Scientific paper

Sulfonamide Derived Esters: Synthesis, Characterization, Density Functional Theory and Biological Evaluation through Experimental and Theoretical Approach

Muhammad Danish,[1,*] Ayesha Bibi,[1] Muhammad Asam Raza,[1,*] Nadia Noreen,[1] Muhammad Nadeem Arshad[2] and Abdullah Mohamed Asiri[2]

[1] *Department of Chemistry, University of Gujrat, Gujrat 50700 Pakistan*

[2] *Chemistry Department, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia*

* *Corresponding author: E-mail: drdanish62@gmail.com and asamgcu@yahoo.com*

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Abstract

A series of new solid esters was synthesized by using greener chemistry strategy involving simple reaction of an alcohol with sulfonamide ligand. Characterization study of these methyl (**1**), ethyl (**2**) isopropyl (**3**) and *n*-butyl (**4**) ester of 4-((4-chlorophenylsulfonamido)methyl)cyclohexanecarboxylic acid was done by using FTIR, NMR mass spectrometry and X-ray crystallography. The compounds were optimized with Gaussian software according to basis set B3LYP/6-31G(d,p) and their different parameters related to structure were calculated. Furthermore, all compounds of the series were screened for their *in vitro* biological applications involving anti-bacterial (*Chromohalobactor salixgens*, *Halomonas halofila*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Shiegella sonnei*), anti-fungal (*Aspergillus niger*), anti-oxidant (DPPH scavenging activity) and enzyme inhibition (acetylcholine esterase and butyrylcholine esterase) study. Sulfonamide based esters were also docked against selected enzymes (AChE and BChE) using MOE software for their mode of binding. Results obtained from these biological evaluations showed that such compounds have potential against targeted activity.

Keywords: Sulfonamide derived esters; DFT; docking studies; enzyme inhibition.

1. Introduction

Infectious diseases due to bacteria or fungi are the major leading causes of morbidity all over the world.[1–4] The development of resistance (antibacterial) in microbes against the present antibiotics is growing now on a daily basis.[5] Consequently, there is an urgent need in this area for new and improved antimicrobial agents having a broad-spectrum activity against the resistant strains. Researchers throughout the world are engaged in synthesizing and designing new drugs having widespread activity to overcome this issue.[6–9] Sulfonamides form the foundation for the first drugs mainly employed as preventive and chemotherapeutic agents against different ailments.[10] Sulfa drugs, having sulfonamide functionality, revolutionized the medicinal field due to their extensive biological activities.[11] Folic acid, an important compound for synthesis of bacterial nucleic acids, is inhibited by sulfonamides which ultimately leads to death of bacterial cells. Green chemistry synthesis is the current requirement as it has no hazardous by-products. Carboxylate ester formation implemented simply by reacting alcohol with carboxylic acid is such an example. Besides their applications in artificial flavoring of food and in perfume making,[12] esters of various compounds are found to show promising biological applications. Variety of carboxylate esters derived from sulfonamides are found to serve in the field of health by giving meritorious biological applications. Ester derived from *para*-tolylbenzene sulfonamide of benzoic acid has been found to show inhibition activity for enzyme lipoxygenase. Carboxylate ester derived from biphenyl sulfonamide is found to show inhibitory behavior toward carbonic anhydrase enzymes as well as its isozymes, including isozymes I, II, XIV, XII and XIV.[13] Moreover, for the treatment of obesity and to control Type 2 diabetes, esters derived from arylsulfonamide served to give such applications.[14] As anti-microbial agents, esters of *N*-substituted sulfonamide are found to give anti-bacterial activity against four bacterial strains, *i.e*. *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus au-*

reus.[15] Due to the presence of amide and sulfonamide functional groups in carboxylate esters of sulfonamide derivatives, they are found to show anti-fungal activity against *Aspergillus niger* and *Candida albicans*.[16]

In current research work new methyl, ethyl, isopropyl and *n*-butyl esters derived from 4-((4-chlorophenylsulfonamido)methyl)cyclohexanecarboxylic acid were aimed to be synthesized. *In vitro* biological study, *i.e.* enzyme inhibition, anti-oxidant study, anti-bacterial and anti-fungal screening were also part of this research work.

2. Experimental

2. 1. Chemical and Instruments

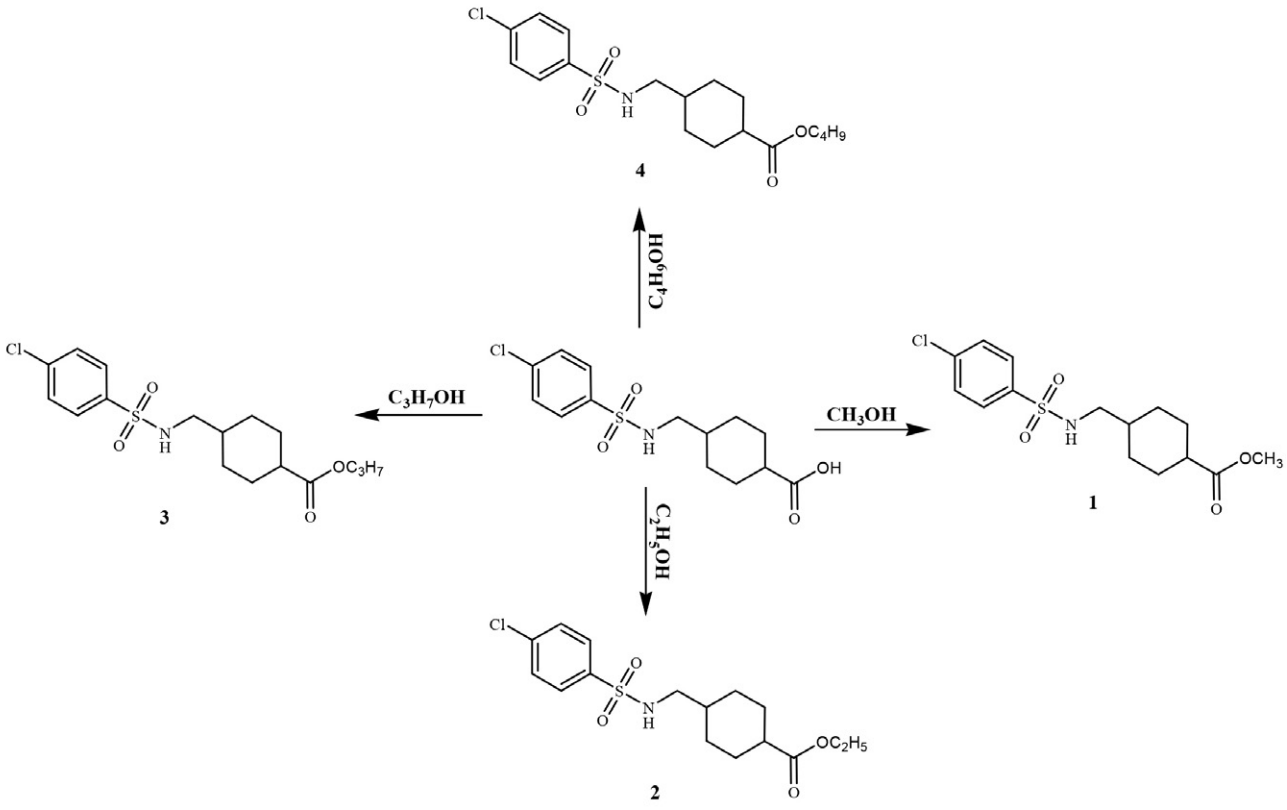
Chemicals like dimethyl sulfoxide, methanol, ethanol, isopropanol and *n*-butanol were purchased from Alfa Aesar and Merck chemical industries. These were of analytical grade. Infrared spectral study of compounds was done in mid IR region (4000–400 cm^{-1}) by KBr disc method using Perkin-Elmer System 100. Mass spectral study was done by ESI-MS technique.

2. 2. Synthesis of Carboxylate Esters of Sulfonamide

20 mL alcohol (methanol for **1**, ethanol for **2**, isopropanol for **3** and *n*-butanol for **4**) was taken and then added 1 mL conc. H_2SO_4 as a catalyst into it. 0.5 g of 4-((4-chlorophenylsulfonamido)methyl)cyclohexanecarboxylic acid (published by our research group) was added in 20 mL alcohol till clear solution was obtained. Then added this alcoholic solution to alcohol-sulfuric acid mixture and refluxed it for about 4–6 hours. Reaction mixture was concentrated at room temperature by slow evaporation process and ester as a solid product was obtained (Scheme 1).[17]

2. 3. Crystallography of 3

The compound **3** was re-crystallized to support the synthesis of series of compounds being presented in this manuscript. Microscope was used for screening of suitable crystal for data collection. The selected single crystal was fixed over a glass fiber tip fascinated in a wax supported by a hollow copper rod with magnetic base. This holder was mounted on Agilent SuperNova (Dual source) Agilent Technologies Diffractometer, equipped with graphite-monochromatic Cu/Mo Kα radiation for data collection. The data collection was accomplished using CrysAlisPro software[18] at 296 K under the Mo Kα radiation. The structure solution was performed using SHELXS-97[19] and refined by full-matrix least-squares methods on F^2 using SHELXL-97 in-built with WinGX.[20] All non-hydrogen atoms were refined anisotropically by full-matrix least-squares methods.[19] Figures were drawn using PLATON



Scheme 1. Synthesis of carboxylate esters of sulfonamide

and *ORTEP*-3.[20] All the aromatic CH hydrogen atoms were positioned geometrically and treated as riding atoms where C–H = 0.93 Å and U_{iso}(H) = 1.2U_{eq}(C) for carbon atoms. The C–H bond distances are 0.96 Å, 0.97 Å and 0.98 Å for methyl, methylene and methine groups, respectively. U_{iso}(H) = 1.5U_{eq}(C) for methyl carbon atoms, while U_{iso}(H) = 1.2U_{eq}(C) for methylene and methine carbon atoms. The N–H = 0.68(7)–0.94(4) Å, hydrogen atoms were located with difference Fourier map and refined with U_{iso}(H) = 1.2U_{eq}(N). The assigned CCDC number is 1861455.

2. 4. Biological Studies

Antibacterial Activity

Antibacterial activity was determined according to the disc diffusion method against four bacterial strains: *Chromohalobacter salexigens, Chromohalobacter israelensis, Halomonas halofila* and *Halomonas salina*.[21] Bacterial medium was prepared and autoclaved for 20 min at 121 °C and 15 psi. 30 mL of the sterilized medium was poured in the petri plates and seeded with respective bacterial strains. 20 µL of sample (5 mg/mL) was applied on disks with the help of a micropipette. Streptomycin and ampicillin were used as reference drugs while solvents were used as negative. After incubation of 24 hours at 37 °C, the zone of inhibition was measured.

Antifungal Activity

Antifungal activity was determined against two different fungal strains: *Aspergillus flavus* and *Aspergillus niger* by using the method of Samina *et al.* (2009) with minor modification.[21] Sterilized medium of 30 mL was poured aseptically in autoclaved petri plates and seeded with the respective fungal strain. After the solidification of the medium disks were placed on it and 20 µL of sample (5 mg/mL) was applied on each disc. The plates were incubated at 25 °C and the zone of inhibition was measured with Vernier caliper after 48 hours.

Antioxidant Activity

Antioxidant activity of synthesized compounds was checked according to the method of Shahwar *et al.* (2012) using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical.[22] DPPH solution was prepared as 0.0025 g/mL in methanol and 100 µL of sample (5 mg/mL) was mixed with 2 mL DPPH solution. Test tubes were kept in dark for half an hour and measured the absorbance at 517 nm using methanol as blank and gallic acid as reference standard. The scavenging of free radicals was calculated using following formula:

$$\% \text{ Inhibition } = = \frac{\text{Absorbance (blank) - Absorbance (test)}}{\text{Absorbance (blank)}} \times 100 \quad (1)$$

Enzyme Inhibition Studies

The AChE and BChE inhibition activities were determined according to the method of Ellman *et al.* (1961)[23] with slight modifications. 100 µL test compound (5 mg/mL) was mixed with 100 µL enzyme (AChE and BChE) and incubated at 37 °C for 10 minutes. After incubation, 0.5 mL buffer (50 mM), 50 µL DTNB followed by the addition of 50 µL substrate acetylthiocholine iodide and butyrylthiocholine iodide for AChE and BChE, respectively. After 30 minutes of incubation at 37 °C, the absorbance was measured at 410 nm using UV/VIS spectrophotometer. All experiments were carried out with their respective controls in triplicate.

The percentage inhibition was calculated by the following formula:

$$\% \text{ age inhibition } = \frac{A - B}{A} x100 \quad (2)$$

Where A is the optical density of blank and B is the optical density of sample.

2. 5. DFT Studies

Quantum chemical calculations were performed with Gaussian 09. The results are visualized with Gauss View 5.0. The geometries of the compounds are optimized without any symmetry constraints using the hybrid functional B3LYP method with 6-31G(d,p) basis set.[24,25] The basis set chosen contains polarization functions on all atoms. The B3LYP method of DFT is quite reliable for the prediction of geometric and electronic properties of neutral and charged species ranging from simple molecular to polymer structures.[26–28] For optimization, the input geometries are taken from the crystal structure (where available) in order to better match with the experimentally obtained structures. Frequency calculations are also performed at the same level in order to confirm these structures as true minima (absence of an imaginary frequency).

2. 6. Docking Studies

Docking experiments were performed *via* Molecular Operating Environment (MOE). Crystal structures of AChE and BChE with PDB codes 1EVE and 1POI, respectively, were selected for these studies. All the water molecules were removed from the protein structure, then hydrogen atoms were added and energy optimization was carried out using default force field. The three-dimensional (3D) structures of compounds were modeled through the builder program implemented in MOE. The geometrical parameters for 3D structures of the compounds were optimized, and partial charges were calculated before docking. The 3D protonation of the downloaded enzymes was done and energy minimization of the retrieved protein molecule was carried out using default parameters of

MOE energy minimization algorithm (gradient: 0.05, Force Field: MMFF94X). The resulting model was subjected to systematic conformational search at default parameters with RMS gradient of 0.01 kcal/mol using Site Finder. For 1EVE (AChE), the active site of the prepared enzyme was defined as the residues within 10 Å of the reference ligand (donepezil). However, for 1POI (BChE), the enzyme was searched for its active site and dummy atoms were created using alpha spheres as centroids. A key tryptophan residue in AChE, Trp84 (TcAChE numbering), is conserved in BChE (Trp82). The backbone and residues were kept fixed and the energy minimization was performed. The lowest energy minimized pose was used for further analysis. Ligand-interaction module of MOE was used to calculate the 2D ligand-enzyme interactions. The view of the docking results and analysis of their surface with graphical representations were done using MOE and discovery studio visualizer.[29]

3. Results and Discussion

The reaction was carried out in acid catalyzed media by simply reacting alcohol with sulfonamide ligand. The reaction gives water as by-product, hence the strategy is a type of green synthesis which is advantageous and environmentally friendly having no hazardous or harmful effects on the environment. The structure and activity of the starting material (acid) has been already published by our group.[30] The reaction was monitored with TLC and after completion of the reaction, structure elucidation was done with FTIR, mass spectrometry and NMR. The exact crystal structure of **3** was confirmed with XRD analysis.

3. 1. Methyl 4-((4-Chlorophenylsulfonamido) methyl)cyclohexanecarboxylate (1)

White amorphous solid; yield: 1.74 g (76%); mp: 96–97 ºC; molecular formula: $C_{15}H_{20}ClNO_4S$; molecular mass: 345.84 g mol^{-1}; IR (KBr, cm^{-1}): ν_{max} 3270 (NH), 2922 (CH), 1320–1158 (SO_2), 1699–1432 (C=O); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.65–7.80 (dd, 4H, aromatic), 3.56 (s, 3H, -CH_3), 2.59 (t, 2H, -CH_2), 0.79–2.24 (m, 10H, cyclohexyl); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 175.8 (C-2), 140.0 (C-4'), 137.5 (C-1'), 129.8 (C-3' and C-5'), 128.8 (C-2' and C-6'), 51.7 (C-1), 48.8 (C-3), 42.6 (C-1"), 37.1 (C-4"), 29.4 (C-2" and C-6"), 28.5 (C-3" and C-5"); EI-MS: *m/z* 344.25 [M – 1].

3. 2. Ethyl 4-((4-Chlorophenylsulfonamido) methyl)cyclohexanecarboxylate (2)

Lustrous white amorphous; yield: 0.81 g (47%); mp: 120–122 °C, molecular formula: $C_{16}H_{22}ClNO_4S$; molecular mass: 359.87 g mol^{-1}; IR (KBr, cm^{-1}): ν_{max} 3298 (NH), 2934 (CH), 1321–1159 (SO_2), 1727 (C=O). ^{1}H NMR ($CDCl_3$): δ 7.5–7.8 (dd, 4H, aromatic), 4.8 (t, 3H, -CH_3), 4.1 (q, 2H, -CH_2), 1.0–2.2 (m, 10H, cyclo-H); ^{13}C NMR ($CDCl_3$): δ 175.7 (C-3), 139.1 (C-4'), 138.5 (C-1'), 129.4 (C-3' and C-5'), 128.5 (C-2' and C-6'), 60.3 (C-1), 49.1 (C-3), 43.1 (C-1"), 37.2 (C-4"), 29.5 (C-2" and C-6"), 28.2 (C-3" and C-5"), 14.2 (C-2); ESI-MS: *m/z* 358.25 [M – 1].

3. 3. Isopropyl 4-((4-Chlorophenylsulfonamido)methyl)cyclohexanecarboxylate (3)

Lustrous white crystalline; yield: 1.97 g (84%); mp: 111–113 °C, molecular formula: $C_{17}H_{24}ClNO_4S$; molecular mass: 373.89 g mol^{-1}; IR (KBr, cm^{-1}): ν_{max} 3263 (NH), 2924 (CH), 1320–1158 (SO_2), 1730–1432 (C=O), ESI-MS: *m/z* 372.25 [M – 1].

3. 4. Butyl 4-((4-chlorophenylsulfonamido) methyl)cyclohexanecarboxylate (4)

Off-white amorphous solid; yield: 1.21g (59%); mp: 106–108 °C; molecular formula: $C_{18}H_{26}ClNO_4S$; molecular mass: 387.92 g mol^{-1}; IR (KBr, cm^{-1}): ν_{max} 3267 (NH), 2931 (CH), 1323–1159 (SO_2), 1725–1431 (C=O); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.65–7.80 (dd, 4H, aromatic), 4.00 (t, 2H, H-4), 2.58 (t, 2H, H-6), 1.54–2.21 (m, 10H, cyclohexyl), 0.87 (t, 3H, H-1), 1.20 (m, 2H, H-2), 1.47 (m, 2H, H-3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 175.4 (C-5), 140.0 (C-4'), 137.5 (C-1'), 129.8 (C-3' and C-5'), 128.8 (C-2' and C-6'), 63.8 (C-4), 48.9 (C-5), 42.8 (C-1"), 37.1 (C-3), 30.6 (C-4"), 29.4 (C-2" and C-6"), 28.5 (C-3" and C-5"), 19.0 (C-2), 14.0 (C-1); ESI-MS: *m/z* 386.33 [M – 1].

3. 5. Crystallography of 3

Molecule **3** is ornamented with the methyl, methylene, methine and aromatic hydrogen atoms along with the NH group. We have observed two independent molecules [(C1–C17) and (C18–C34)] per asymmetric unit cell (Figure 1). The crystallographic parameters are given in Table 1, while the selected bond lengths and bond angles are provided in Supplementary Data (Tables S1 and S2). The cyclohexane ring adopted the chair conformation in each independent molecule and the root mean square (r.m.s) deviations for the fitted atoms of this ring are 0.2342(4) Å and 0.2298(4) Å in molecule 1 and molecule 2, respectively. The puckering parameters were determined for the cyclohexane rings in each independent molecule and the parameters in black and white are Q = 0.5738, θ = 0.69 and φ = 22.3039 for the ring II (C8–C13), while Q = 0.5628, θ = 1.55 and φ = 40.732 for the ring IV (C25–C30). The geometry around the S atom is distorted tetrahedral which is typical behavior of sulfonamide functional group.[31–34] The dihedral angle between the aromatic ring I (C1–C6) and cyclohexane ring II (C8–C13)

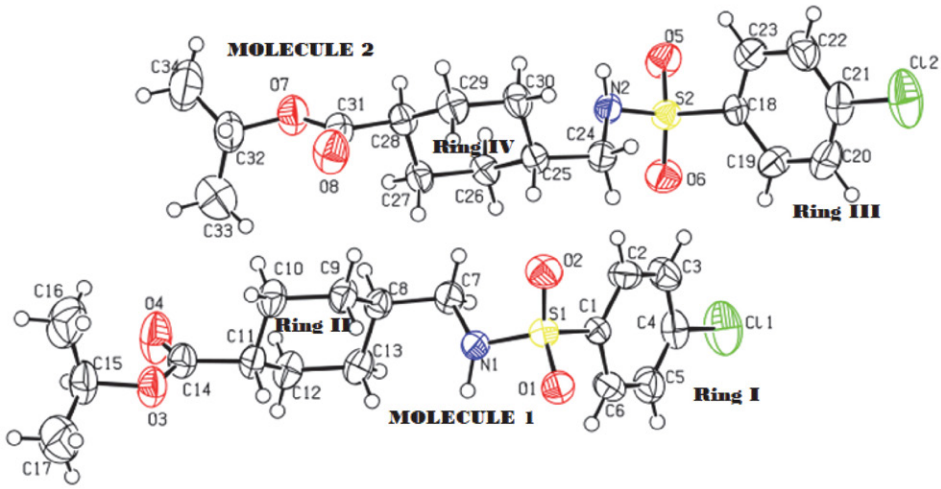


Figure 1. *ORTEP* diagram of **3** with thermal ellipsoids were drawn at 50% probability level

is 44.649(3)° but the same angle between the ring III (C18–C23) and ring IV (C25–C30) is 45.663(3)°. The carboxylate and the isopropyl groups are almost perpendicular in each molecule as the dihedral angles are 78.772(7)° and 78.906(72)° in molecule 1 and molecule 2, respectively. The carboxylate groups are twisted at dihedral angles of 68.738(3)° and 60.883(4)° with respect to the plane produced from the fitted atoms of cyclohexane rings in molecule 1 and molecule 2, respectively. Classical hydrogen bonding of N–H···O type connects the molecule along *b*-axis to generate the infinite long chains. The N1 acts as donor *via* H1N to the O1 following the symmetry operation 1 – *x*, ½ + *y*, –*z*. The N2 acts as donor in the molecule 2 *via* H2N to O5 oxygen atom, where the symmetry operation is 1 – *x*, ½ + *y*, 1 – *z* as shown in Figure 2, Table 2.

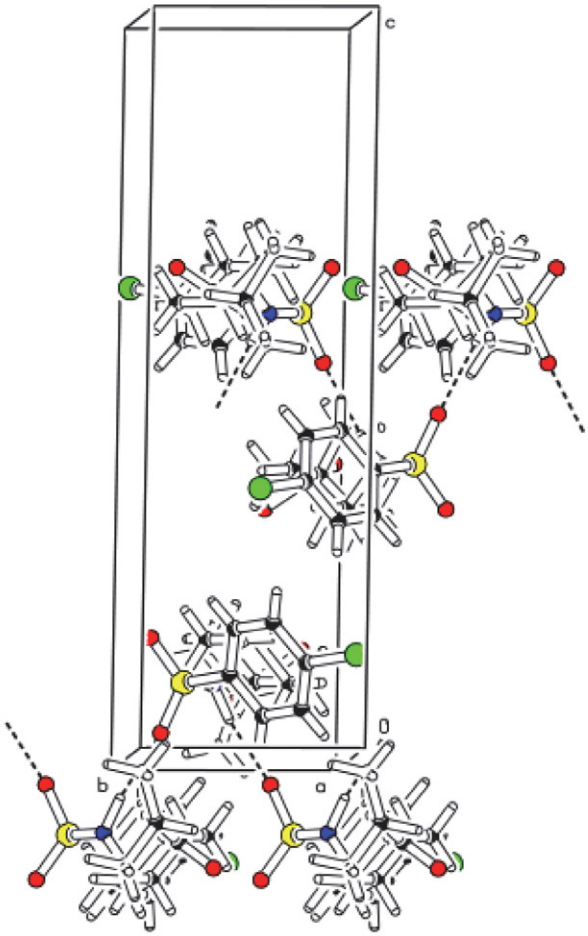


Figure 2. A unit cell view for **3** showing the intermolecular hydrogen bonding and formation of long chains along *b*-axis.

Table 1. Crystal data and structure refinement for **3**

CCDC number	1861455
Empirical formula	$C_{17}H_{24}ClNO_4S$
Formula weight	373.88
Temperature/K	296(2)
Crystal system	monoclinic
Space group	$P2_1$
a/Å	16.9426(13)
b/Å	5.8454(3)
c/Å	20.5910(18)
α/°	90
β/°	112.829(9)
γ/°	90
Volume/Å^3	1879.5(3)
Z	4
ρ_{calc}mg/mm^3	1.321
μ/mm^{-1}	0.334
F(000)	792.0
Reflections collected	11000
Independent reflections	7495[R(int) = 0.0379]
Data/restraints/parameters	7495/1/442
Goodness-of-fit on F^2	1.026
Final R indexes [I ≥ 2σ (I)]	R_1 = 0.0729, wR_2 = 0.1776
Final R indexes [all data]	R_1 = 0.0986, wR_2 = 0.2039
Largest diff. peak/hole / e Å^{-3}	1.11/–0.31
Flack Parameters	0.02(12)

Table 2. Hydrogen Bonds for **3**

D	H	A	*d*(D–H)/Å	*d*(H–A)/Å	*d*(D–A)/Å	D–H–A/°
N1	H1N	O1[i]	1.01(6)	2.02(6)	2.992(6)	161(5)
N2	H2N	O5[ii]	0.87(6)	2.12(6)	2.977(6)	169(6)

[i] 1 – *x*, –½ + *y*, –*z* [ii] 1 – *x*, ½ + *y*, 1 – *z*

3. 6. Antimicrobial Studies

Bacteria are necessary for life function but pathogenic bacterial species are major cause of various infections in human bodies. For the treatment of infections, different antibiotics are being sold in the market and one among them is sulfa drug. Our group is also synthesizing different sulfonamide compounds and evaluating their antibacterial potential using *in vitro* model. Here we aimed to check the anti-bacterial potential of **1, 2, 3** and **4** against six bacterial strains, *i.e. C. salixgens*, *H. halofila, S. aureus, B. subtilis, S. sonnei, E. coli.* Results obtained showed that except *C. salixgens*, all bacteria were inhibited by synthesized compounds. *Aspergillus niger* (*A. niger*) is the fungus that causes aspergillosis; a lung disease, for the person having extremely weak immune system.[35] It is also a common cause of fungal ear infection known as otomycosis in tropical areas.[36] For the treatment of these diseases, synthesis of anti-fungal agents is obvious. In current research work compounds **1, 2, 3** and **4** were screened for their anti-fungal potential against *A. niger*. It was found from results that **2** and **3** are more active against fungal strain (see Table 3).

3. 7. Antioxidant Potential

Reactive oxygen species (ROS) are free radicals involving hydrogen peroxide, hydroxyl ion, superoxide, hydrogen peroxide and hydroxyl free radical (·OH, OH^-, $\cdot O_2^-$, $\cdot O_2^{-2}$, H_2O_2). Diseases caused by the overproduction of ROS in body involve chronic inflammation and autoimmune diseases, infectious diseases, cancer, sensory impairment, neurological disorders, fibrotic and cardiovascular diseases.[37] In order to treat the mentioned health disorders, use of antioxidants is imperative. The antioxidant potential of all synthesized esters has been evaluated using standard protocols and results are presented in Table 4. All compounds exhibited moderate to good activity except **1** (24.1±0.4), furthermore maximum radical scavenging activity was shown by **3** comparable to standard.

3. 8. Acetylcholine/Butyrylcholine Esterase Study

Acetylcholine esterase (AChE) is mainly involved in the transmission of neurotransmitter acetylcholine in brain. AChE hydrolyzes the acetylcholine into choline and acetate group. Over-activity of AChE causes deficiency of acetylcholine, hence leads to Alzheimer's disease (AD). In order to treat Alzheimer's disease, AChE activity must be inhibited. Butyrylcholine esterase also belongs to the same class of enzyme and is actively involved in Alzheimer's disease.[38] Our research group members are working on the synthesis of different compounds and evaluating their enzyme inhibition potential. This work is a continuation of our previous research, in which we aimed to check the inhibitory potential of **1, 2, 3** and **4** against both enzymes

Table 4. Enzyme inhibition and antioxidant potential of synthesized esters

Compound	Enzyme Inhibition (%)		Antiradical Scavenging (%)
	AChE	BChE	
1	47.3 ± 0.5	55.4 ± 0.7	24.1 ± 0.4
2	41.7 ± 0.9	32.2 ± 0.4	61.4 ± 1.2
3	60.4 ± 1.4	47.7 ± 1.1	77.9 ± 1.3
4	58.1 ± 1.1	54.5 ± 0.9	63.3 ± 0.9
Gallic acid	–	–	91.1 ± 0.9

Table 3. Antimicrobial potential of synthesized esters **1**–**4**

Compound	Zone of inhibition (mm) Bacterial strains						Fungal strain
	C. salixgens	*H. halofila*	*E. coli*	*S. aureus*	*B. subtilis*	*S. sonnei*	*A. niger*
1	NIL	12.4 ± 1.1	11.1 ± 0.5	10.0 ± 0.7	NIL	25.4 ± 0.7	NIL
2	NIL	7.3 ± 0.7	15.4 ± 0.4	12.1 ± 0.9	17.5 ± 1.1	30.1 ± 1.1	4.5 ± 0.1
3	NIL	13.7 ± 0.9	11.3 ± 0.8	12.4 ± 1.3	15.1 ± 0.8	12.5 ± 0.6	6.5 ± 0.3
4	NIL	14.1 ± 1.2	NIL	8.5 ± 0.8	NIL	15.3 ± 1.0	NIL
Ampicillin	NIL	15.6 ± 0.7	33.5 ± 1.1	39.2 ± 1.0	41.3 ± 1.3	31.5 ± 1.1	–
Fungone	–	–	–	–	–	–	30.2 ± 0.8

Chromohalobactor salixgens (*C. salixgens*), *Halomonas halofila* (*H. halofila*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus (S. aureus)*, *Bacillus subtilis (B. subtilis)*, *Shiegella sonnei (S. sonnei).*

Table 5. Docking results of synthesized esters

Compound	Docking Score			Binding Affinity (kcal/mol)		
	AChE	BChE		AChE	BChE	
	1EVE	1POI	4BDS	1EVE	1POI	4BDS
1	−10.2116	−9.8558	−11.6188	−6.9708	−5.2680	−5.4057
2	−10.9667	−10.5598	−11.2390	−6.5568	−5.4028	−4.7353
3	−12.6362	−10.8013	−12.4252	−6.5128	−5.0774	−4.7111
4	−11.7883	−10.4357	−12.1566	−7.3311	−5.6446	−5.5252

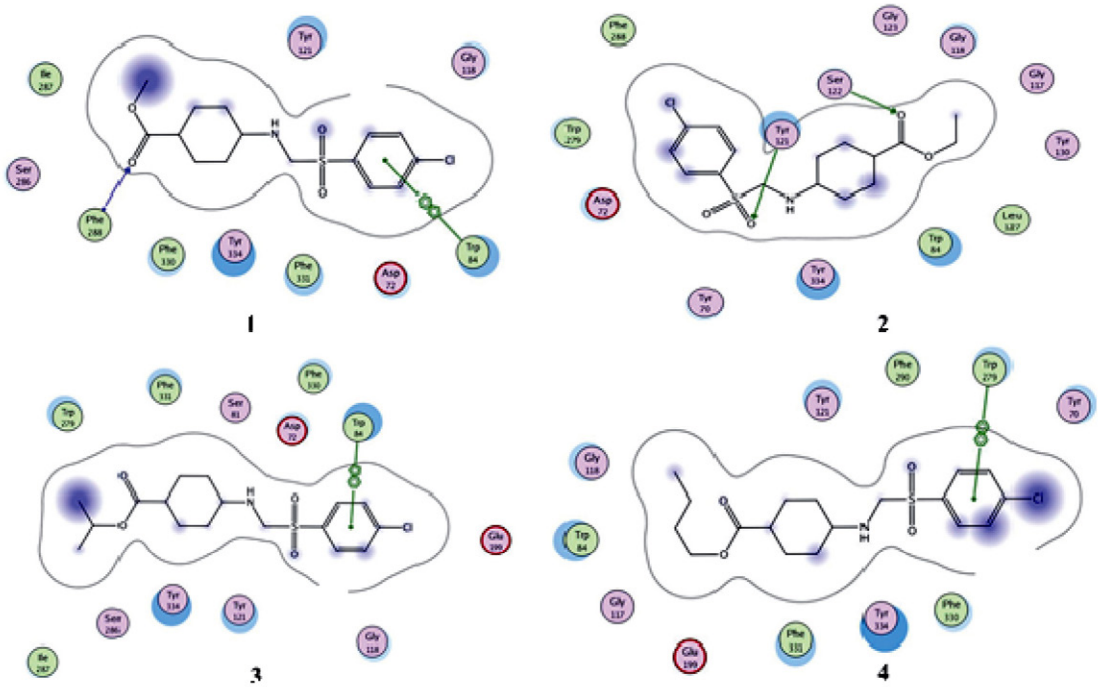


Figure 3. Interactions of synthesized esters with AChE (1EVE)

(AChE and BChE). As shown in Table 4, all the synthesized compounds gave moderate to good enzyme inhibition activity. The order of enzyme inhibition against AChE was found to be: **3** > **4** > **1** > **2**, while **1** > **4** > **3** > **2** against BChE.

All four synthesized esters **1**–**4** were docked with AChE (1EVE) and BChE (1POI and 4BDS) by downloading their respective PDB files from the internet source using MOE software. The solvent molecules were eliminated and structures of enzyme and compounds were minimized before docking. The interaction of molecules with different amino acid residues at the active site are shown in Figure 3. Esters showed different types of interactions with residues such as hydrogen bonding, van der Waals, π–π interaction; among these some are weak while others bind the inhibitor to the active site rigidly. In AChE, inhibitors (esters **1**–**4**) showed interactions with Phe288, Trp84, Tyr121, Ser122, Trp279, Phe290, Arg289, Phe331, Tyr334, His440, Asp72, Ser31, Tyr130, Gly123 and Gly118. Maximum interaction was demonstrated by **3** with docking score and binding affinity −12.6362, −6.5128, respectively, while others have close results (Table 5). Ester **1** interacts with Trp84 at anionic site, His440 at catalytic triad and Phe288 located at acyl pocket. Phen290, Trp84, Tyr334 and Phe288 are the amino acid residues exhibiting different interactions with **2**. In case of **3**, the amino acid residues at peripheral anionic site (PAS) Tyr70, Asp72, Tyr334, Tyr130 interacted with inhibitor, while Trp84 and Tyr130 located at anionic site also stabilized the molecule. The **4** ester also interacted with Trp84, Tyr121, Tyr 334 and His440 located at anionic, PAS and catalytic triad of the AChE (IEVE), as shown in Figure 4.

The synthesized inhibitors were also docked with BChE using PDB files; 1POI and 4BDS. It is evident from the results that **3** exhibited the highest binding score −10.8013 and −12.4252 against 1POI and 4BDS, respectively. The order (1POI) of remaining esters with respect to the binding score was **2** > **4** > **1**, while in 4BDS it was **4** > **1** > **2** as shown in Table 7. The inhibitors showed the interactions with Trp82, Thr120, Ser198, Gly116, Try332, His438, Gly117, Trp231, Val288, Glu197, Leu286 and Pro285 in the case of 1POI, while in 4BDS Trp82, Thr120, His438,

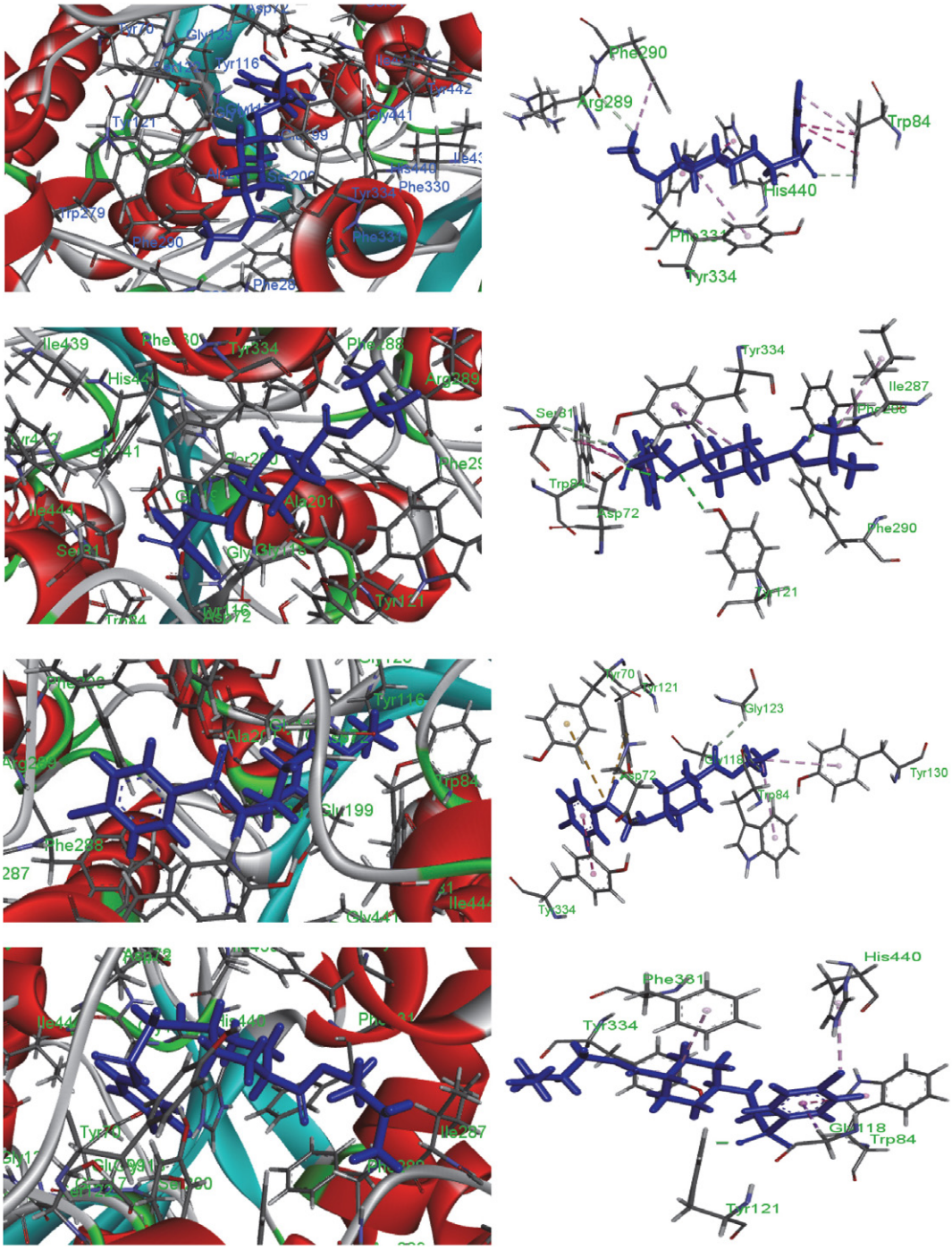


Figure 4. Best docking-poses of the synthesized compounds in the binding site of 1EVE

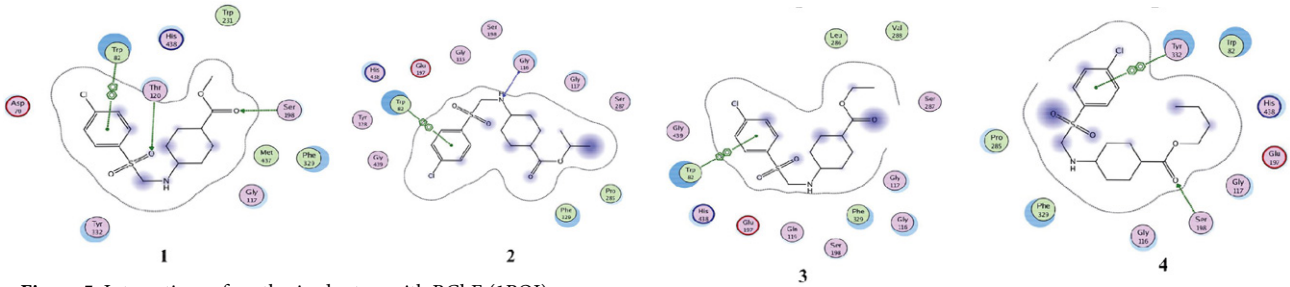


Figure 5. Interactions of synthesized esters with BChE (1POI)

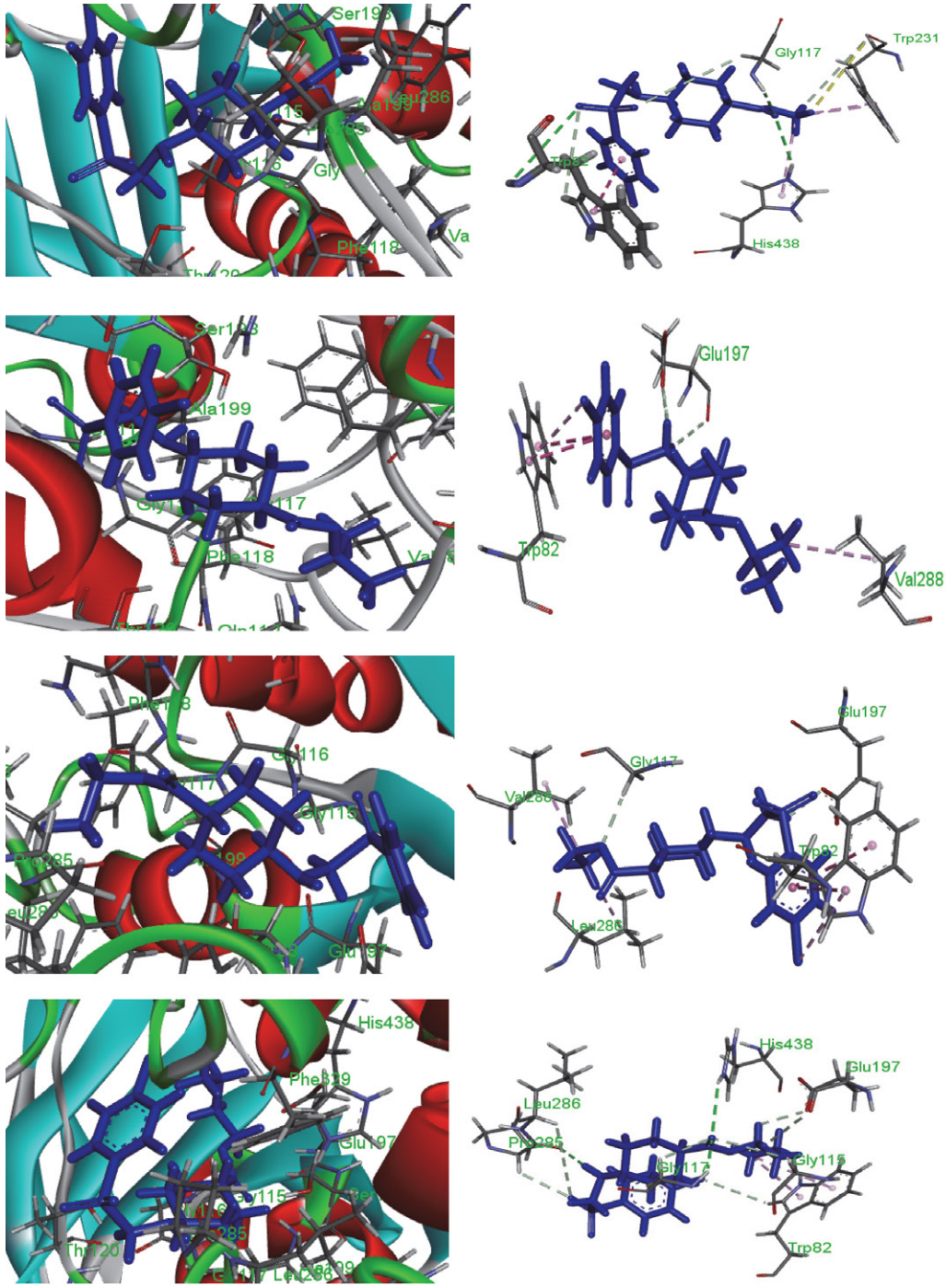


Figure 6. Best docking-poses of the synthesized compounds in the binding site of 1POI

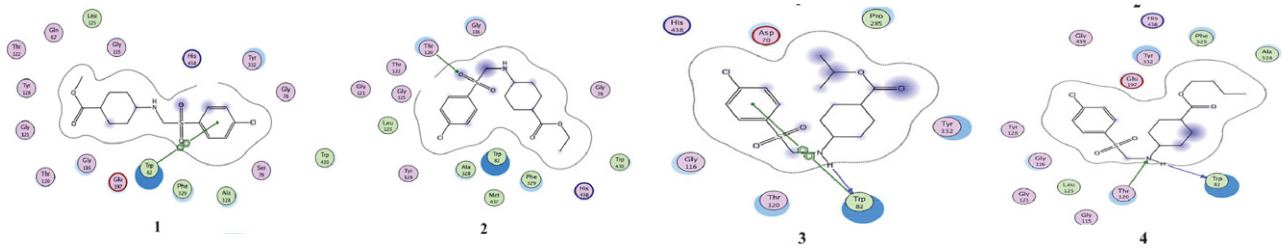


Figure 7. Interactions of synthesized esters with BChE (4BDS)

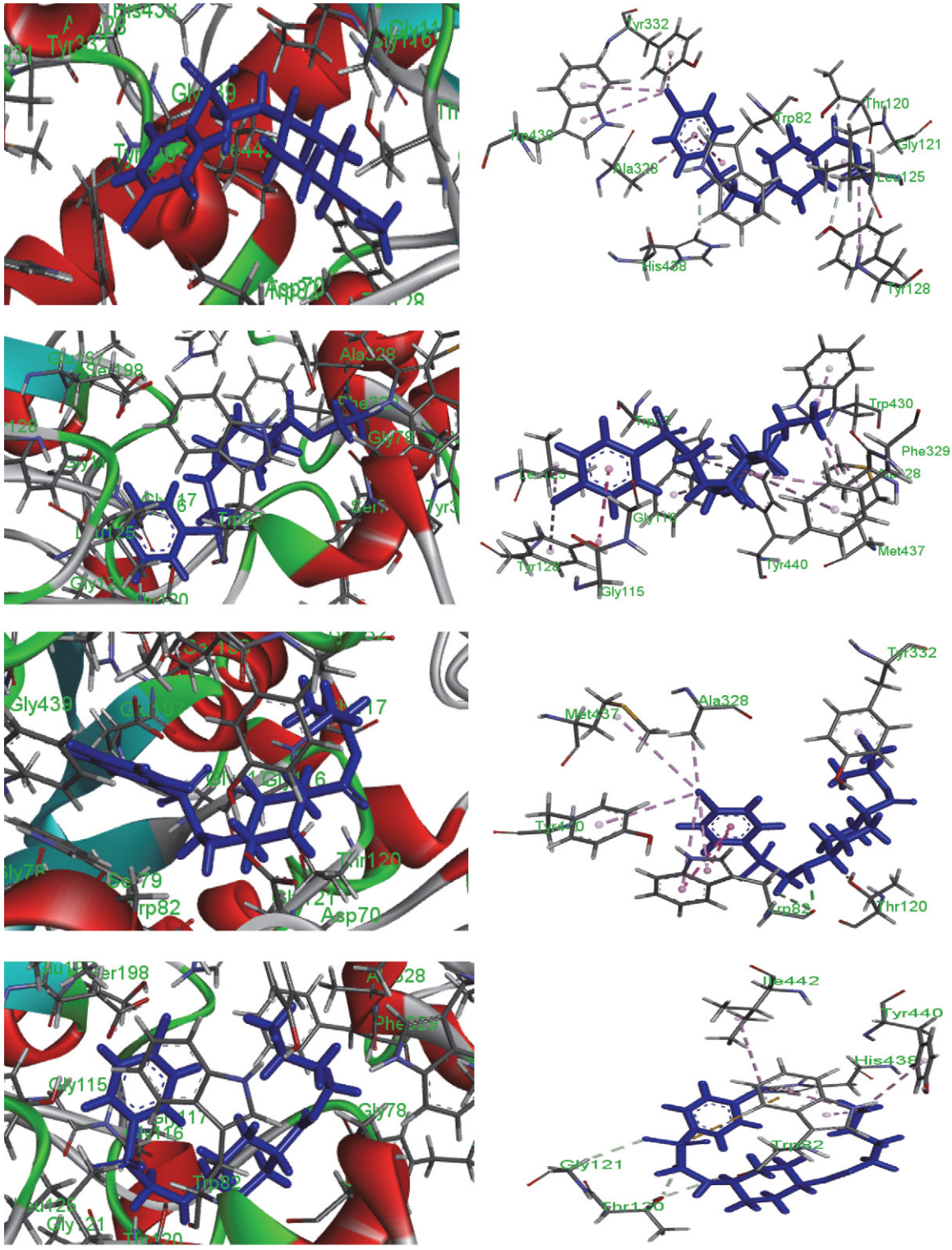


Figure 8. Best docking-poses of the synthesized compounds in the binding site of 4BDS

Gly117, Tyr440, Tyr332 and Ala328 are major residues which bind to the inhibitors (Figures 5–8). Ester **1** showed hydrogen binding with Gly117 and Trp231, while hydrophobic interaction with His438 and Trp82. Ester **2** demonstrated π–π interaction with Trp82 at anioic site, while hydrogen bonding with Val288 located at acyl pocket of 1POI. Similarly, Val288 and Trp82 provide the major interactions of ester **3** with the enzyme. It was observed that different residues such as Trp82, Glu197, His438, Gly117 etc. interact with hydrogen bonds with the inhibitor **4**. On

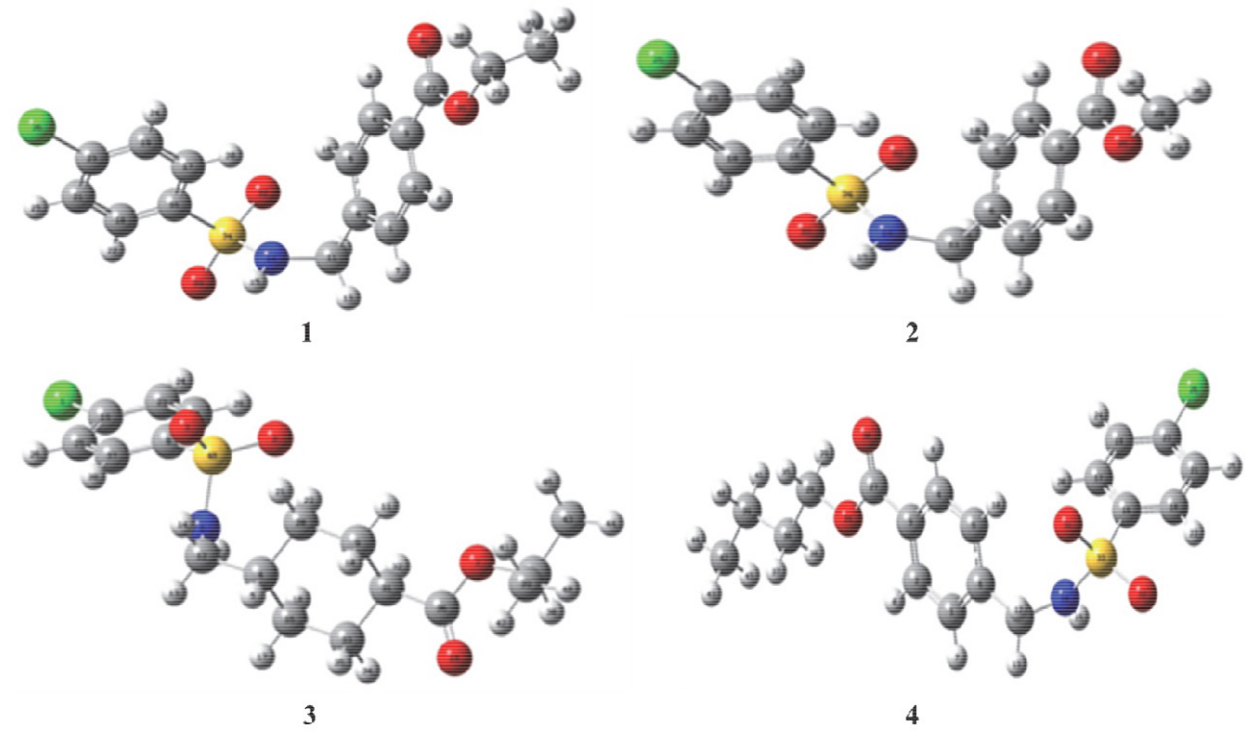


Figure 9. Optimized structures of synthesized compounds

Table 6. Global chemical reactivity indices of synthesized esters

Esters	1	2	3	4
μ (chemical potential)	−0.1402	−0.1405	−0.1540	−0.1398
η (chemical hardness)	0.2138	0.2139	0.2312	0.2137
X (electronegativity)	0.1402	0.1405	0.1540	0.1398
HOMO	−0.3540	−0.3544	−0.3852	−0.3535
LUMO	0.0736	0.0734	0.0772	0.0739
(LUMO–HOMO)	0.4276	0.4278	0.4625	0.4274
Energy (Hartree)	−1816.3955	−1777.5706	−1858.7156	−1894.0337
Ω	−0.1402	0.1405	0.154	−0.0193
IP (ionization potential)	0.3540	0.3544	0.3852	0.3535
EA (electron affinity)	−0.0736	−0.0734	−0.0772	−0.0739
Dipole moment	5.8484	5.8946	2.9289	6.2044
Nuclear repulsion energy	2111.4097	1983.1852	2359.5095	2341.0539
Gibbs free energy	−1816.1263	−1777.3305	−1858.3458	−1893.7086
Enthalpy	−1816.0521	−1777.2589	−1858.2654	−1893.6267

the active site of 4BDS, all four esters mainly interact with Trp82, Thr120, Tyr440 and **1**, **2** and **3** are further stabilized by interaction with Ala328.

3. 9. Computational Studies

DFT study of the targeted compounds was carried out using Gaussian software while optimized structures were visualized in Gauss view 5. The structures of all compounds were optimized using basis set B3LYP and bond lengths and bond angels of **3** were compared with experimental data (XRD results). It was clear from the results that there is a close resemblance between experimental and theoretical results. HOMO and LUMO were also drawn and energy gap between these was calculated and it was found that **1**, **2** and **4** have very small difference in the energy gap ranging from 0.4274 to 0.4278, while **3** has 0.4625 as shown in Table 6 and Figure 10. Others parameters such as chemical potential, chemical hardness, electronegativity, Hartree energy, ionization potential, electron affinity, dipole moment, nuclear repulsion energy, Gibbs free energy were also calculated and are presented in Table 6. It was clear from

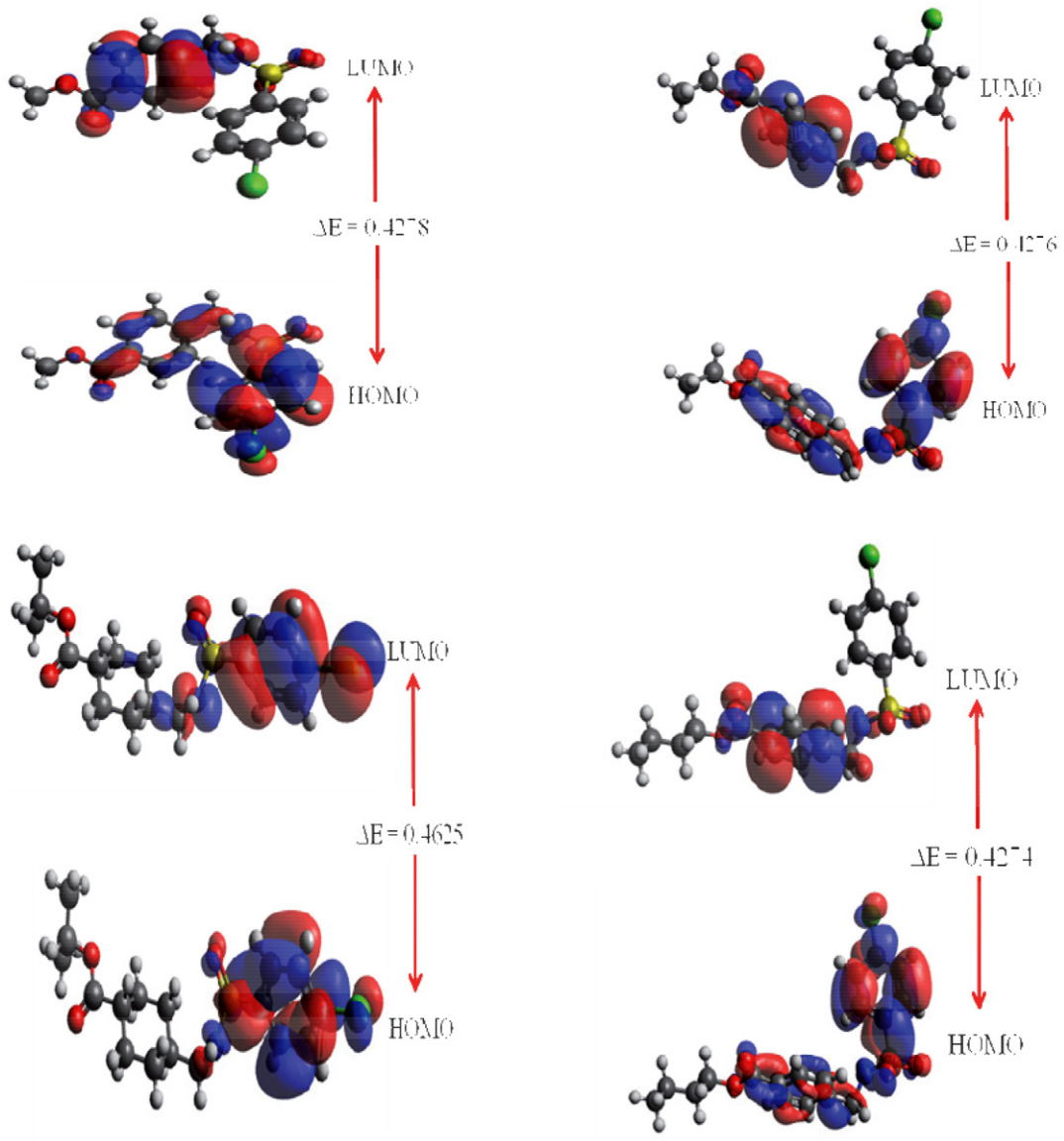


Figure 10. HOMO-LUMO energy diagram of synthesized esters

the results that there is a slight variation among the values of calculated parameters which suggested the presence of the same functionality and having similar physical and chemical properties of the molecules.

4. Conclusion

In current research work, a series of methyl (**1**), ethyl (**2**), isopropyl (**3**) and butyl (**4**) esters of 4-((4-chlorophenylsulfonamido)methyl)cyclohexanecarboxylic acid has been synthesized. Characterization of these compounds was done by FT-IR and mass spectrometry and NMR techniques while **3** was confirmed with X-ray crystallography. All compounds were screened for their biological applications involving anti-bacterial, anti-fungal, enzyme inhibition and anti-oxidant studies. Results showed that synthesized molecules have biological potential against tested activities. HOMO and LUMO was drawn after optimizing the structures with Gaussian and computational analysis was done to check binding mode of compound **3**.

Conflict of Interest

All authors declared that they have no conflict of interest.

Acknowledgment

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Supplementary Data

Selected bond lengths and bond angles for ester **3** are provided (Tables S1 and S2).

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Povzetek

S pomočjo zelene sintezne strategije smo z enostavno reakcijo med alkoholom in sulfonamidnim ligandom pripravili serijo novih trdnih estrov. Karakterizacijo tako pripravljenega metilnega (**1**), etilnega (**2**), izopropilnega (**3**) in *n*-butilnega (**4**) estra 4-((4-klorofenilsulfonamido)metil)cikloheksankarboksilne kisline smo izvedli s pomočjo FTIR, NMR masne spektrometrije in rentgenske kristalografije. Spojine smo optimizirali s pomočjo Gaussian programskega paketa z baznim setom B3LYP/6-31G(d,p) in izračunali nekaj parametrov, ki so povezani s strukturo. Za vse spojine smo *in vitro* izvedli nekatere biološke študije, vključno z antibakterijskim delovanjem (*Chromohalobactor salixgens*, *Halomonas halofila*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* in *Shiegella sonnei*), delovanjem proti glivam (*Aspergillus niger*), antioksidacijskim delovanjem (aktivnost uničevanja DPPH) in encimsko inhibicijo (acetilholin esteraza in butirilholin esteraza). Da bi ugotovili način vezave, smo sulfonamidne estre sidrali v izbrana encima (AChE in BChE) s pomočjo MOE programske opreme. Rezultati bioloških študij kažejo, da pripravljene spojine izkazujejo potencialno aktivnost.