bearing hydrogen donating groups showed good Antioxidant activities.
- Good enzyme inhibition activity against AChE and BChE were shown by amide derivatives of benzohydrazides.
- Molecular docking evaluation also supported the experimental results for enzyme inhibition.

Declaration of Interest

The authors declare that there is no conflict of interest.