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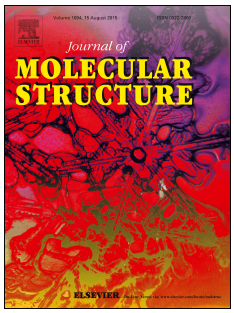

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Sulfonamide Hybrid Schiff Bases of Anthranilic acid: Synthesis, Characterization and their Biological Potential

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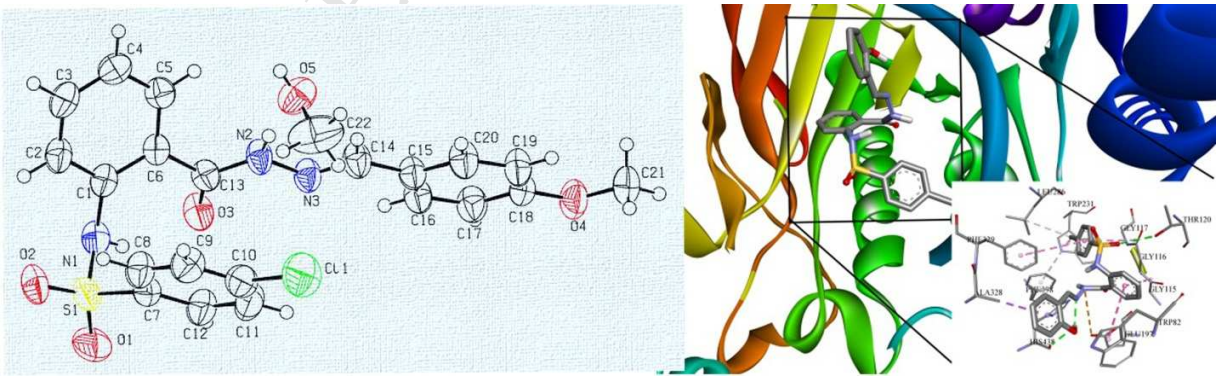
Abstract:

In the present work, the novel Schiff bases (03-20) of 4-chloro-*N*-[2-(hydrazinocarbonyl) phenyl]benzenesulfonamide (02) were synthesized by reacting it with various aldehydes. 4-chloro-*N*-[2-(hydrazinocarbonyl) phenyl]benzenesulfonamide (02) was synthesized by reacting methyl 2-{[(4-chlorophenyl)sulfonyl]amino}benzoate (01) with hydrazine. All synthesized compounds (01-20) were characterized by using FTIR, NMR and Mass spectrometry and by single crystal X-ray diffraction (XRD) analysis techniques. The synthesized compounds were screened for their enzyme inhibition potential against AChE and BChE enzymes. Molecular docking studies were carried out to demonstrate putative binding modes. Antioxidant potential of the synthesized compounds was also determined. Enzyme inhibition assay revealed that compounds 02 and 12 showed maximum inhibition against AChE enzyme with percentage inhibition of 91% and 83% respectively, while compounds 12 and 07 showed highest inhibition against BChE with percentage inhibition of 92% and 81% respectively. Molecular docking studies supported the results of enzyme inhibition assay with binding energy values of -8.49 Kcal mol^{-1} against AChE and -8.39 Kcal mol^{-1} against BChE for compound 12. Antioxidant studies also showed good results with percentage scavenging of 96% for both compounds 02 and 19 investigated by DPPH scavenging method.

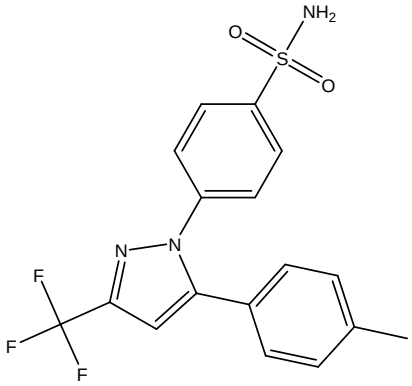
Keywords: sulfonamide, mental disorders, docking studies, hydrazine, methyl anthranilate, DPPH

Graphical Abstract:

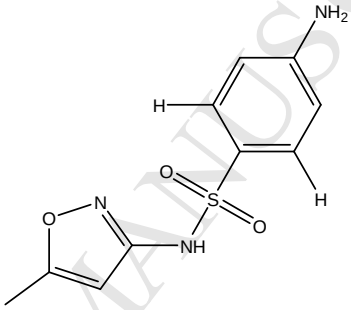


1. Introduction

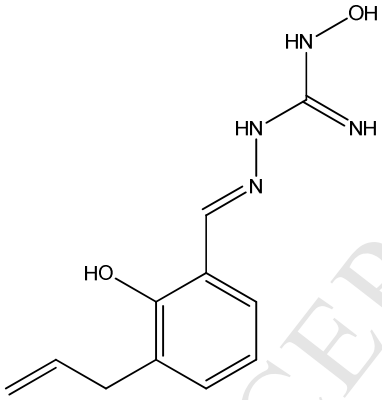
The revolutions in the field of research have explored many secret areas that have the potential to resolve many known problems of the advanced world. Many achievements have been gained in medical sciences, such as cure of many fetal diseases. The importance of developing new drugs, synthesizing new materials having active pharmacophore is a better strategy. Sulfonamide and Schiff base are the two well-known moieties present in active drugs (Fig. 1) [1].



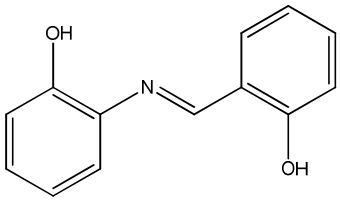
Celecoxib



Sulphamethoxazole



3-Hydroxyguanidine antiviral schiff base



(*E*)-2-((2-hydroxybenzylidene)amino)phenol

Fig. 1. Biologically active drugs having Sulfonamide and Schiff base moieties.

This research work leads to the synthesis of compounds having sulfonamide and Schiff base moieties. Sulfonamides are widely used as antimicrobial [2, 3], anticancer [4, 5], anti-inflammatory [6] and antiviral agents as well as HIV protease inhibitors [7]. Some sulfonamide derivatives are also well recognized as an antimetabolite [8]. Sulfonamides have also shown very good cytotoxic effects against breast cancer cells [9]. Sulfonamides were the first effective

chemotherapeutic agents which were used competently to check and treat the bacterial infection in human beings [10-13]. Clinically sulfonamides are used to treat several urinary tract infections and gastrointestinal infections [14]. Some sulfonamide derivatives were screened for their antioxidant activity, which showed good results [15]. Sulfonamide being very good phamacophore have also shown very good inhibition against AChE and BChE enzymes [16-18].

Schiff bases also form a group of very important compounds having applications in biological, industrial, pharmaceutical and many other fields of science. Schiff bases have antibacterial, antifungal [19-21], and anti-tumor activity [22]. Schiff base derivatives of different compounds which can act as anticancer and anti HIV agents have attracted the attention of modern researchers [23]. These are also used for the treatment of mental disorders and leprosy [24].

By keeping in mind the immense biological significance of sulfonamides and Schiff bases, sulfonamide hybrid Schiff bases (3-20) were synthesized and further investigated their antioxidant and enzyme inhibition potential against AChE and BChE enzymes. Likewise, to develop structure activity relationship (SAR) for these synthesized compounds, the effects of various substituents on the enzyme inhibition activity were studied.

2. Materials and Methods

Analytical grade Hydrazine hydrate, 4-chlorobenzenesulfonyl chloride, methyl anthranilate and aldehydes were obtained from Aldrich (USA). NMR (^{1}H and ^{13}C) were noted using JEOL- 500 MHz DELTA2_NMR Spectrometer using DMSO-d_6 as solvent. The selected single crystal was analyzed by Agilent Technologies Diffractometer (Agilent SuperNova-Dual source). For data collection, it is equipped with micro-focus Cu/Mo Kα radiation. The data collection was accomplished using CrysAlisPro software at 296 K. SHELXS–97 methods was used for structure solution and refinement [25]. PLATON and ORTEP [26] in built with WinGX were used for figures generation.

All the X-H (X = C, N and O) hydrogen atoms were positioned geometrically and treated as riding atoms with C–H = 0.93 Å and Uiso(H) = 1.2 Ueq(C) for aromatic carbon atoms. The methyl hydrogens were positioned geometrically with C_{methyl}-H = 0.96 Å and Uiso(H) = 1.5 Ueq(C). The N-H and O-H hydrogen with N-H = 0.83 (3) - 0.92 (3) Å, O-H = 0.76 Å were

refined using riding model with Uiso (H) = 1.2 Ueq(N) and Uiso (H) = 1.5 Ueq(O). The Crystal data was placed at the Cambridge Crystallographic Data Centre. The deposition number which is assigned to it is 1866050. This is known as CCDC number for molecule (09). This Crystal data can be obtained without any charges on application to CCDC 12 Union Road, Cambridge CB21 EZ, UK. (Fax: (+44) 1223 336-033; e-mail: data_request@ccdc.cam.ac.uk).

2.1. Synthesis of methyl 2-{[(4-chlorophenyl)sulfonyl]amino}benzoate (01)

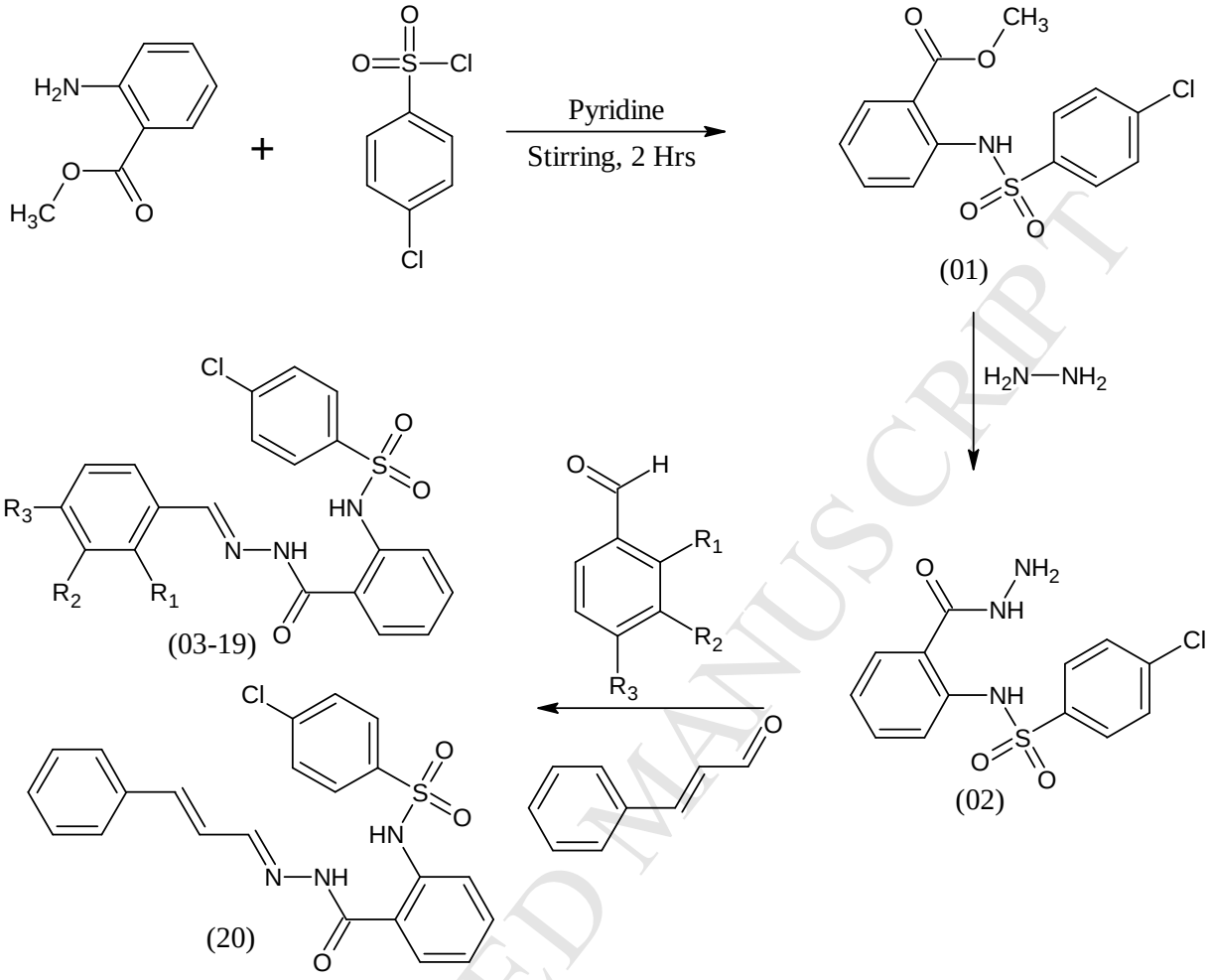
Weighed amount of methyl anthranilate was dissolved in pyridine (10 mL). Solution of 4-chlorobenzenesulphonyl chloride in pyridine was added into the solution of methyl anthranilate. Reaction mixture was allowed to stir at room temperature. The pH of this mixture was retained up to 7 by continuous addition of sodium bicarbonate into the reaction mixture. The progress of the reaction was monitored through thin layer chromatography. The reaction mixture was filtered to remove excess salts. White crystals of the product appeared in the filtrate which were collected and washed with cold methanol. (Scheme 1)

2.2. Synthesis of 4-chloro-N-[2-(hydrazinocarbonyl)phenyl]benzene sulfonamide (02)

Weighed amount of methyl 2-{[(4-chlorophenyl)sulfonyl]amino}benzoate (01) was dissolved in acetonitrile followed by addition of excess hydrazine and refluxed the mixture for two hours. After the completion of the reaction, acetonitrile was removed under vacuum. Extra hydrazine was removed by performing ethyl acetate /water extraction. Ethyl acetate layer was evaporated under vacuum to get the pure product as white solid. The product was recrystallized in ethanol to get colorless crystals of the product. (Scheme 1)

2.3. General procedure for the synthesis of Schiff bases of 4-chloro-N-[2-(hydrazinocarbonyl) phenyl]benzenesulfonamide (03-20)

Stoichiometric amounts of different aldehydes and 4-chloro-*N*-[2-(hydrazinocarbonyl) phenyl]benzenesulfonamide (02) were dissolved in methanol (15 mL). The mixtures were allowed to reflux for 2 hours. The progress of the reactions was monitored using thin layer chromatography. Upon completion of reactions, solvents were evaporated under reduced pressure. The products were washed with cold ethanol and recrystallized by methanol to obtain the products in good yield (above 90%). (Scheme 1)



Where

Compounds	R_1	R_2	R_3
03	H	H	Cl
04	H	H	Br
05	H	H	$N(CH_3)_2$
06	H	H	H
07	H	H	NO_2
08	OH	H	OH
09	H	H	OCH_3
10	H	H	$CH(CH_3)_2$
11	CH_3	H	CH_3

12	OH	H	H
13	CF_3	H	H
14	H	H	CF_3
15	H	F	H
16	H	H	F
17	H	H	COOH
18	COOH	H	H
19	H	OCH_3	OH

Scheme 1. Synthesis of methyl 2-{[(4-chlorophenyl)sulfonyl]amino}benzoate (01) and 4-chloro-*N*-[2-(hydrazinocarbonyl)phenyl]benzenesulfonamide (02) and their Schiff base derivatives (03-20).

2.4. Characterization

The spectral data for all the synthesized compounds is represented here

Methyl 2-{[(4-chlorophenyl)sulfonyl]amino}benzoate (01)

Yield: 81.3%; M.F: $C_{14}H_{12}ClNO_4S$; M.P: 97±1°C; FTIR (KBr pellet, cm^{-1}): 1255.22 (C-O, stretch), 1346.19 (S=O, stretch), 1676.95 (ester C=O, stretch), 3133.49 (NH, stretch). PNMR (300MHz, DMSO-d_6: δ = 3.67 (3H, s, CH_3O), 7.07-7.72 (8H, m, Aromatic CH), 10.15 (1H, s, NH-SO_2). ^{13}C NMR (DMSO-d_6): 51.1(CH_3O), 110.1 - 147.9(benzene ring), 168.5(C=O). MS (ESI), 70 eV: m/z (%), 324 (100, $[M]^{-\cdot}$), 310 (10), 266 (25), 175 (25).

4-Chloro-*N*-[2-(hydrazinocarbonyl)phenyl] benzene sulfonamide (02)

Yield: 70.4%; M.F: $C_{13}H_{12}ClN_3O_3S$; M.P: 157±1°C; FTIR (KBr pellet, cm^{-1}): 1337.86 (S=O, stretch), 1630.37 (C=O, stretch), 3272.20 (SO_2N-H, stretch), 3334.70 (CON-H, Stretch), 3390.13 (NH_2 sym. stretch), 3556.32 (NH_2 anti-sym. stretch). PNMR (300 MHz, DMSO- d_6: δ = 4.77 (2H, NH_2, amine), 7.12-7.76 (8H, m, Aromatic CH), 10.10 (1H, s, NH-SO_2), 11.60 (1H, NH-NH_2). ^{13}C NMR (DMSO-d_6): 116.4 - 145.5, (benzene rings), 164.9 (NHCO). MS (ESI), 70 eV: m/z (%), 324(100, $[M]^{-\cdot}$), 310 (10), 266 (20), 175 (20).

4-Chloro-*N*-[2-({(2*E*)-2-[(4-chlorophenyl)methylidene]hydrazino}carbonyl)phenyl]benzene sulfonamide (03)

Yield: 85%; M.F: $C_{20}H_{15}Cl_2N_3O_3S$; M.P: 201-202°C; FTIR (KBr pellet, cm^{-1}): 1336.95 (S=O, stretch), 1599.56 (N=CH, Stretch), 1651.94 (C=O, stretch), 3319.65 (NH, stretch). PNMR (300 M Hz, DMSO- d_6: δ=7.35 -7.79 (12H, m, Aromatic CH), 8.15 (1H, s, N=CH), 10.16 (1H, s, NH-SO_2), 11.44 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 116.8 - 146.7, (benzene ring), 142.5 (CH=N), 162.9 (NHCO). MS (ESI), 70 eV: m/z (%), 446 (100, $[M]^{-\cdot}$), 418 (05), 309 (10), 182 (05).

N-[2-({(2*E*)-2-[(4-bromophenyl)methylidene]hydrazino}carbonyl)phenyl]-4-chlorobenzene sulfonamide (04)

Yield: 87.1%; M.F: $C_{20}H_{15}ClBrN_3O_3S$; M.P: 208-209°C; FTIR (KBr pellet, cm^{-1}): 1333.15 (S=O, stretch), 1590.20 (N=CH, Stretch), 1646.50 (C=O, stretch), 3335.65 (NH, stretch). PNMR (300 MHz, DMSO-d_6: δ = 7.24-7.81 (12H, m, Aromatic CH), 8.25 (1H, s, N=CH), 10.37 (1H, s, NH-SO_2), 11.40 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 121.7 - 141.8 (benzene ring), 142.6 (CH=N), 163.7 (NHCO). MS (ESI), 70 eV : m/z (%), 491.02 (100), $[M]^{-\cdot}$), 464 (10), 311 (10), 268 (05).

4-Chloro-*N*-[2-({(2*E*)-2-[(4-*N*, *N*-dimethylaminophenyl)methylidene]hydrazino}carbonyl) phenyl] benzenesulfonamide (05)

Yield: 86%; M.F: $C_{22}H_{21}ClN_4O_3S$; M.P: 147 °C; FTIR (KBr pellet, cm^{-1}): 1338.44 (S=O, stretch), 1594.03 (N=CH, Stretch), 1631.74 (C=O, stretch), 3202.25 (NH, stretch). PNMR (300 MHz, DMSO-d_6 :δ= 2.85 (6H, s, $2CH_3$, CH_3-N), 6.78-7.83 (12H, m, Aromatic CH), 8.35 (1H, s, N=CH), 10.78 (1H, s, NH-SO_2), 11.83 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 40.3 (**C**H_3N), 114.5 - 151.7 (benzene ring), 143.6 (CH=N), 163.7 (NHCO). MS (ESI), 70 eV : m/z (%), 447 (100), $[M]^{-\cdot}$), 428 (10), 309 (10), 266 (05).

4-Chloro-*N*-(2-{[(2*E*)-2-(phenylmethylidene)hydrazino]carbonyl}phenyl)benzenesulfonamide(6)

Yield: 81%; M.F: $C_{20}H_{16}ClN_3O_3S$; M.P: 145-146°C; FTIR (KBr pellet, cm^{-1}): 1337.75 (S=O, stretch), 1601.89 (N=CH, Stretch), 1646.95 (C=O, stretch), 3362.50 (NH, stretch). PNMR (300 MHz, DMSO-d_6: δ = 7.19-7.86 (13H, m, Aromatic CH), 8.08 (1H, s, N=CH), 10.14 (1H, s, NH-

SO_2), 11.43 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 115.7 - 144.9 (benzene ring), 140.9 (CH=N), 163.2 (NHCO). MS (ESI), 70 eV : m/z (%), 412.25 (100), $[M]^{-\cdot}$), 384.42 (10), 370.08 (10), 309 (10), 266(12).

4-Chloro-*N*-[2-({(2*E*)-2-[(4-nitrophenyl)methylidene]hydrazino}carbonyl)phenyl]benzene sulfonamide (07)

Yield: 88%; M.F: $C_{20}H_{15}ClN_4O_5S$; M.P: 250-251°C; FTIR (KBr pellet, cm^{-1}): 1334.93 (S=O, stretch), 1595.43(N=CH, Stretch), 1656.36 (C=O, stretch), 3313.90 (NH, stretch). PNMR (300 MHz, DMSO- d_6: δ = 7.23-8.27 (12H, m, Aromatic CH), 8.51 (1H, s, N=CH), 10.49 (1H, s, NH-SO_2), 11.57 (1H, s, NH-N).^{13}C NMR (DMSO-d_6: 163.1 (NHCO), 115.8 - 150.7 (benzene ring), 141.9(CH=N), 163.2 (NHCO). MS (ESI), 70 eV : m/z (%), 457.7 (15), $[M]^{-\cdot}$), 430.8 (60), 309 (12), 266 (05).

4-Chloro-*N*-[2-({(2*E*)-2-[(2,4-dihydroxyphenyl)methylidene]hydrazino}carbonyl)phenyl] benzenesulfonamide (08)

Yield: 76.7%; M.F: $C_{20}H_{16}ClN_3O_5S$; M.P: 264-265°C; FTIR (KBr pellet, cm^{-1}): 1323.98 (S=O, stretch), 1605.32 (N=CH, Stretch), 1652.85 (C=O, stretch), 3286.95 (NH, stretch), 3460.41 (OH, stretch). PNMR(300 MHz, DMSO- d_6: δ= 6.19-7.84 (11H, m, Aromatic CH), 8.67 (1H,s, N=CH), 10.87 (1H, s, NH-SO_2), 11.16 (2H, s, 2OH), 12.14 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 116.1 - 162.2 (benzene rings), 142.0 (CH=N), 163.1 (NHCO). MS (ESI), 70 eV: m/z (%), 444.67 (15, $[M]^{-\cdot}$), 416.86 (10), 309 (13), 266 (05).

4-Chloro-*N*-[2-({(2*E*)-2-[(4-methoxyphenyl)methylidene]hydrazino}carbonyl)phenyl]benzene sulfonamide (09)

Yield: 70%; M.F: $C_{21}H_{18}ClN_3O_4S$; M.P: 142°C; FTIR (KBr pellet, cm^{-1}): 1337.05 (S=O, stretch), 1599.07 (N=CH, Stretch), 1641.85 (C=O, stretch), 3197.69 (NH, stretch). PNMR(300 MHz, DMSO- d_6: δ= 3.82 (3H, s, OCH_3), 6.97-7.70 (12H, m, Aromatic CH), 8.30 (1H, s, N=CH), 10.80 (1H, s, NH-SO_2), 11.73 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 55.9 (CH_3O), 116.3 - 163.0 (benzene rings), 144.7 (CH=N), 163.1 (NHCO). MS (ESI), 70 eV : m/z (%), 442 (100), $[M]^{-\cdot}$), 310 (10), 265 (05).

4-Chloro-*N*-[2-({(2*E*)-2-[(4-isopropylphenyl)methylidene]hydrazino}carbonyl)phenyl]benzene sulfonamide (10)

Yield: 71.3%; M.F: $C_{23}H_{22}ClN_3O_3S$; M.P: 173-174°C; FTIR (KBr pellet, cm^{-1}): 1340.25 (S=O, stretch), 1595.64 (N=CH, Stretch), 1631.90 (C=O, stretch), 3213.31 (NH, stretch). PNMR (300 MHz, DMSO- d_6: δ = 1.29 (6H, d, $2CH_3$-CH), 3.12 (1H, sep, CH-CH_3), 7.22-7.86 (12H, m, Aromatic CH), 8.29 (1H, s, N=CH), 10.23 (1H, s, NH-SO_2), 11.38 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 23.4 ($2CH_3$C), 36.3 (CH-CH_3), 116.7 - 150.9 (benzene rings), 141.0 (CH=N), 163.3 (NHCO). MS (ESI), 70 eV: m/z (%), 454.8 (100), $[M]^{+\cdot}$), 426.94 (20), 311 (10), 266 (05).

4-chloro-*N*-[2-({(2*E*)-2-[(2,4-dimethylphenyl)methylidene]hydrazino}carbonyl)phenyl]benzene sulfonamide(11)

Yield: 80.4%; M.F: $C_{22}H_{20}ClN_3O_3S$; M.P: 216-217 °C; FTIR (KBr pellet, cm^{-1}): 1337.63 (S=O, stretch), 1593.45 (N=CH, Stretch), 1625.73 (C=O, stretch), 3179.77 (HN, stretch). PNMR (300 MHz, DMSO- d_6: δ = 2.35 (6H, s, $2CH_3$), 6.99-7.75 (11H, m, Aromatic CH), 8.31 (1H, s, N=CH), 10.11 (1H, s, NH-SO_2), 11.49 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 18.2-24.6 ($2CH_3$), 115.9 - 145.5 (benzene rings), 142.6 (CH=N), 163.1 (NHCO). MS (ESI), 70 eV : m/z (%), 441 (100), $[M]^{-\cdot}$), 413 (10), 224 (12), 309 (15), 266 (10).

4-chloro-*N*-[2-({(2*E*)-2-[(2-hydroxyphenyl)methylidene]hydrazino}carbonyl)phenyl]benzene sulfonamide (12)

Yield: 70.25%; M.F: $C_{20}H_{16}ClN_3O_4S$; M.P: 223-224 °C; FTIR (KBr pellet, cm^{-1}): 1335.82 (S=O, stretch), 1609.25 (N=CH, Stretch), 1639.28 (C=O, stretch), 3271.79 (HN, stretch), 3341.66 (OH, stretch). PNMR (300 MHz, DMSO- d_6: δ = 6.92-7.71 (12H, m, Aromatic CH), 8.58 (1H, s, N=CH), 10.75 (1H, s, NH-SO_2), 11.10 (1H, s, 1OH), 12.10 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 116.4 - 162.2 (benzene rings), 141.8 (CH=N), 162.6 (NHCO). MS (ESI), 70 eV : m/z (%), 428.88 (100), $[M]^{-\cdot}$), 401 (10), 211 (15), 309 (20), 267 (100).

4-chloro-*N*-(2-{[(2*E*)-2-{[2-(trifluoromethyl)phenyl]methylidene}hydrazino]carbonyl}phenyl) benzenesulfonamide (13)

Yield: 78.4%; M.F: $C_{21}H_{15}ClF_3N_3O_3S$; M.P: 224 °C; FTIR (KBr pellet, cm^{-1}): 1335.52 (S=O, stretch), 1628.95 (C=O, stretch), 1597.50 (N=CH, Stretch), 3198.39 (HN, stretch). PNMR (300

MHz, DMSO- d_6: δ = 6.98 - 7.78 (12H, m, Aromatic CH), 8.39 (1H, s, N=CH), 10.19 (1H, s, NH-SO_2), 11.46 (1H, s, NH-N). ^{13}C NMR (DMSO- d_6): 119.7 – 142.7 (benzene rings), 114.5 (1CF_3), 142.2 (CH=N), 161.9 (NHCO). MS (ESI), 70 eV : m/z (%), 480.88 (100), $[M]^{-\cdot}$), 453 (10), 264 (20), 309 (15), 266 (10).

4-chloro-*N*-(2-{[(2*E*)-2-{[4-(trifluoromethyl)phenyl]methylidene}hydrazino]carbonyl}phenyl) benzenesulfonamide (14)

Yield: 77.3%; M.F: $C_{21}H_{15}ClF_3N_3O_3S$; M.P: 193 °C; FTIR (KBr pellet, cm^{-1}): 1323.56 (S=O, stretch), 1603.45 (N=CH, Stretch), 1659.60 (C=O, stretch), 3325.42 (HN, stretch). PNMR (300 MHz, DMSO- d_6: δ = 6.87 - 7.79 (12H, m, Aromatic CH), 8.26 (1H, s, N=CH), 10.18 (1H, s, NH-SO_2), 11.47 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 112.6 - 146.5 (benzene rings), 120.7 (CF_3), 140.8 (CH=N), 162.5 (NHCO). MS (ESI), 70 eV : m/z (%), 480.25 (100), $[M]^{-\cdot}$), 456.08 (15), 412.17 (10), 324.17 (15).

4-chloro-*N*-[2-({(2*E*)-2-[(3-fluorophenyl)methylidene]hydrazino}carbonyl)phenyl]benzene sulfonamide (15)

Yield: 81.4%; M.F: $C_{20}H_{15}ClFN_3O_3S$; M.P: 196 °C; FTIR (KBr pellet, cm^{-1}): 1333.42 (S=O, stretch), 1597.50 (N=CH, Stretch), 1640.80 (C=O, stretch), 3334.96 (HN, stretch). PNMR (300 MHz, DMSO- d_6: δ = 6.80-7.94 (12H, m, Aromatic CH), 8.22 (1H, s, N=CH), 10.35 (1H, s, NH-SO_2), 11.44 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 113.6 - 160.5 (benzene rings), 141.2 (CH=N), 164.1 (NHCO). MS (ESI), 70 eV : m/z (%), 430.01(100), $[M]^{-\cdot}$).

4-chloro-*N*-[2-({(2*E*)-2-[(4-fluorophenyl)methylidene]hydrazino}carbonyl)phenyl]benzene sulfonamide (16)

Yield: 80.4%; M.F: $C_{20}H_{15}ClFN_3O_3S$; M.P: 187 °C; FTIR (KBr pellet, cm^{-1}): 1334.25 (S=O, stretch), 1602.25 (N=CH, Stretch), 1654.36 (C=O, stretch), 3323.28 (HN, stretch). PNMR (300 MHz, DMSO-d_6: δ = 6.89 - 7.80 (12H, m, Aromatic CH), 8.13 (1H, s, N=CH), 10.11 (1H, s, NH-SO_2), 11.45 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 117.1 - 163.6 (benzene rings), 144.1 (CH=N), 162.8 (NHCO). MS (ESI), 70 eV : m/z (%), 430.02(100), $[M]^{+\cdot}$).

4-[(*E*)-{[(2-{[(4-chlorophenyl)sulfonyl]amino}phenyl)carbonyl]hydrazono}methyl]benzoic acid (17)

Yield: 80.4%; M.F: $C_{21}H_{16}ClN_3O_5S$; M.P: 267 °C; FTIR (KBr pellet, cm^{-1}): 1343.56 (S=O, stretch), 1612.69 (N=CH, Stretch), 1654.23 (C=O, stretch), 1680.65 (carboxyl C=O, stretch), 3100.28 (carboxyl OH, stretch), 3269.36 (HN, stretch). PNMR (300 MHz, DMSO- d_6: δ = 6.89 - 8.14 (12H, m, Aromatic CH), 8.34 (1H, s, N=CH), 10.20 (1H, s, NH-SO_2), 11.05 (1H, s, COOH), 11.71 (1H, s, NH-N). ^{13}C NMR (DMSO- d_6): 115.6 - 147.2 (benzene rings), 142.7 (CH=N), 165.1 (NHCO), 173.2 (COOH). MS (ESI), 70 eV : m/z (%), 456.89 (100), [M] $^{-\cdot}$), 429 (15), 240 (10).

2-[(*E*)-{[(2-{[(4-chlorophenyl)sulfonyl]amino}phenyl)carbonyl]hydrazono}methyl]benzoic acid (18)

Yield: 80.4%; M.F: $C_{21}H_{16}ClN_3O_5S$; M.P: 234 °C; FTIR (KBr pellet, cm^{-1}): 1340.23 (S=O, stretch), 1601.50 (N=CH, Stretch), 1634.84 (C=O, stretch), 1712.18 (carboxyl C=O, stretch), 3182.88 (carboxyl OH, stretch), 3301.75 (HN, stretch). PNMR (300 MHz, DMSO-d_6: δ = 6.99-7.79 (12H, m, Aromatic CH), 8.15 (1H, s, N=CH), 10.09 (1H, s, NH-SO_2), 11.12 (1H, s, COOH), 11.69 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 119.3 - 143.8 (benzene rings), 147.5 (CH=N), 163.1 (NHCO), 169.4 (COOH). MS (ESI), 70 eV : m/z (%), 456.89 (100), [M]$^{-\cdot}$), 429 (10), 240 (15).

4-chloro-*N*-[2-({(2*E*)-2-[(4-hydroxy-3-methoxyphenyl)methylidene]hydrazino}carbonyl)phenyl] benzenesulfonamide (19)

Yield: 80.4%; M.F: $C_{21}H_{18}ClN_3O_5S$; M.P: 229 °C; FTIR (KBr pellet, cm^{-1}): 1339.60 (S=O, stretch), 1586.37 (N=CH, Stretch), 1630.21 (C=O, stretch), 3181.95 (HN, stretch), 3455.80 (OH, stretch). PNMR (300 MHz, DMSO- d_6: δ = 3.78 (3H, s, OCH_3), 6.61-7.89 (11H, m, Aromatic CH), 8.31 (1H, s, N=CH), 10.63 (1H, s, NH-SO_2), 11.09 (1H, s, OH), 11.98 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 56.3 (OCH_3), 116.7 - 151.5 (benzene rings), 144.6 (CH=N), 163.2 (NHCO). MS (ESI), 70 eV : m/z (%), 459 (15), [M] $^{-\cdot}$), 431 (05), 242 (10), 311 (15).

4-Chloro-*N*-[2-({(2*E*)-2-[(2*E*)-3-phenylprop-2-en-1-ylidene]hydrazino}carbonyl)phenyl]benzene sulfonamide (20)

Yield: 70.4%; M.F: $C_{22}H_{18}ClN_3O_3S$; M.P: 199 °C; FTIR (KBr pellet, cm^{-1}): 1340.09 (S=O, stretch), 1599.02 (N=CH, Stretch), 1643.03 (C=O, stretch), 3240.15 (HN, stretch). PNMR (300 MHz, DMSO-d_6: δ = 7.28-7.69 (13H, m, Aromatic CH), 7.89 (1H, s, N=CH), 10.71 (1H, s, NH-

SO_2), 11.94 (1H, s, NH-N). ^{13}C NMR (DMSO-d_6): 116.7 - 141.6 (benzene rings), 135.8 (CH=N), 137.9 (CH=CH), 165.18 (NH**C**O). MS (ESI), 70 eV : m/z (%), 438.8 (15), $[M]^{+\cdot}$), 411 (20), 309 (10), 266 (15).

2.5. Biological activity

2.5.1. Enzyme Inhibition Essay

Ryan and Elman method [27] was used to evaluate enzyme inhibition with little modification. 50 µL of the enzyme (AChE/BChE) was taken and mixed with 50 µL of each sample (50µM) (01-20). The mixture of each sample was allowed to stand for 10 minutes. Then 50 µL of substrate i.e. butyrylthiocholine chloride (0.2mM) for BChE and acetylthiocholine iodide(0.71 mM) for AChE was added to each sample along with 50 µL(0.5 mM) of DTNB [5,5´-dithio-bis(2-nitrobenzoic acid)] and 500 µL of phosphate buffer at pH 8 and shaked well. This mixture was then incubated at 37 °C for 30 minutes. 5-thio-2- nitrobenzoate anion was formed due to the hydrolysis of the substrate which gave the yellow coloration of the mixture. Spectrophotometric method was used to monitor the yellow coloration produced due to the hydrolysis of the substrate by measuring absorbance at 412 nm and 400 nm for BChE and AChE, respectively. Following formula was used to calculate percentage enzyme inhibition for all the samples.

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

Where A =Absorption value of enzyme having test compounds

B = Absorption value of the enzyme without any test compounds

Every experiment was performed three times and average value was utilized. Donepzil was used as reference drug.

2.5.2. Antioxidant assay

DPPH scavenging methodology [28] was utilized to find the antioxidant potential of the synthesized compounds (01-20). 2 mL of 0.2mM ethanolic solution of DPPH was taken and 100 uL of 50 uM solution of the samples was added into it. Incubation of the mixture was done at 37

^{o}C for 20 minutes. Then Spectrophotometric method was used to find the disappearance of DPPH coloration by taking absorbance at λ = 516 nm. 1mM ascorbic acid was utilized as positive control. Following equation was used to calculate the free radical scavenging (%age) for all the samples

$$\%age\ Scavenging = \left(\frac{A° - AT}{A°}\right) \times 100$$

Where

AT = Absorbance of the sample solution, A^{o} = Absorbance of the DPPH solution,

2.6. Molecular Docking studies

Molecular docking studies were carried out to check enzyme and ligand interactions. Crystal structure of enzymes AChE and BChE was acquired from (RCSB) protein data bank. ACD Chem sketch software was utilized to sketch the molecular structures of the ligands and 3D Pro 12.0 software was utilized to obtain 3D optimization of the ligands and SYBYL mol2 file format was used to save the optimized structures. Process of docking was performed using Auto Dock Tool v 1.5.6., 100 different configurations were attained. One of the best conformations of the compound was selected after visualizing all the conformations using Discovery Studio Visualizer 4.0.

3. Results and Discussion

3.1. Chemistry

Sulfonamide based Schiff bases of anthranilic acid were synthesized according to Scheme 1. Their structures were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and X-ray-Crystallography.

3.1.1. FT-IR studies

Functional groups identification of all the synthesized compounds was done using FTIR spectra. The presence of imine linkage was confirmed with the peaks in the range of $\bar{\upsilon}$ = 1586.37 cm^{-1}- 1612.69 cm^{-1} (N=CH) and 1625.73 cm^{-1}- 1656.36 cm^{-1} (C=O). CH aromatic stretching vibrations lies in the region 3100-3040 and other CH peaks in the region 2940-2800 cm^{-1}.

3.1.2. ^{1}H NMR Studies

^{1}H NMR spectra of synthesized compounds confirmed their structures. Compound (01) was converted to compound (02), peak of OCH_3 at 3.67ppm disappeared and new peaks appeared at δ = 4.77ppm, corresponding to NH_2. Similarly when compound (02) was further converted to its Schiff base derivatives (03-20), peaks appeared in the range of δ = 7.89ppm to 8.58ppm corresponding to their N=CH bond. Peaks corresponding to aromatic protons were appeared between 6.19ppm - 8.27ppm, depending upon the type of substituents attached to aromatic ring. Electron donating effects of dimethyl amino, hydroxyl and methoxyl groups increased the electron density at ortho and para positions that caused the shifting of the peak of the corresponding H to field up (6.19ppm - 6.92ppm) in compounds (05, 08, 12, 09 and 19) as compared to 7.19ppm values protons of non-substituted ring (compound 06). In contrast, electron withdrawing group (NO_2 group) caused the shifting of chemical shifts downfield (higher ppm values up to 8.27ppm in compound 07. In compounds 17 and 18, signal of carboxyl hydrogen appear at 11.05ppm – 11.12ppm, because carboxyl proton is highly de-shielded. In compounds 09 and 19, peaks appear in the range of δ = 3.78ppm - 3.82ppm corresponding to protons of methoxyl groups. All the spectral data for Proton NMR are in good agreement with the structures of the synthesized compounds.

3.1.3. ^{13}C NMR studies

^{13}C NMR spectra also supported the molecular structures of the series. Peak of carbonyl carbon (C=O) of ester group at 168.5ppm in compound (01) has been shifted towards lower ppm values at 164.9ppm in compound (02) due to the formation of amide linkage, which is in accordance with the literature values. In compounds (03-20), peak for imine carbon (N=C-H) appeared in the range of 135.8ppm – 147.5ppm. Variation of the peaks of aromatic carbons in the range of 114.5ppm - 169.5ppm depending upon the nature of substituents is also in accordance with the structures of the synthesized compounds as described in the spectral data in experimental section.

3.1.4. Mass spectroscopic studies

The molecular masses of the synthesized compounds were established by Electron Spray Ionization (ESI) technique. The mass spectrum of all the compounds showed characteristic peaks corresponding to [M-H] ions in negative mode and [M+H] peaks in positive mode. The

compounds (2-20) contain chlorine atom which is confirmed by its [M+2] isotopic peak (33%). While in compound 03, that [M+2] peak was enhanced up to 66% due to the presence of two chlorine atoms. All the spectral data for MS (ESI) are in good accordance with the structures of the synthesized compounds.

3.1.5. XRD studies

The molecules in the unit cell of the target compound (09) arranged themselves to generate the monoclinic crystal system with one molecule in an asymmetric unit cell and a methanol molecule as solvent as shown in Fig. 2 (a). The crystallographic parameters are given in Table 1 while selected bond lengths and bond angles are provided in Tables 2 and 3. The geometry around the central S atom of sulfonamide group adopted the distorted tetrahedral geometry [29-32]. There are the three aromatic rings in the molecule. The central aromatic ring (C1-C6) is oriented at dihedral angle of 54.227 (7)° and 46.482 (8)° with respect to the 4-chlorophenyl (C7-C12) and 4-methoxyphenyl (C15-C20) aromatic rings. The donor-nitrogen atom N1 at the position (x, y, z) connects with the acceptor atom O3 via intra-molecular hydrogen bonding interaction and produced six membered ring motifs as shown in Fig. 2 (b) [33]. The root mean square deviation for this ring (C1/C6/C13/N1/H1N/O3) is 0.1129(3)° where the most deviations were observed from the C13 = 0.11861 Å and O3 = 0.1611Å atoms. This ring is twisted with the dihedral angle of 9.411(3)° with its fused ring (C1-C6) and its dihedral angle with the 4-methoxyphenyl (C15-C20) aromatic ring is 50.001 (3)°. The N2-H2N...O5 interaction along-with O5-H1O...O3 type intermolecular hydrogen bonding connects the molecules along a-axis to generate the infinite long chains as shown in Fig. 3 (a). Another non-classical interaction, in which C11 acts as hydrogen atom donor and O2 acts as acceptor atom following the symmetry operation -x, y+1/2, -z+3/2, connects the chains and give rise to formation of two-dimensional network along *ab*-plane as shown in Fig. 3 (b) and Table 4.

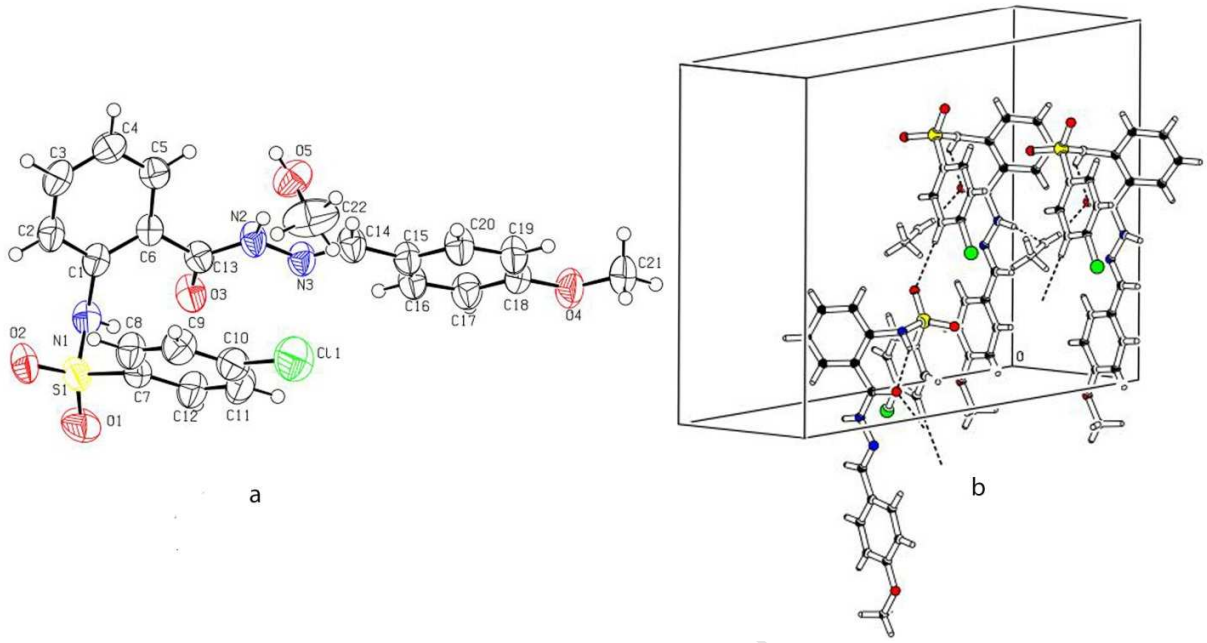


Fig. 2. a) *ORTEP* diagram of compound 09, thermal ellipsoids were drawn at 50% probability level. b) A unit cell view showing the intra- and inter-molecular hydrogen bonding interactions in compound 09.

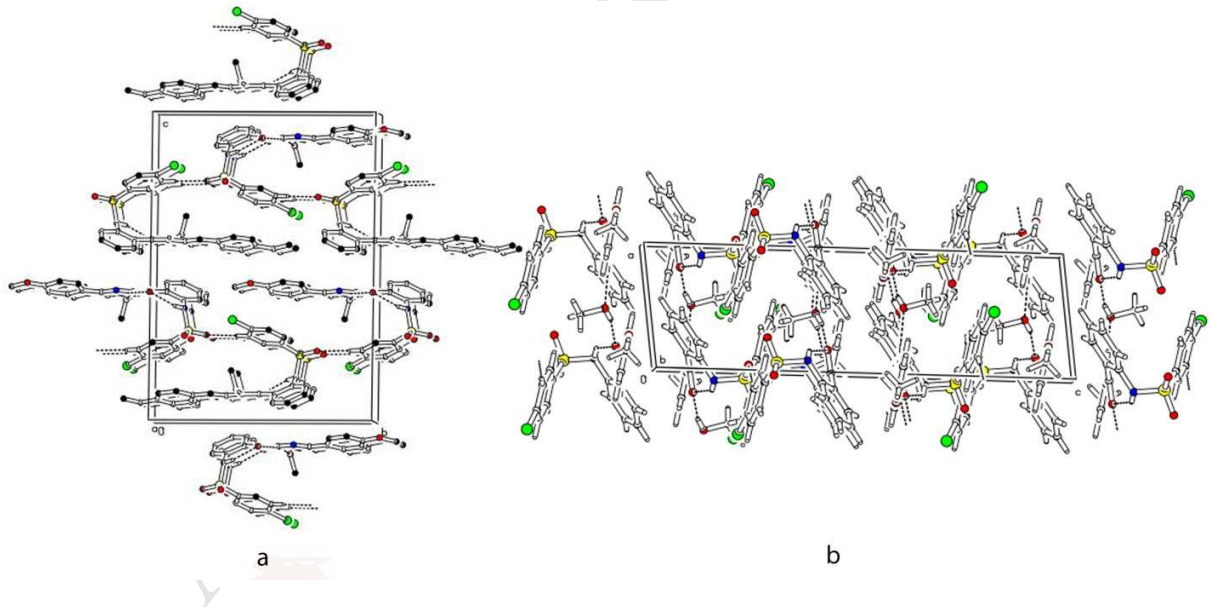


Fig. 3. a)Formation of long chains along the diagonal of *b*-axes for compound 09. b) A view showing the long chain generation along a-axis for compound 09.

Table 1

Crystal data and structure refinement for compound 09

Crystal data and structure refinement for compound 09

CCDC number	1866050
Empirical formula	$C_{22}H_{22}ClN_3O_5S$
Formula weight	475.93
Temperature/K	296(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	6.6203(5)
b/Å	15.8815(15)
c/Å	22.143(2)
α/°	90
β/°	97.386(8)
γ/°	90
Volume/Å^3	2308.8(4)
Z	4
ρ_{calc} mg/mm^3	1.369
μ/mm^{-1}	0.294
F(000)	992.0
Crystal size/mm^3	0.48 × 0.42 × 0.33
2θ range for data collection	6.128 to 58.134°
Index ranges	$-8 \le h \le 8$, $-21 \le k \le 11$, $-29 \le l \le 29$
Reflections collected	12501
Independent reflections	5462[R(int) = 0.0446]
Data/restraints/parameters	5462/0/299
Goodness-of-fit on F^2	1.018
Final R indexes [I>=2σ (I)]	R_1 = 0.0597, wR_2 = 0.1336
Final R indexes [all data]	R_1 = 0.1243, wR_2 = 0.1701
Largest diff. peak/hole / e Å^{-3}	0.21/-0.34

Table 2

Bond Lengths for compound 09.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
C1	C2	1.379(4)	C13	O3	1.243(3)
C1	C6	1.405(4)	C14	C15	1.439(4)
C1	N1	1.434(4)	C14	N3	1.276(4)
C2	C3	1.377(4)	C15	C16	1.398(4)

C3	C4	1.369(5)	C15	C20	1.389(4)
C4	C5	1.373(4)	C16	C17	1.370(4)
C5	C6	1.395(4)	C17	C18	1.387(4)
C6	C13	1.479(4)	C18	C19	1.374(4)
C7	C8	1.384(4)	C18	O4	1.363(4)
C7	C12	1.376(4)	C19	C20	1.374(4)
C7	S1	1.759(3)	C21	O4	1.416(4)
C8	C9	1.374(5)	C22	O5	1.379(5)
C9	C10	1.363(5)	N1	S1	1.640(3)
C10	C11	1.378(5)	N2	N3	1.387(3)
C10	Cl1	1.735(3)	O1	S1	1.420(2)
C11	C12	1.376(5)	O2	S1	1.429(2)
C13	N2	1.341(4)			

Table 3

Bond Angles for compound 09.

Atom	**Atom**	**Atom**	**Angle/˚**	**Atom**	**Atom**	**Atom**	**Angle/˚**
C2	C1	C6	120.1(3)	O3	C13	N2	122.0(3)
C2	C1	N1	118.5(3)	N3	C14	C15	123.9(3)
C6	C1	N1	121.4(2)	C16	C15	C14	123.7(3)
C3	C2	C1	120.4(3)	C20	C15	C14	119.2(3)
C4	C3	C2	120.5(3)	C20	C15	C16	117.0(3)
C3	C4	C5	119.7(3)	C17	C16	C15	120.9(3)
C4	C5	C6	121.4(3)	C16	C17	C18	120.6(3)
C1	C6	C13	121.1(2)	C19	C18	C17	119.7(3)
C5	C6	C1	117.9(3)	O4	C18	C17	115.7(3)
C5	C6	C13	121.0(3)	O4	C18	C19	124.6(3)
C8	C7	S1	120.1(2)	C18	C19	C20	119.3(3)
C12	C7	C8	120.4(3)	C19	C20	C15	122.5(3)
C12	C7	S1	119.4(2)	C1	N1	S1	119.82(19)
C9	C8	C7	119.7(3)	C13	N2	N3	119.4(2)
C10	C9	C8	119.4(3)	C14	N3	N2	115.0(2)
C9	C10	C11	121.6(3)	C18	O4	C21	117.9(3)
C9	C10	Cl1	119.2(3)	N1	S1	C7	104.99(14)
C11	C10	Cl1	119.1(3)	O1	S1	C7	109.40(15)
C12	C11	C10	119.0(3)	O1	S1	N1	105.09(14)
C7	C12	C11	119.8(3)	O1	S1	O2	120.28(14)
N2	C13	C6	115.8(2)	O2	S1	C7	108.23(14)

O3	C13	C6	122.2(3)	O2	S1	N1	107.80(14)

Table 4

Hydrogen bond interactions in compound 09

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C11	H11	O2[1]	0.93	2.46	3.391(4)	176.9
C14	H14	O5	0.93	2.47	3.286(4)	145.9
N1	H1	O3	0.83(3)	2.06(3)	2.745(3)	141(3)
N2	H2A	O5	0.92(3)	1.96(3)	2.874(3)	174(3)
O5	H5A	O3[2]	0.76(4)	2.13(4)	2.853(3)	160(4)

[1]1+X,1+Y,+Z; [2]1-X,1-Y,1-Z; [3]1-X,1-Y,2-Z

3.2. Antioxidant activity

Antioxidant potential of the compounds can be determined by carrying DPPH free radical scavenging activity. This technique gives antioxidant potential information in relatively shorter times. The compounds possessing the ability to reduce the DPPH pledge this radical to form 1,1-diphenyl-2-picrylhydrazine [34]. Deep violet color of DPPH is due to the presence of an odd electron in it, which results in strong absorption at 517 nm [35]. The compounds possessing antioxidant potential cause the pairing of the odd electron of DPPH, which result in decrease in absorption at the above said wavelength. The results are interpreted in terms of %age scavenging activity as compared to ascorbic acid chosen as standard and are shown in Table 5. All the compounds showed moderate antioxidant activities except 2, 8 and 19 which showed very good antioxidant activities. These compounds showed good activities due to the availability of $–NH_2$ and –OH groups in their structures to provide hydrogen atoms to show antioxidant potential [36].

Table 5

Percentage scavenging of compounds 01-20 at 1ug/µl concentration

Compounds	***Absorbance***	***Percentage scavenging***
01	1.60	21
02	0.08	96

03	1.316	35
04	1.59	21
05	0.963	53
06	1.496	28
07	1.477	29
08	0.182	91
09	1.655	19
10	1.587	22
11	1.638	20
12	0.525	74
13	1.704	16
14	1.667	18
15	1.603	21
16	1.343	34
17	1.601	19
18	0.826	59
19	0.08	96
20	1.742	14
+ve Control (Ascorbic acid)	0.09	95.5
DPPH	2.031	

3.3. Enzyme inhibition studies

All the synthesized compounds (01-20) were checked in vitro for their inhibitory potential against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes using spectrophotometric method.

The results are interpreted as %age inhibition of the AChE and BChE using Donepzil as standard drug and are reported in Table 6. It is clear from the results that most of the compounds showed very good inhibition against both the enzymes AChE and BChE. The most active compound was compound 12 which showed very good inhibition potential against both the enzymes, due to the presence of -OH group as substituent. This -OH group forms strong H-bond associations with the active parts of both the enzymes. Compound 02 also showed very good inhibitions against both the enzymes due to the presence of hydrazide linkage in it, which leads to formation of strong association with the active pockets of both the enzymes through hydrogen bonding. Compound 08 also showed very good inhibition potential against both the enzymes, AChE and BChE due to the presence of two -OH groups as substituents which lead to increased H- bond interactions with the active sites of the enzymes. Compound 17 also depicted very good activities due to the

presence of –COOH group at the aromatic ring, which leaded to strong electrostatic forces of attraction with the active sites of the enzymes. Compounds 07, 15 and 16 showed good inhibition against BChE enzyme, due to the presence of NO_2 group and F atom as substituents in the compound. These groups also cause the formation of H-bonds with the active sites of the enzymes. Overall, most of the compounds showed good enzyme inhibition activities against both the enzymes AChE and BChE. These outcomes have been additionally proved by *In-silico* studies (molecular docking). The results depict that these sulfonamide based Schiff bases can be further studied to develop new scaffolds to cure Alzheimer disease.

Table 6

Percentage inhibition of compounds 01-20 against AChE and BChE at 50uM concentration

Compounds	***Percentage Inhibition against AChE***	***Percentage Inhibition against BChE***
01	55	77
02	91	75
03	61	31
04	63	26
05	12	51
06	77	51
07	50	81
08	67	69
09	51	62
10	72	52
11	48	34
12	83	92
13	53	58
14	51	36
15	53	79
16	58	60
17	63	70
18	55	36
19	53	25
20	50	43
Donepzil	95	92

3.4. Molecular Docking Studies

To establish SAR studies, molecular docking studies of all the compounds (01-20) was carried out to find protein ligand interactions by checking the binding interactions of the compound with

the active pockets of the enzymes. To carry out molecular docking studies, Templates taken were human BChE (PDB ID: 4BDS) and AChE (PDB ID: 4BDT) X-ray structures. After molecular docking studies, the minimum binding energies of the compounds (01-20) calculated are given in Table 7.

Table 7

Binding energies of selected conformations against human AChE and BChE

Compound No	*h AChE Lowest Binding Energy ΔG in kJ mol^{-1}*	*h BChE Lowest Binding Energy ΔG in kJ mol^{-1}*
01	-9.06	-8.25
02	-8.79	-8.48
03	-11.27	-9.81
04	-10.41	-9.08
05	-9.03	-8.43
06	-9.89	-9.47
07	-8.02	-8.98
08	-7.66	-8.65
09	-9.75	-8.81
10	-10.69	-9.64
11	-8.97	-9.35
12	-8.49	-8.39
13	-5.54	-8.67
14	-8.75	-9.44
15	-9.88	-9.41
16	-8.97	-8.80
17	-4.5	-8.18
18	-8.37	-8.25
19	-9.77	-8.79
20	-9.68	-8.77
Standard	−10 (HUW)	−6.83 (THA)

The molecular docking studies revealed that most stable conformations of all the compounds showed various types of interactions with the amino acids of the active pockets of BChE and AChE and are well accommodated there. For example visualization of the most potent compound 12 in the active pocket of human AChE enzyme manifested many important bonding interactions as depicted in Fig. 4. Compound 12 establishes H-bonding interactions with amino acid residues Ser-203 utilizing –OH group of aromatic ring, Tyr-337 using sulfonamide oxygen and aromatic ring and with His-447 through –OH group of aromatic ring. Trp-86 shows various types of interactions with this compound including π-π-stacked interaction, π-sulfur interactions

with sulfonamide S, as well as π-sigma interactions with aromatic ring. This compound also shows hydrophobic π-π T-shaped interactions with PHE-338 and hydrophobic π-alkyl interactions with Trp-439, Tyr-449, Tyr-337 and Trp-86. Moreover, amide π-stacked interactions are being shown by this compound utilizing Gly-121 and Gly-122.

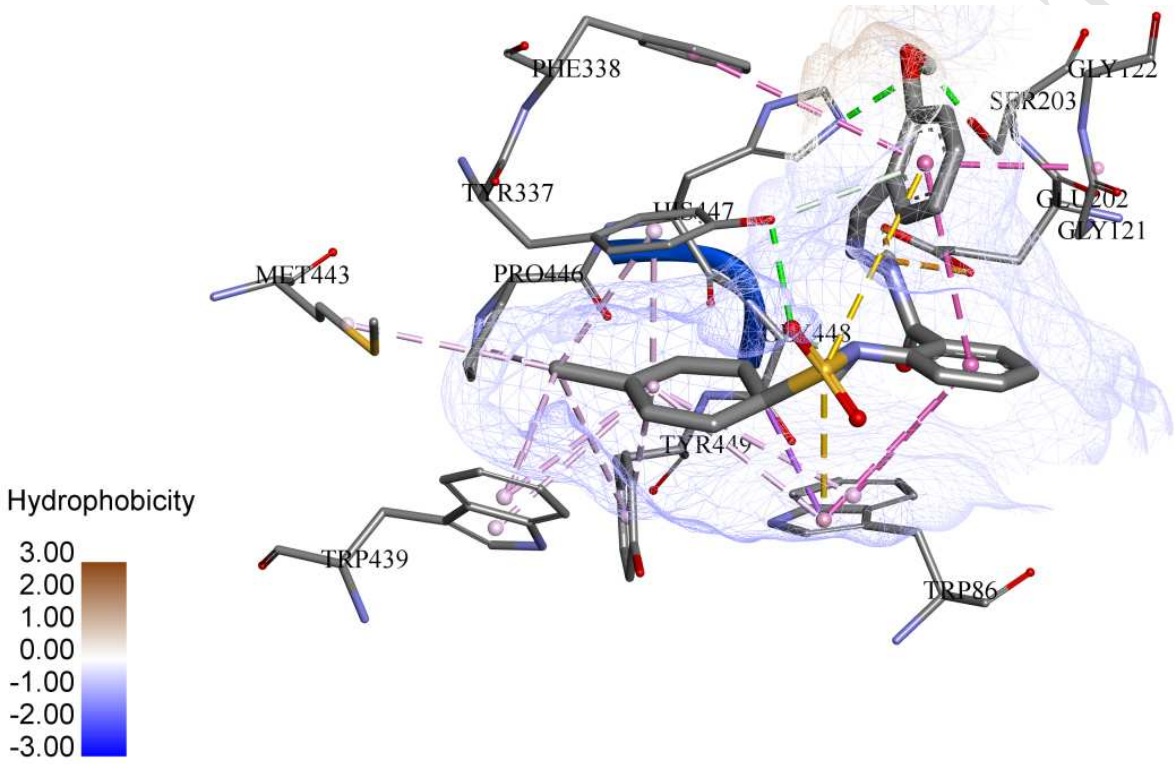


Fig. 4. Putative binding approaches of compound 12 inside AChE enzyme.

Similarly, the conception of the compound 12 in the active pocket of BChE enzyme unveiled many significant interactions. The compound 12 forms H-bonding interactions with amino acid residue of catalytic triad of BChE, His-438 using Hydrogen atom of Hydroxyl group and with Thr-120 by utilizing sulfonamide oxygen atom. It also depicts π-alkyl interaction with amino acids Trp-231 and Phe-398 using Cl atom. The catalytic amino acid Leu-286 shows hydrophobic alkyl interactions. Π-π T-shaped interactions are shown by Trp-82 and Phe-329. Phenyl ring of the compound 12 also showed amide-pi stacking interactions with Gly-115 and Gly-116 amino acid residues of BChE. This compound also makes π-sigma type of interactions with amino acid Ala-328. Glu-197 amino acid residue of BChE shows electrostatic interactions with electron deficient nitrogen of hydrazide portion. The assumed binding type of the compound 12 can be found in Fig. 5.

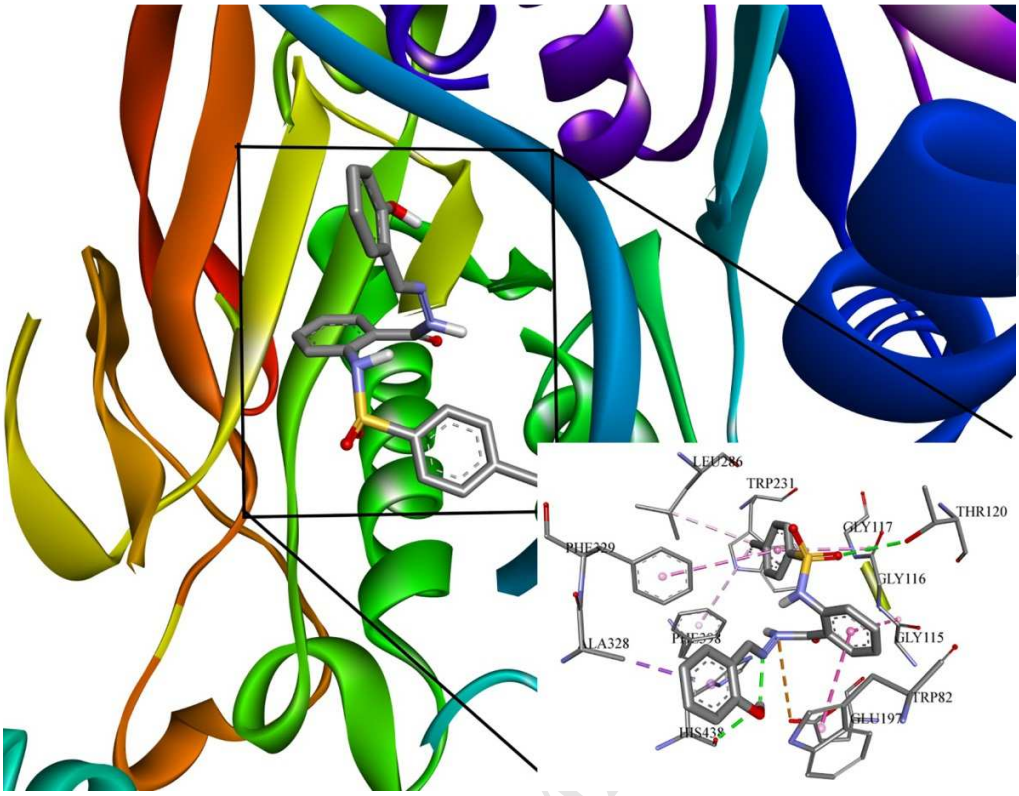


Fig. 5. Interactions of the compound 12 with BChE at 3D space; Interactions with specific amino acid residues are shown in the box. The 3D ribbon represents the enzyme; stick model is the lowest energy conforms of the inhibitor 12 along with amino acids of BChE interacting with it.

Moreover, conception of the active compound 08 depicts H-bonding interactions with amino acid residues of anionic sub site of AChE, as hydroxyl group of aromatic ring establishes H-bonding with 'O' atom of Trp-86. Similarly, H-bond interactions are shown by this compound with amino acid residues Tyr-337 using aromatic ring and Tyr-341 using sulfonamide oxygen. This derivative shows π alkyl type interactions with Met-443, Tyr-449, Trp-439, Tyr-337, Pro-446 and Trp-86 of AChE. Trp-86 and Trp-439 residues of AChE show π-sigma interactions with under observed compound. Tyr-341 establishes π-sulfur interactions with sulfur atom of sulfonamide portion of the compound. Glu-202 and Trp-86 residues of AChE show electrostatic π- cation type interaction with partial positively charged nitrogen of hydrazide portion of the displayed compound as portrayed in Fig. 6.

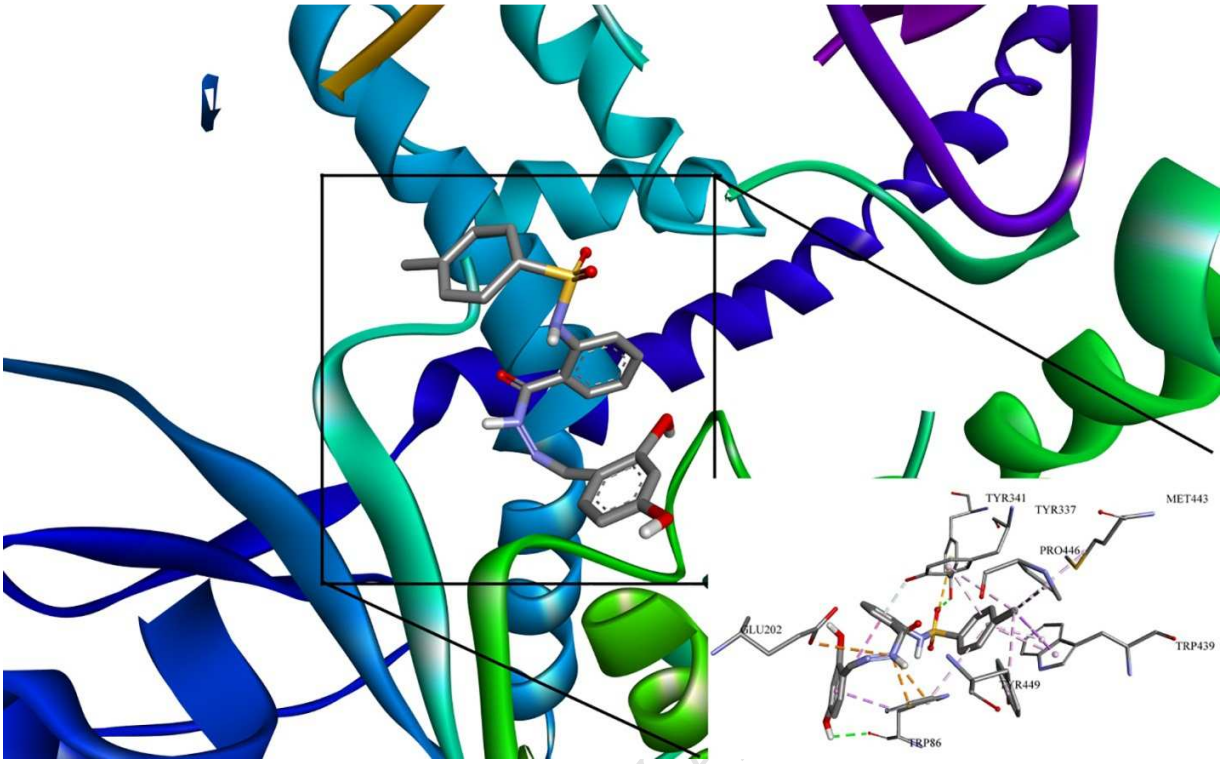


Fig. 6. Interactions of the compound 08 with AChE at 3D space; Interactions with specific amino acid residues are shown in the box. The 3D ribbon represents the enzyme; stick model is the lowest energy conforms of the inhibitor 08 along with amino acids of AChE interacting with it.

The visualization of one of the compounds 08 inside active pocket of BChE enzyme revealed many important bonding linkages. This compound develops H-bonding interactions with catalytic amino acid residues His-438, Ser-198, Leu-286 and Gly-116. Amino acid residue Tyr-332 also develops π-sigma interaction with this compound. This compound also forms π-π stacked interactions with amino acid residues, His-438, Tyr-332 and Trp-82 and Phe-329 using its aryl ring. The presumed bonding approach of this compound is presented in Fig. 7.

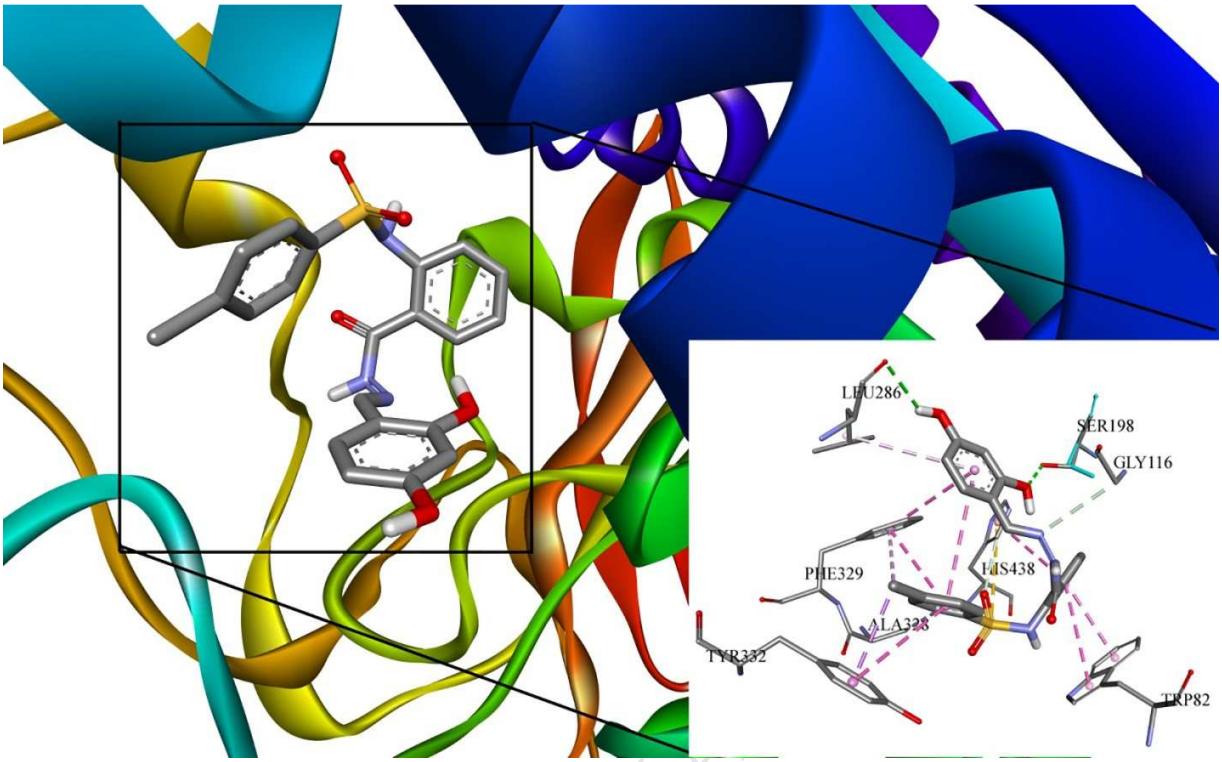


Fig. 7. Interactions of the compound 08 with BChE at 3D space; Interactions with specific amino acid residues are shown in the box. The 3D ribbon represents the enzyme; stick model is the lowest energy conforms of the inhibitor 08 along with amino acids of BChE interacting with it.

Similarly, docking evaluation of compound 07 against AChE showed many different types of associations. Gly-126 shows hydrogen bonding with oxygen of the nitro group. Similarly Trp-86 shows hydrogen bonding with sulfonamide oxygen atom, Tyr-337 also shows H-bonding with aromatic ring of ligand 07. This compound also shows π-alkyl interactions with Tyr-449 and Tyr-337. Amino acid residues Trp-439 and Tyr-337 exhibit hydrophobic π-π stacked bonding interactions with the under study compound. Additionally, this shows π –π T-shaped interaction with Trp-86 amino acid residue. Π-sulfur type of associations is shown by Met-443 with ligand 07. Amide π-stacked interactions are shown by Gly-120 and Gly-121 with aromatic ring of the compound. Glu-202 and Trp-86 show electrostatic type of interactions with partially positive nitrogen of the hydrazide part of the ligand. The bonding mode of compound 07 has been exposed in Fig. 8.

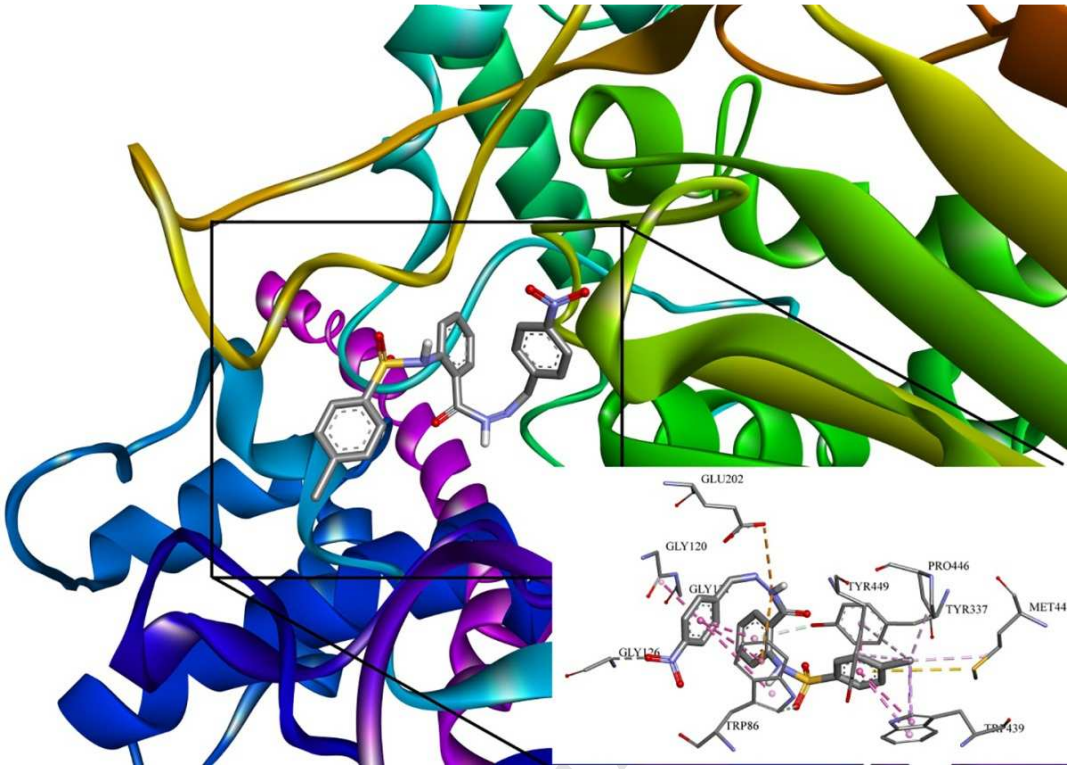


Fig. 8. Interactions of the compound 07 with AChE at 3D space; Interactions with specific amino acid residues are shown in the box. The 3D ribbon represents the enzyme; stick model is the lowest energy conforms of the inhibitor 07 along with amino acids of AChE interacting with it.

In continuation to our earlier discussion, it is found that one of the active compounds, compound 07 showed several significant interactions inside active pocket of BChE enzyme. It forms H-bonding type interactions with amino acid residues Asp-70, Tyr-32, Ala-199, Gly-116, Gly-117 and Pro-285. π - Alkyl interactions are also shown by compound 07 with amino acid residues His-438, Ala-328 and Trp-82. Trp-82 residue also forms π-sigma interactions with chlorine atom attached to aromatic ring. Phe-329 and Trp-82 are also involved in π-π-T-shaped interactions. All the interactions of compound 07 with active pocket of BChE are shown in Fig. 9.

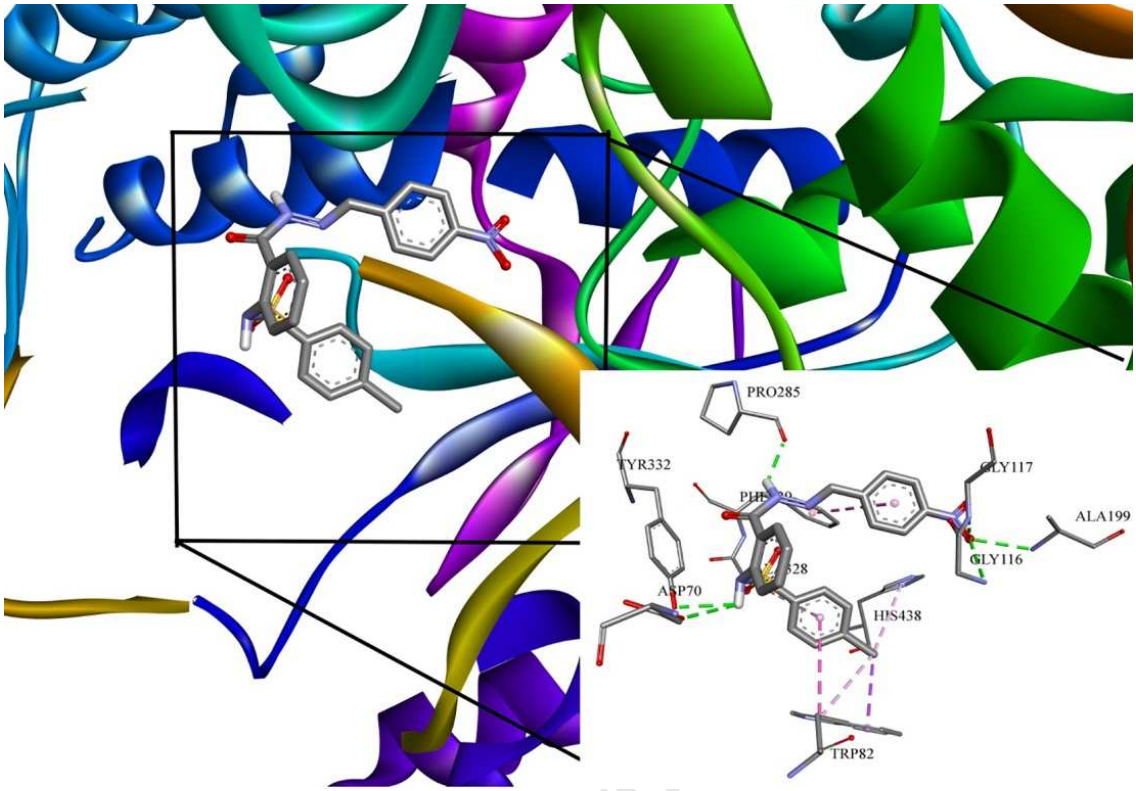


Fig. 9. Interactions of the compound 07 with BChE at 3D space; Interactions with specific amino acid residues are shown in the box. The 3D ribbon represents the enzyme; stick model is the lowest energy conforms of the inhibitor 07 along with amino acids of BChE interacting with it.

3.5. Conclusion

In conclusion, efficient synthetic method, characterization, antioxidant potential and enzyme inhibition assay against AChE and BChE of anthranilic acid based sulfonamides has been described. Sulfonamide derivative was firstly synthesized starting from methyl anthranilate, which was further derivatized to give hydrazide based Schiff bases. These synthesized compounds were then evaluated for antioxidant and enzyme inhibition potential against AChE and BChE. The results showed that most of the compounds showed good enzyme inhibition activity against AChE and BChE. The experimental findings were further supported by molecular docking studies. The molecular docking results are coinciding with the experimental results. These findings therefore encouraged to synthesize new hybrid compounds having Schiff base and sulfonamide moieties together which can be optimized to be used as potent AChE and BChE inhibitors.

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- The sulfonamides bearing hydrogen donating groups showed good Antioxidant activities.
- Enzyme inhibition activity against AChE and BChE showed excellent results due to the ability of these compounds to form H-bondings in the active pockets of enzymes.
- *In-silico* molecular docking studies also supported the experimental results for enzyme inhibition.