BJPS

Brazilian Journal of Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902020000118092

Article

Biological activity of synthesized 5-{1-[(4-chlorophenyl)sulfonyl]piperidin-4-yl}-2-mercapto-1,3,4-oxadiazole derivatives demonstrated by *in silico* and BSA binding studies

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We synthesized a series of compounds bearing pharmacologically important 1,3,4-oxadiazole and piperidine moieties. Spectral data analysis by ^{1}H-NMR, ^{13}C-NMR, IR and EI-MS was used to elucidate the structures of the synthesized molecules. Docking studies explained the different types of interaction of the compounds with amino acids, while bovine serum albumin (BSA) binding interactions showed their pharmacological effectiveness. Antibacterial screening of these compounds demonstrated moderate to strong activity against *Salmonella typhi* and *Bacillus subtilis* but only weak to moderate activity against the other three bacterial strains tested. Seven compounds were the most active members as acetyl cholinesterase inhibitors. All the compounds presented displayed strong inhibitory activity against urease. Compounds **7l**, **7m**, **7n**, **7o**, **7p**, **7r**, **7u**, **7v**, **7x** and **7v** were highly active, with respective IC_{50} values of 2.14±0.003, 0.63±0.001, 2.17±0.006, 1.13±0.003, 1.21±0.005, 6.28±0.003, 2.39±0.005, 2.15±0.002, 2.26±0.003 and 2.14±0.002 µM, compared to thiourea, used as the reference standard (IC_{50} = 21.25±0.15 µM). These new urease inhibitors could replace existing drugs after their evaluation in comprehensive *in vivo* studies.

Keywords: 1,3,4-Oxadiazole. Acetylcholinesterase (AChE) inhibition. Antibacterial activity. Piperidine. Sulfonamide. Urease inhibition.

INTRODUCTION

1,3,4-Oxadiazole is a pharmacologically important heterocyclic core, and a number of biological activities have been described for derivatives with this moiety. Among these bioactivities, the most important ones are antibacterial (Li *et al.*, 2014), anti-inflammatory (Omar, Mahfouz, Rahman, 1996), anticancer (Kumar *et al.*, 2009), antihepatitis (Tan *et al.*, 2006), antitumor (Zhang *et al.*, 2014) and antiproliferative (El-Din *et al.*, 2015), among many other activities. Another key bioactive heterocyclic core is piperidine. The various bioactivities

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of this nucleus have been evaluated *via* the synthesis of a number of piperidine derivatives (Sanchez-Sancho, Herrandón, 1998). Compounds bearing the piperidine nucleus are associated with anesthetic activity, treatment of cocaine abuse, and controlling plasma glucose and insulin (Nithiya, Karthik, Jayabharathi, 2011). Among the different functionalities, sulfamoyl is one of the most important ones regarding pharmacological and therapeutic potential. The pharmacological behavior of this moiety is mainly related to antibacterial action, enzyme inhibition, cancer chemotherapy, hypoglycemic activity, diuretic action and many other uses (Aziz-ur-Rehman *et al*., 2011).

The synthesized compounds were evaluated for antibacterial activity, enzyme inhibitory activity (acetylcholinesterase (AChE) and urease) and bovine serum albumin (BSA) binding. The bacterial strains taken into account were *Salmonella Typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*, which are involved in enteric fever (Bhattacharya, Das, Choudhury, 2011), food poisoning (Vogt, Dippold, 2005), chronic infection (Pressler *et al*., 2011), pathogen adherence to extracellular matrix or plasma proteins (Harris, Foster, Richards, 2002) and skin allergy or hypersensitivity (Barbe *et al*., 2009), respectively. AChE (EC 3.1.1.7), a serine hydrolase, is known to hydrolyze the neurotransmitter acetylcholine leading to termination of nerve impulse in cholinergic synapses and Alzheimer's disease (Tougu, 2001). Urease (EC 3.5.1.5), urea amidohydrolase, is actively involved in pyelonephritis, peptic ulceration, kidney stones, hepatic encephalopathy, urolithiasis and urinary catheter incrustation (Lodhi *et al*., 2007). Blood plasma proteins act carriers for the transportation of drugs and other substances throughout the body (Wani *et al*., 2018). The most abundant plasma protein is albumin which binds with metabolites and ligands. The pharmacokinetic and pharmacodynamic properties can be understood through the binding interaction of serum albumin and drug molecules. BSA is widely used in this regard because of high resemblance in structure with human serum albumin (HSA) (Wani *et al*., 2017a; Wani *et al*., 2017b; Wani *et al*., 2017c).

Chemists and pharmacologists have been working extensively on the synthesis of new molecules to combat and control various diseases. In this regard, two heterocyclic cores, 1,3,4-oxadiazole and piperidine, and the sulfamoyl functionality of sulfonamides have been introduced as bioactive structures. The notable pharmacological behavior of these three functionalities prompted us to synthesize some new molecules bearing all these three moieties. As an extension of our previous work (Nafeesa *et al*., 2015), the synthesized compounds were evaluated for their pharmacological potential as antibacterial agents and AChE and urease inhibitors.

MATERIAL AND METHODS

General

The IR spectra were recorded using KBr pellet method on a Jasco-320-A spectrometer with wave number in cm^{-1}. Mass spectra (EI-MS) were recorded on a JMS-HX-110 spectrometer with a data system. Proton and carbon nuclear magnetic resonance spectra were recorded in $CDCl_3$ solvent on a Bruker spectrometer operating at 300 and 400 MHz with chemical shifts in ppm and coupling constant in hertz (Hz). Purity of synthesized compounds was confirmed using thin layer chromatography (TLC), carried out on pre-coated silica gel G-25-UV_{254} aluminum plates, run under different solvent systems with varying proportions of ethyl acetate and *n*-hexane and visualized using a 254-nm UV lamp. Melting points of the synthesized compounds were recorded on a Griffin and George meting point apparatus with open capillary tube and were uncorrected.

Preparation of ethyl 1-[(4-chlorophenyl) sulfonyl] piperidin-4-carboxylate (3)

Ethyl isonipecotate (**2**; 0.003 mol) was dispersed in 40 mL of distilled water in a 250-mL round-bottom (RB) flask followed by addition of 4-chlorophenylsulfonyl chloride (**1**; 0.003 mol). During gradual addition of **1**, the mixture was maintained at pH 10.0 with 18% aqueous Na_2CO_3. The mixture was stirred for 4 hours and monitored by TLC. Dilute HCl (2-3 mL) was added with continuous stirring to adjust pH at 6.0, and the mixture allowed to stand for 20-30 minutes. The precipitates were filtered out, washed with cold distilled water and dried.

Preparation of 1-[(4-chlorophenyl)sulfonyl] piperidin-4-carbohydrazide (4)

Compound **3** (0.0025 mol) was dissolved in 35 mL of methanol in a 250-mL RB flask, and 80% hydrazine hydrate (12 mL) was added, followed by refluxing for

3 hours. The reaction was monitored by TLC. Excess solvent was evaporated off and the precipitate was filtered out, washed with cold distilled water and dried.

Preparation of 5-{1-[(4-chlorophenyl)sulfonyl] piperidin-4-yl}-2-mercapto-1,3,4-oxadiazole (5)

Compound **4** (0.002 mol) was dissolved in 35 mL of ethanol in a 250-mL RB flask followed by the addition of KOH (0.002 mol) and refluxing for 30 minutes. After the addition of CS_2 (0.004 mol), the reaction mixture was refluxed for 6 hours and monitored by TLC. Excess chilled distilled water (70 mL) was added and pH was adjusted to 6 with dilute HCl (3-4 mL) to obtain the precipitate. The precipitate was filtered out, washed with cold distilled water and dried.

General procedure for the synthesis of 5-{1-[(4-chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(alkyl/aralkyl)thio]-1,3,4-oxadiazole (7a-z)

Compound **5** (0.0015 mol) was dissolved in *N,N*-dimethyl formamide (DMF, 15 mL) in a 50-mL RB flask and lithium hydride (0.0015 mol) was added. The reaction mixture was stirred for 30 minutes. Equimolar alkyl/aralkyl halides, **6a-z**, were then added to the reaction mixture, which was further stirred for 4-5 hours. The reaction was monitored by TLC. Excess cold distilled water was added to obtain the precipitated final compounds, **7a-z**, which were recovered by filtration, washed with distilled water and dried.

Biological activity assays

Antibacterial activity assay

Antibacterial activity was determined under aseptic conditions in sterile 96-well microplates. The method is based on the increase in the absorbance of the broth medium with microbial cell population during log phase growth (Nafeesa *et al*., 2015). Ciprofloxacin was used as the reference standard.

Acetyl cholinesterase inhibition assay

AChE inhibitory activity was determined by following the reported method (Mahernia *et al.,* 2015) with minor modifications. A mixture of Na_2HPO_4 buffer (pH 7.7), 10 µL of test compound (0.5 mM $well^{-1}$) and 10 µL AChE (0.005 mM) was prepared. Absorbance of the mixture at 405 nm was recorded, and the mixture was then incubated for 15 min at 35 ºC. The enzyme reaction was started by the addition of 10 µL of 0.5 mM acetylthiocholine iodide as substrate and 10 µL of 0.5 mM DTNB. After 15 minutes of incubation, the absorbance was measured at 405 nm by using a Synergy HT 96-well plate reader (BioTek, USA). Eserine (0.5 mM) was used as a standard for the positive control. Percent inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Text}}{\text{Control}} \times 100$$

where Test is the activity in the presence of compound of concern, while Control is the activity in the absence of inhibitor.

Urease inhibition assay

A 985-µL reaction mixture containing test compound (0 to 100 µL), urea (850 µL) and phosphate buffer (100 mM, pH 7.4) was prepared to evaluate urease inhibitory activity using a previously reported method (Chapdelaine, Tremblay, Dube, 1978). The reaction was started by the addition of 15 µL of urease enzyme and allowed to proceed for 60 minutes at 37 °C. A volume of 500 µL of solution A containing 0.5 g phenol and 2.5 mg sodium nitroprusside in 50 mL of distilled water and equi-volume of solution B containing 250 mg sodium hydroxide and 820 µL of 5% sodium hypochlorite in 50 mL of distilled water was used to measure the extent of reaction after incubation for another 30 minutes. Urease activity before inhibition was taken as 100% control activity. The formula used to calculate % inhibition was similar as for AChE.

Statistical analysis

The results are presented as mean ± SEM for triplicate calculations after statistical analysis executed in MS Excel 2010. Minimum inhibitory concentration (MIC) for antibacterial activity and concentration for 50% inhibition (IC_{50}) for AChE inhibition were determined using suitable dilutions for each sample, and the results were obtained using EZ-Fitz software (Perrella Scientific Inc., USA). GraphPad Prism 5 software was used to calculate the IC_{50} values for urease.

Computational studies

Initial processing of proteins and ligand preparation

To study the interaction of ligands in the active site of AChE by using chemoinformatics, the co-crystal 3D-structure recombinant human AChE (Ghersi, Sanchez, 2011) in complex with donepezil (PDB entry: 4EY7) was obtained from the RCSB Protein Data Bank (PDB). All protein structures from PDB were further processed prior to docking studies using structure preparation tools included in biopolymer module of *SYBYL-X 1.3*. Missing hydrogens were added, charges were applied and atom types were assigned according to AMBER 7 FF99 force field followed by the energy minimization using the Powell algorithm with a convergence gradient of 0.5 kcal (mol Å)$^{-1}$ for 1000 cycles. Moreover, the 3D structures of the inhibitors studied were constructed using the SKETCH module in Sybyl-X 1.3 followed by energy minimization using Tripos force field with Gasteiger-Hückel charge.

Molecular docking

The molecular docking studies were performed with the Surflex-Dock module of SybylX-1.3 (Jain, Dock, 2007). The experimentally determined active conformation of donepezil in the AChE active site was used as initial conformation to define the potential binding pocket by protomol (an idealized active site) generation (Chohan *et al.,* 2016a). The defined active site can be used to generate several putative poses of ligand (Chohan *et al.,* 2016b). These putative poses of ligand were ranked using the Hammerhead scoring function (Holt, Chaires, Trent, 2008). All parameters determining the extent of protomol were kept at default (threshold = 0.50 and bloat=0). Finally, the synthesized compounds were then individually docked in the idealized active site of AChE with the "whole" molecular alignment algorithm, and twenty top-ranked docked poses were finally saved for each inhibitor.

Protein drugs interaction studies

BSA binding interactions using fluorescence measurements

For the determination of the BSA quenching constant, fluorometric titration of a solution of BSA (3 mL, 3 μM) in phosphate buffer (20 mM, pH 7.4) with the synthesized compounds (1 mg/mL in DMSO solvent) was carried out. The solutions were excited at 295 nm and the emission of the BSA solution with and without test compound was recorded at 336 nm and 298 K. For site-selective binding studies, fluorometric titration of BSA solution (3 mL, 3 μM) with and without site markers (ibuprofen or warfarin) was carried out with the solutions of synthesized compounds (1 mg/mL in DMSO). The solutions were scanned using an excitation wavelength of 295 nm at 298 K (Wu *et al.,* 2011).

Spectral characterization

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-(allylthio)-1,3,4-oxadiazole (7a)

Off-white amorphous solid; Yield: 79 %; M.P.: 102-103 ºC; Mol. formula: $C_{16}H_{18}ClN_3O_3S_2$; Mol. weight: 399 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3130 (Ar C-H), 1678 (Al C=C), 1658 (C=N), 1594 (Ar C=C), 1517 (N=O), 1446 (S=O), 1228, 1025 (C-O-C), 1148 (C-N), 655 (C-Cl), 622 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.86 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 5.99-5.90 (m, 1H, H-2"'), 5.30 (dd, J = 16.8, 0.8 Hz, 1H, H_a-3"'), 5.18 (d, J = 10.4 Hz, 1H, H_b-3"'), 3.81 (d, J = 7.2 Hz, 2H, H-1"'), 3.66-3.63 (m, 2H, H_e-2' & H_e-6'), 2.92-2.85 (m, 1H, H-4'), 2.63 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.09 (m, 2H, H_e-3' & H_e-5'), 2.02-1.92 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 401 $[M+2]^+$, 399 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-(ethylthio)-1,3,4-oxadiazole (7b)

Light brown crystalline solid; Yield: 83 %; M.P.: 137-138 ºC; Mol. formula: $C_{15}H_{18}ClN_3O_3S_2$; Mol. weight: 387 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3030 (Ar C-H), 1688 (C=N), 1599 (Ar C=C), 1519 (N=O), 1466 (S=O), 1220, 1029 (C-O-C), 1168 (C-N), 658 (C-Cl), 632 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.86 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 3.66-3.63 (m, 2H, H_e-2' & H_e-6'), 3.20 (q, J = 7.6 Hz, 2H, H-1"'), 2.93-2.85 (m, 1H, H-4'), 2.62 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.09 (m, 2H, H_e-3' & H_e-5'), 2.01-1.94 (m, 2H, H_a-3' & H_a-5'), 1.43 (t, J = 7.6 Hz, 3H, H-2"'); EI-MS (*m/z*): 389 $[M+2]^+$, 387 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(1-propyl)thio]-1,3,4-oxadiazole (7c)

White amorphous solid; Yield: 85 %; M.P.: 111-112 ºC; Mol. formula: $C_{16}H_{20}ClN_3O_3S_2$; Mol. weight: 401 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3130 (Ar C-H), 1682 (C=N), 1565 (Ar C=C), 1520 (N=O), 1386 (S=O), 1228, 1026 (C-O-C), 1158 (C-N), 678 (C-Cl), 612 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.67 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.51 (d, J = 8.8 Hz, 2H, H-3" & H-5"), 3.70-3.67 (m, 2H, H_e-2' & H_e-6'), 3.18 (t, J = 7.2 Hz, 2H, H-1"'), 2.71-2.67 (m, 1H, H-4'), 2.56 (dt, J = 12.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.13-2.09 (m, 2H, H_e-3' & H_e-5'), 1.92 (dq, J = 10.8, 3.6 Hz, 2H, H_a-3' & H_a-5'), 1.34 (sex, J = 7.2 Hz, 3H, H-2"'), 1.03 (t, J = 7.6 Hz, 3H, H-3"'); EI-MS (*m/z*): 403 $[M+2]^+$, 401 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-propyl)thio]-1,3,4-oxadiazole (7d)

Off-white amorphous solid; Yield: 77 %; M.P.: 156-157 ºC; Mol. formula: $C_{16}H_{20}ClN_3O_3S_2$; Mol. weight: 401 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3067 (Ar C-H), 1673 (C=N), 1582 (Ar C=C), 1525 (N=O), 1476 (S=O), 1170, 1026 (C-O-C), 1142 (C-N), 662 (C-Cl), 638 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.69 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.51 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 3.85 (sep, J = 6.8 Hz, 1H, H-1"'), 3.71-3.64 (m, 2H, H_e-2' & H_e-6'), 2.72-2.65 (m, 1H, H-4'), 2.56 (dt, J = 12.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.13-2.09 (m, 2H, H_e-3' & H_e-5'), 1.99-1.86 (m, 2H, H_a-3' & H_a-5'), 1.45 (d, J = 6.8 Hz, 6H, H-2"' & H-3"'); EI-MS (*m/z*): 403 $[M+2]^+$, 401 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(1-butyl)thio]-1,3,4-oxadiazole (7e)

White amorphous solid; Yield: 76 %; M.P.: 113-114 ºC; Mol. formula: $C_{17}H_{22}ClN_3O_3S_2$; Mol. weight: 415 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3080 (Ar C-H), 1688 (C=N), 1578 (Ar C=C), 1545 (N=O), 1456 (S=O), 1235, 1019 (C-O-C), 1161 (C-N), 660 (C-Cl), 622 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 3.67-3.62 (m, 2H, H_e-2' & H_e-6'), 3.19 (t, J = 7.6 Hz, 2H, H-1"'), 2.89-2.84 (m, 1H, H-4'), 2.63 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.10 (m, 2H, H_e-3' & H_e-5'), 2.02-1.92 (m, 2H, H_a-3' & H_a-5'), 1.74 (qui, J = 7.6 Hz, 2H, H-2"'), 1.44 (sex, J = 7.2 Hz, 2H, H-3"'), 0.92 (t, J = 7.6 Hz, 3H, H-4"'); EI-MS (*m/z*): 417 $[M+2]^+$, 415 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 57 $[C_4H_9]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-butyl)thio]-1,3,4-oxadiazole (7f)

White amorphous solid; Yield: 80 %; M.P.: 105-106 ºC; Mol. formula: $C_{17}H_{22}ClN_3O_3S_2$; Mol. weight: 415 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3036 (Ar C-H), 1685 (C=N), 1592 (Ar C=C), 1514 (N=O), 1465 (S=O), 1225, 1022 (C-O-C), 1163 (C-N), 653 (C-Cl), 631 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.70 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.52 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 4.96-4.77 (m, 1H, H-1"'), 3.70-3.68 (m, 2H, H_e-2' & H_e-6'), 2.70-2.68 (m, 1H, H-4'), 2.57 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.13-2.10 (m, 2H, H_e-3' & H_e-5'), 2.01-1.96 (m, 2H, H-2"'), 1.97-1.87 (m, 2H, H_a-3' & H_a-5'), 1.44 (d, J = 6.8 Hz, 3H, H-4"'), 1.01 (t, J = 7.2 Hz, 3H, H-3"'); EI-MS (*m/z*): 417 $[M+2]^+$, 415 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 57 $[C_4H_9]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(1-pentyl)thio]-1,3,4-oxadiazole (7g)

White amorphous solid; Yield: 84 %; M.P.: 115-116 ºC; Mol. formula: $C_{18}H_{24}ClN_3O_3S_2$; Mol. weight: 429 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3026 (Ar C-H), 1675 (C=N), 1582 (Ar C=C), 1534 (N=O), 1445 (S=O), 1215, 1042 (C-O-C), 1193 (C-N), 673 (C-Cl), 641 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.69 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.51 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 3.66-3.63 (m, 2H, H_e-2' & H_e-6'), 3.18 (t, J = 7.6 Hz, 2H, H-1"'), 2.89-2.84 (m, 1H, H-4'), 2.63 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.10 (m, 2H, H_e-3' & H_e-5'), 2.02-1.95 (m, 2H, H_a-3' & H_a-5'), 1.75 (qui, J = 7.6 Hz, 2H, H-2"'), 1.41-1.29 (m, 4H, H-3"' & H-4"'), 0.88 (t, J = 7.2 Hz, 3H, H-5"'); EI-MS (*m/z*): 431 $[M+2]^+$, 429 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 71 $[C_5H_{11}]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-pentyl)thio]-1,3,4-oxadiazole (7h)

White amorphous solid; Yield: 78 %; M.P.: 98-99 ºC; Mol. formula: $C_{18}H_{24}ClN_3O_3S_2$; Mol. weight: 429 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3126 (Ar C-H), 1685 (C=N), 1682 (Ar C=C), 1530 (N=O), 1385 (S=O), 1225, 1031 (C-O-C), 1143 (C-N), 773 (C-Cl), 631 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.70 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.52 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 4.95-4.80 (m, 1H, H-1"'), 3.71-3.67 (m, 2H, H_e-2' & H_e-6'), 2.69-2.65 (m, 1H, H-4'), 2.57 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.13-2.10 (m, 2H, H_e-3' & H_e-5'), 2.02-1.97 (m, 2H, H-2"'), 1.97-1.87 (m, 2H, H_a-3' & H_a-5'), 1.75-1.61 (m, 2H, H-3"'), 1.45 (d, J = 7.2 Hz, 3H, H-5"'), 0.92 (t, J = 7.2 Hz, 3H, H-4"'); EI-MS (*m/z*): 431 $[M+2]^+$, 429 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 71 $[C_5H_{11}]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(1-heptyl)thio]-1,3,4-oxadiazole (7i)

Off-white amorphous solid; Yield: 75 %; M.P.: 123-124 ºC; Mol. formula: $C_{20}H_{28}ClN_3O_3S_2$; Mol. weight: 457 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3122 (Ar C-H), 1682 (C=N), 1688 (Ar C=C), 1538 (N=O), 1382 (S=O), 1228, 1036 (C-O-C), 1148 (C-N), 772 (C-Cl), 633 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 3.67-3.62 (m, 2H, H_e-2' & H_e-6'), 3.18 (t, J = 7.2 Hz, 2H, H-1"'), 2.89-2.84 (m, 1H, H-4'), 2.63 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.10 (m, 2H, H_e-3' & H_e-5'), 2.02-1.95 (m, 2H, H_a-3' & H_a-5'), 1.76 (qui, J = 7.2 Hz, 2H, H-2"'), 1.40 (qui, J = 7.6 Hz, 2H, H-3"'), 1.31-1.26 (m, 6H, H-4"' to H-6"'), 0.85 (t, J = 6.8 Hz, 3H, H-7"'); EI-MS (*m/z*): 459 $[M+2]^+$, 457 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 99 $[C_7H_{15}]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(1-octyl)thio]-1,3,4-oxadiazole (7j)

Off-white amorphous solid; Yield: 86 %; M.P.: 127-128 ºC; Mol. formula: $C_{21}H_{30}ClN_3O_3S_2$; Mol. weight: 471 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3129 (Ar C-H), 1688 (C=N), 1680 (Ar C=C), 1540 (N=O), 1389 (S=O), 1238, 1038 (C-O-C), 1138 (C-N), 782 (C-Cl), 639 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 3.66-3.63 (m, 2H, H_e-2' & H_e-6'), 3.18 (t, J = 7.6 Hz, 2H, H-1"'), 2.89-2.84 (m, 1H, H-4'), 2.63 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.09 (m, 2H, H_e-3' & H_e-5'), 2.02-1.93 (m, 2H, H_a-3' & H_a-5'), 1.75 (qui, J = 7.2 Hz, 2H, H-2"'), 1.40 (qui, J = 7.6 Hz, 2H, H-3"'), 1.27-1.24 (m, 8H, H-4"' to H-7"'), 0.85 (t, J = 6.8 Hz, 3H, H-8"'); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ (ppm) 168.3 (C-5), 164.9 (C-2), 139.5 (C-1"), 134.8 (C-4"), 129.5 (C-3" & C-5"), 129.0 (C-2" & C-6"), 45.0 (C-2' & C-6'), 32.5 (C-6"'), 32.2 (C-4'), 31.7 (C-5"'), 29.1 (C-4"'), 29.0 (C-3"'), 28.9 (C-2"'), 28.5 (C-1"'), 28.3 (C-3' & C-5'), 22.6 (C-7"'), 14.0 (C-8"'); EI-MS (*m/z*): 473 $[M+2]^+$, 471 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 113 $[C_8H_{17}]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-(benzylthio)-1,3,4-oxadiazole (7k)

Off-white amorphous solid; Yield: 76 %; M.P.: 131-132 ºC; Mol. formula: $C_{20}H_{20}ClN_3O_3S_2$; Mol. weight: 429 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3134 (Ar C-H), 1673 (C=N), 1610 (Ar C=C), 1524 (N=O), 1323 (S=O), 1228, 1012 (C-O-C), 1148 (C-N), 761 (C-Cl), 624 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.8 Hz, 2H, H-2" & H-6"), 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.37 (br.d, J = 6.4 Hz, 2H, H-2"' & H-6"'), 7.30-7.28 (m, 3H, H-3"' to H-5"'), 4.04 (s, 2H, H-7"'), 3.64-3.61 (m, 2H, H_e-2' & H_e-6'), 2.88-2.83 (m, 1H, H-4'), 2.62 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.12-2.08 (m, 2H, H_e-3' & H_e-5'), 2.00-1.93 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 431 $[M+2]^+$, 429 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 91 $[C_7H_7]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-methylbenzyl)thio]-1,3,4-oxadiazole (7l)

White amorphous solid; Yield: 77 %; M.P.: 125-126 ºC; Mol. formula: $C_{21}H_{22}ClN_3O_3S_2$; Mol. weight: 463 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3138 (Ar C-H), 1676 (C=N), 1618 (Ar C=C), 1525 (N=O), 1327 (S=O), 1224, 1017 (C-O-C), 1142 (C-N), 769 (C-Cl), 628 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.32 (d, J = 7.6 Hz, 1H, H-6"'), 7.20-7.12 (m, 3H, H-3"' to H-5"'), 4.44 (s, 2H, H-7"'), 3.66-3.63

(m, 2H, H_e-2' & H_e-6'), 2.89-2.84 (m, 1H, H-4'), 2.63 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.13-2.09 (m, 2H, H_e-3' & H_e-5'), 2.01-1.94 (m, 2H, H_a-3' & H_a-5'), 2.39 (s, 3H, CH_3-2'''); EI-MS (*m/z*): 465 $[M+2]^+$, 463 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 105 $[C_8H_9]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(4-fluorobenzyl)thio]-1,3,4-oxadiazole (7m)

White amorphous solid; Yield: 75%; M.P.: 146-147ºC; Mol. formula: $C_{20}H_{19}ClFN_3O_3S_2$; Mol. weight: 467 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3137 (Ar C-H), 1672 (C=N), 1619 (Ar C=C), 1522 (N=O), 1326 (S=O), 1227, 1013 (C-O-C), 1147 (C-N), 1056 (C-F), 720 (C-Cl), 626 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.8 Hz, 2H, H-3" & H-5"), 7.37 (dis. dd, $J_{(b,a\ \&\ b,\ ^{19}F)}$ = 8.8, 5.6 Hz, $2H_b$, H-2''' & H-6'''), 6.98 (br.t, $J_{(a,b\ \&\ a,\ ^{19}F)}$ = 8.8 Hz, $2H_a$, H-3''' & H-5'''), 4.38 (s, 2H, H-7'''), 3.66-3.62 (m, 2H, H_e-2' & H_e-6'), 2.88-2.83 (m, 1H, H-4'), 2.62 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.12-2.08 (m, 2H, H_e-3' & H_e-5'), 1.97-1.93 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 469 $[M+2]^+$, 467 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 109 $[C_7H_6F]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-chlorobenzyl)thio]-1,3,4-oxadiazole (7n)

White amorphous solid; Yield: 81%; M.P.: 121-122ºC; Mol. formula: $C_{20}H_{19}Cl_2N_3O_3S_2$; Mol. weight: 483 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3127 (Ar C-H), 1682 (C=N), 1609 (Ar C=C), 1532 (N=O), 1396 (S=O), 1237, 1023 (C-O-C), 1167 (C-N), 780 (C-Cl), 646 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.8 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.53 (dd, J = 7.2, 1.2 Hz, 1H, H-6'''), 7.38 (dd, J = 8.0, 0.8 Hz, 1H, H-3'''), 7.23-7.19 (m, 2H, H-4''' & H-5'''), 4.51 (s, 2H, H-7'''), 3.65-3.62 (m, 2H, H_e-2' & H_e-6'), 2.87-2.82 (m, 1H, H-4'), 2.62 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.12-2.08 (m, 2H, H_e-3' & H_e-5'), 2.00-1.91 (m, 2H, H_a-3' & H_a-5'); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ (ppm) 168.7 (C-5), 164.1 (C-2), 139.5 (C-1"), 134.8 (C-4"), 134.4 (C-1'''), 133.6 (C-2'''), 131.4 (C-4'''), 129.7 (C-5'''), 129.6 (C-3'''), 129.5 (C-3" & C-5"), 128.9 (C-2" & C-6"), 127.0 (C-6'''), 44.9 (C-2' & C-6'), 34.5 (C-7'''), 32.2 (C-4'), 28.2 (C-3' & C-5'); EI-MS (*m/z*): 487 $[M+4]^+$, 485 $[M+2]^+$, 483 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 125 $[C_7H_6Cl]^+$, 111 $[C_6H_4Cl]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(3-chlorobenzyl)thio]-1,3,4-oxadiazole (7o)

White amorphous solid; Yield: 82%; M.P.: 176-177ºC; Mol. formula: $C_{20}H_{19}Cl_2N_3O_3S_2$; Mol. weight: 483 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3122 (Ar C-H), 1680 (C=N), 1600 (Ar C=C), 1538 (N=O), 1398 (S=O), 1239, 1026 (C-O-C), 1168 (C-N), 782 (C-Cl), 643 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.37 (s, 1H, H-2'''), 7.29-7.23 (m, 3H, H-4''' to H-6'''), 4.36 (s, 2H, H-7'''), 3.66-3.63 (m, 2H, H_e-2' & H_e-6'), 2.87-2.83 (m, 1H, H-4'), 2.62 (dt, J = 12.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.12-2.08 (m, 2H, H_e-3' & H_e-5'), 1.97-1.93 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 487 $[M+4]^+$, 485 $[M+2]^+$, 483 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 125 $[C_7H_6Cl]^+$, 111 $[C_6H_4Cl]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(4-chlorobenzyl)thio]-1,3,4-oxadiazole (7p)

White amorphous solid; Yield: 85%; M.P.: 182-183ºC; Mol. formula: $C_{20}H_{19}Cl_2N_3O_3S_2$; Mol. weight: 483 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3125 (Ar C-H), 1683 (C=N), 1608 (Ar C=C), 1534 (N=O), 1392 (S=O), 1230, 1028 (C-O-C), 1166 (C-N), 780 (C-Cl), 642 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.67 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.48 (d, J = 8.8 Hz, 2H, H-3" & H-5"), 7.35 (d, J = 8.4 Hz, 2H, H-2''' & H-6'''), 7.28 (d, J = 8.4 Hz, 2H, H-3''' & H-5'''), 4.37 (s, 2H, H-7'''), 3.67-3.62 (m, 2H, H_e-2' & H_e-6'), 2.87-2.81 (m, 1H, H-4'), 2.61 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.13-2.08 (m, 2H, H_e-3' & H_e-5'), 1.99-1.93 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 487 $[M+4]^+$, 485 $[M+2]^+$, 483 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 125 $[C_7H_6Cl]^+$, 111 $[C_6H_4Cl]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-bromobenzyl)thio]-1,3,4-oxadiazole (7q)

White amorphous solid; Yield: 84 %; M.P.: 118-119 ºC; Mol. formula: $C_{20}H_{19}ClBrN_3O_3S_2$; Mol. weight: 526 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3125 (Ar C-H), 1683

(C=N), 1602 (Ar C=C), 1534 (N=O), 1390 (S=O), 1231, 1023 (C-O-C), 1169 (C-N), 782 (C-Cl), 647 (C-S), 625 (C-Br); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.55 (d, J = 8.0 Hz, 1H, H-3"'), 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.25-7.21 (m, 2H, H-5"' & H-6"'), 7.14 (dt, J = 7.6, 1.2 Hz, 1H, H-4"'), 4.52 (s, 2H, H-7"'), 3.65-3.62 (m, 2H, H_e-2' & H_e-6'), 2.87-2.82 (m, 1H, H-4'), 2.62 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.12-2.08 (m, 2H, H_e-3' & H_e-5'), 1.99-1.90 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 530 $[M+4]^+$, 528 $[M+2]^+$, 526 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 169 $[C_7H_6Br]^+$, 111 $[C_6H_4Cl]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(4-bromobenzyl)thio]-1,3,4-oxadiazole (7r)

White amorphous solid; Yield: 83 %; M.P.: 173-174 ºC; Mol. formula: $C_{20}H_{19}ClBrN_3O_3S_2$; Mol. weight: 526 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3128 (Ar C-H), 1688 (C=N), 1605 (Ar C=C), 1539 (N=O), 1393 (S=O), 1234, 1027 (C-O-C), 1166 (C-N), 722 (C-Cl), 649 (C-S), 629 (C-Br); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.67 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.48 (d, J = 8.8 Hz, 2H, H-3" & H-5"), 7.43 (d, J = 8.0 Hz, 2H, H-2"' & H-6"'), 7.28 (d, J = 8.4 Hz, 2H, H-3"' & H-5"'), 4.35 (s, 2H, H-7"'), 3.67-3.62 (m, 2H, H_e-2' & H_e-6'), 2.87-2.81 (m, 1H, H-4'), 2.61 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.13-2.08 (m, 2H, H_e-3' & H_e-5'), 1.99-1.93 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 530 $[M+4]^+$, 528 $[M+2]^+$, 526 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 169 $[C_7H_6Br]^+$, 111 $[C_6H_4Cl]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-phenylethyl)thio]-1,3,4-oxadiazole (7s)

Off-white amorphous solid; Yield: 82 %; M.P.: 108-109 ºC; Mol. formula: $C_{21}H_{22}ClN_3O_3S_2$; Mol. weight: 463 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3155 (Ar C-H), 1643 (C=N), 1592 (Ar C=C), 1544 (N=O), 1450 (S=O), 1241, 1033 (C-O-C), 1182 (C-N), 680 (C-Cl), 624 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.28 (d, J = 7.2 Hz, 2H, H-2"' & H-6"'), 7.23-7.20 (m, 3H, H-3"' to H-5"'), 3.66-3.63 (m, 2H, H_e-2' & H_e-6'), 3.43 (t, J = 7.6 Hz, 2H, H-8"'), 3.08 (t, J = 7.6 Hz, 2H, H-7"'), 2.89-2.84 (m, 1H, H-4'), 2.66-2.61 (m, 2H, H_a-2' & H_a-6'), 2.14-2.10 (m, 2H, H_e-3' & H_e-5'), 2.00-1.95 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 465 $[M+2]^+$, 463 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 105 $[C_8H_9]^+$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(3-phenylpropyl)thio]-1,3,4-oxadiazole (7t)

White amorphous solid; Yield: 80 %; M.P.: 116-117 ºC; Mol. formula: $C_{22}H_{24}ClN_3O_3S_2$; Mol. weight: 477 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3152 (Ar C-H), 1642 (C=N), 1590 (Ar C=C), 1542 (N=O), 1452 (S=O), 1244, 1032 (C-O-C), 1180 (C-N), 684 (C-Cl), 626 (C-S); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.69 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.29-7.15 (m, 5H, H-2"' to H-6"'), 3.66-3.63 (m, 2H, H_e-2' & H_e-6'), 3.20 (t, J = 7.2 Hz, 2H, H-9"'), 2.89-2.84 (m, 1H, H-4'), 2.75 (t, J = 7.5 Hz, 2H, H-7"'), 2.66-2.61 (m, 2H, H_a-2' & H_a-6'), 2.16-2.12 (m, 2H, H_e-3' & H_e-5'), 2.11 (qui, J = 7.2 Hz, 2H, H-8"'), 2.00-1.95 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 479 $[M+2]^+$, 477 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 119 $[C_9H_{11}]^+$, 111 $[C_6H_4Cl]^+$, 105 $[C_8H_9]^+$, 77 $[C_6H_5]^+$, 65 $[C_5H_5]^+$, 51 $[C_4H_3]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(3-nitrobenzyl)thio]-1,3,4-oxadiazole (7u)

White amorphous solid; Yield: 74 %; M.P.: 122-123 ºC; Mol. formula: $C_{20}H_{19}ClN_4O_5S_2$; Mol. weight: 494 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3157 (Ar C-H), 1645 (C=N), 1592 (Ar C=C), 1548 (N=O), 1457 (S=O), 1246, 1038 (C-O-C), 1183 (C-N), 689 (C-Cl), 623 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.26 (s, 1H, H-2"'), 8.13 (d, J = 8.4 Hz, 1H, H-4"'), 7.78 (d, J = 7.6 Hz, 1H, H-6"'), 7.68 (d, J = 8.8 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.0 Hz, 2H, H-3" & H-5"), 7.47 (t, J = 7.2 Hz, 1H, H-5"'), 4.47 (s, 2H, H-7"'), 3.68-3.65 (m, 2H, H_e-2' & H_e-6'), 2.88-2.82 (m, 1H, H-4'), 2.59 (dt, J = 12.0, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.13-2.08 (m, 2H, H_e-3' & H_e-5'), 1.98-1.90 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 496 $[M+2]^+$, 494 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 136 $[C_7H_6NO_2]^+$, 111 $[C_6H_4Cl]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(4-nitrobenzyl)thio]-1,3,4-oxadiazole (7v)

Off-white amorphous solid; Yield: 79 %; M.P.: 166-167 ºC; Mol. formula: $C_{20}H_{19}ClN_4O_5S_2$; Mol. weight: 494

gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3156 (Ar C-H), 1648 (C=N), 1594 (Ar C=C), 1546 (N=O), 1458 (S=O), 1242, 1034 (C-O-C), 1187 (C-N), 680 (C-Cl), 625 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.81 (d, J = 7.6 Hz, 1H, H-3''' & H-5'''), 7.68 (d, J = 8.8 Hz, 2H, H-2'' & H-6''), 7.64 (t, J = 7.6 Hz, 1H, H-2''' & H-6'''), 7.49 (d, J = 8.0 Hz, 2H, H-3'' & H-5''), 4.46 (s, 2H, H-7'''), 3.69-3.65 (m, 2H, H_e-2' & H_e-6'), 2.86-2.81 (m, 1H, H-4'), 2.61 (dt, J = 12.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.09 (m, 2H, H_e-3' & H_e-5'), 1.97-1.92 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 496 $[M+2]^+$, 494 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 136 $[C_7H_6NO_2]^+$, 111 $[C_6H_4Cl]^+$, 65 $[C_5H_5]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-iodoethyl)thio]-1,3,4-oxadiazole (7w)

White amorphous solid; Yield: 78 %; M.P.: 211-212 ºC; Mol. formula: $C_{15}H_{17}ClIN_3O_3S_2$; Mol. weight: 513 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3150 (Ar C-H), 1640 (C=N), 1598 (Ar C=C), 1541 (N=O), 1455 (S=O), 1247, 1039 (C-O-C), 1188 (C-N), 690 (C-Cl), 627 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.0 Hz, 2H, H-2'' & H-6''), 7.49 (d, J = 8.4 Hz, 2H, H-3'' & H-5''), 3.81 (t, J = 7.2 Hz, 2H, H-1'''), 3.71-3.65 (m, 2H, H_e-2' & H_e-6'), 3.32 (t, J = 7.2 Hz, 2H, H-2'''), 2.69-2.64 (m, 1H, H-4'), 2.57 (dt, J = 12.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.15-2.10 (m, 2H, H_e-3' & H_e-5'), 1.96-1.89 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 515 $[M+2]^+$, 513 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 155 $[C_2H_4I]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-chloroethyl)thio]-1,3,4-oxadiazole (7x)

White amorphous solid; Yield: 83 %; M.P.: 157-158 ºC; Mol. formula: $C_{15}H_{17}Cl_2N_3O_3S_2$; Mol. weight: 421 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3058 (Ar C-H), 1664 (C=N), 1602 (Ar C=C), 1508 (N=O), 1385 (S=O), 1178, 1030 (C-O-C), 1086 (C-N), 634 (C-Cl), 606 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.66 (d, J = 8.4 Hz, 2H, H-2'' & H-6''), 7.51 (d, J = 8.4 Hz, 2H, H-3'' & H-5''), 3.79 (t, J = 7.2 Hz, 2H, H-1'''), 3.72-3.67 (m, 2H, H_e-2' & H_e-6'), 3.64 (t, J = 7.2 Hz, 2H, H-2'''), 2.73-2.68 (m, 1H, H-4'), 2.56 (dt, J = 12.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.08 (m, 2H, H_e-3' & H_e-5'), 1.97-1.92 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 423 $[M+2]^+$, 421 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 63 $[C_2H_4Cl]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-bromoethyl)thio]-1,3,4-oxadiazole (7y)

Off-white amorphous solid; Yield: 82 %; M.P.: 170-171 ºC; Mol. formula: $C_{15}H_{17}ClBrN_3O_3S_2$; Mol. weight: 464 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3054 (Ar C-H), 1665 (C=N), 1604 (Ar C=C), 1502 (N=O), 1382 (S=O), 1173, 1033 (C-O-C), 1085 (C-N), 638 (C-Cl), 608 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.67 (d, J = 8.0 Hz, 2H, H-2'' & H-6''), 7.49 (d, J = 8.0 Hz, 2H, H-3'' & H-5''), 3.71 (t, J = 7.2 Hz, 2H, H-1'''), 3.70-3.64 (m, 2H, H_e-2' & H_e-6'), 3.62 (t, J = 7.2 Hz, 2H, H-2'''), 2.70-2.66 (m, 1H, H-4'), 2.57 (dt, J = 12.4, 2.0 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.09 (m, 2H, H_e-3' & H_e-5'), 1.95-1.87 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 466 $[M+2]^+$, 464 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 107 $[C_2H_4Br]^+$.

5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(1,3-dioxalan-2-ylmethyl)thio]-1,3,4-oxadiazole (7z)

Light-bluish amorphous solid; Yield: 82 %; M.P.: 118-119 ºC; Mol. formula: $C_{17}H_{20}ClN_3O_5S_2$; Mol. weight: 445 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3056 (Ar C-H), 1667 (C=N), 1608 (Ar C=C), 1504 (N=O), 1387 (S=O), 1175, 1032 (C-O-C), 1080 (C-N), 636 (C-Cl), 602 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, J = 8.4 Hz, 2H, H-2'' & H-6''), 7.49 (d, J = 8.4 Hz, 2H, H-3'' & H-5''), 5.24 (t, J = 4.0 Hz, 1H, H-2'''), 4.01-3.98 (m, 2H, H_e-4''' & H_e-5'''), 3.90-3.86 (m, 2H, H_a-4''' & H_a-5'''), 3.65-3.61 (m, 2H, H_e-2' & H_e-6'), 3.44 (d, J = 4.0 Hz, 2H, H-6'''), 2.90-2.85 (m, 1H, H-4'), 2.65 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.14-2.09 (m, 2H, H_e-3' & H_e-5'), 2.02-1.95 (m, 2H, H_a-3' & H_a-5'); EI-MS (*m/z*): 447 $[M+2]^+$, 445 $[M]^+$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 87 $[C_4H_7O_2]^+$.

RESULTS AND DISCUSSION

The different *S*-substituted derivatives, **7a-z**, of 5-{1-[(4-chlorophenyl)sulfonyl]piperidin-4-yl}-2-mercapto-1,3,4-oxadiazole (**5**) were synthesized by the protocol outlined in Figure 1. The different alkyl/aralkyl groups are listed in Table I. The bioactivity analysis included antibacterial activity against gram-positive

and gram-negative bacteria, and inhibitory activity against AChE and urease enzymes. Computational and BSA binding studies were also carried out to see the interaction of synthesized compounds with amino acid residues and binding capacity with this specific protein, respectively.

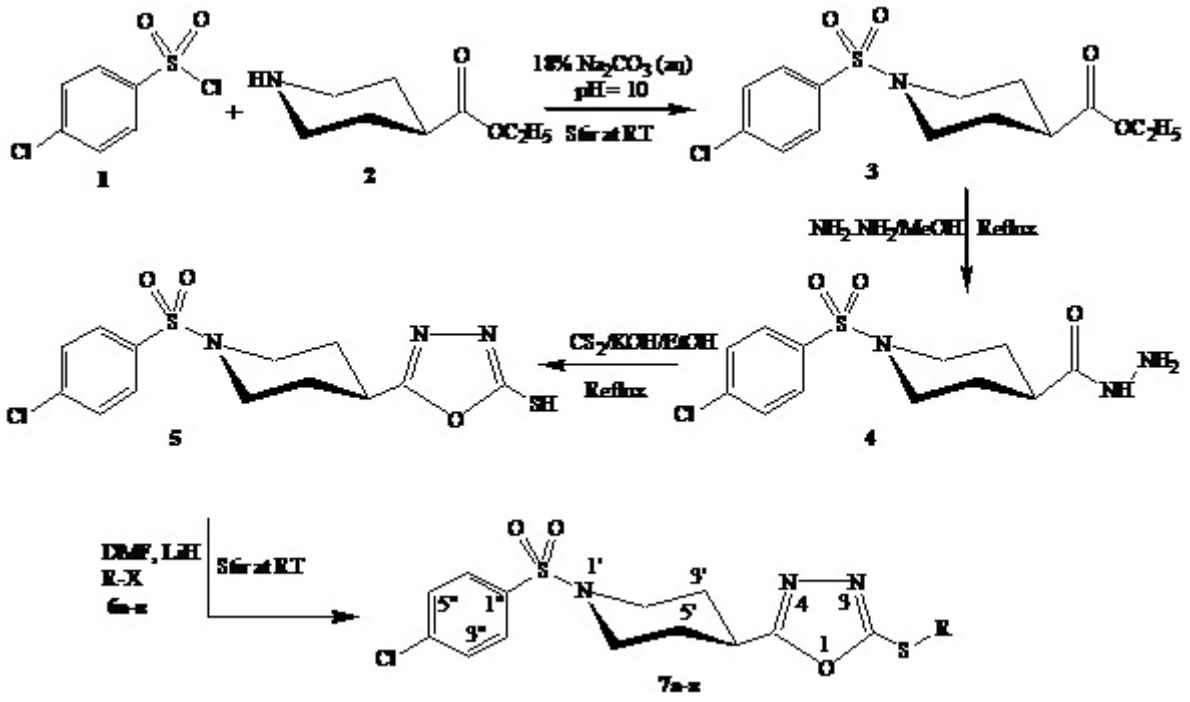

FIGURE 1 - Synthesis of 5-{1-[(4-chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(alkyl/aralkyl)thio]-1,3,4-oxadiazole (**7a-z**).

TABLE I - Different alkyl/aralkyl groups

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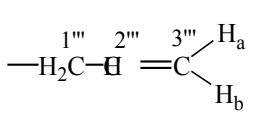
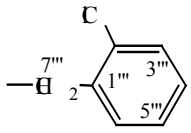
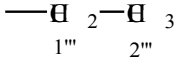
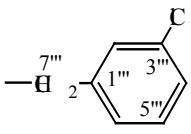
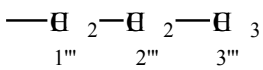
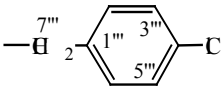
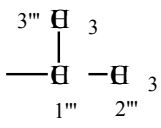
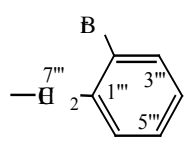
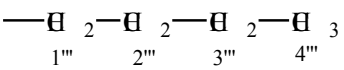
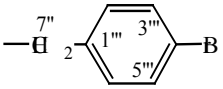
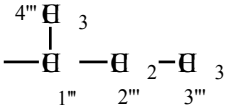
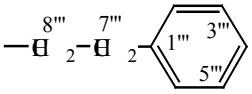
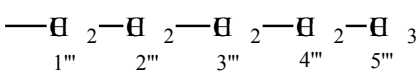
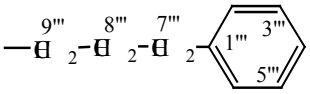
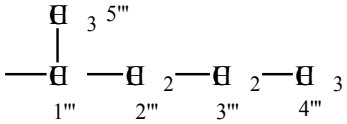
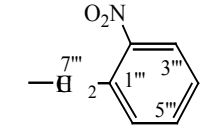
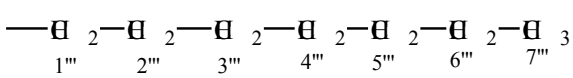
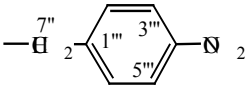
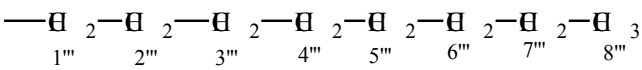
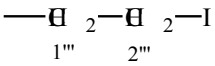
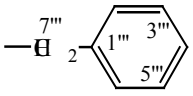
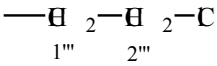
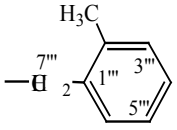
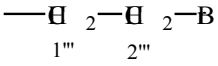
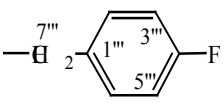
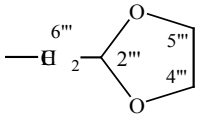
7a		7n	
7b		7o	
7c		7p	
7d		7q	

(continuing)

TABLE I - Different alkyl/aralkyl groups

Com.	R	Com.	R
7e		7r	
7f		7s	
7g		7t	
7h		7u	
7i		7v	
7j		7w	
7k		7x	
7l		7y	
7m		7z	

Chemistry

Compound **7n** is selected for single compound discussion. It was obtained as a white amorphous solid. The % yield was 81 %, and the melting point was determined to be 121-122 ºC. The molecular formula, $C_{20}H_{19}Cl_2N_3O_3S_2$, and molecular mass, 483 $gmol^{-1}$, were established through ^{1}H-NMR and EI-MS spectra. The IR spectrum showed absorptions for the main functionalities present in the molecule, namely 3127 (Ar C-H), 1682 (C=N), 1609 (Ar C=C), 1532 (N=O), 1396 (S=O), 1237, 1023 (C-O-C), 1167 (C-N), 710 (C-Cl) and 646 (C-S). The mass spectrum displayed different fragments that confirmed the molecular structure of all the synthesized compounds. The fragments at *m/z* 286 and 284 demonstrated the partial cleavage of the 1,3,4-oxadiazole ring. The fragment at *m*/*z* 175 was assigned to the 4-chlorophenylsulfonyl

group and that at *m/z* 111 to the 4-chlorophenyl group. All these fragments were shown by all the compounds. The compound **7n** specifically showed two fragments at *m/z* 125 and 65 for 2-chlorobenzyl as substituted tropyllium cation and cyclopentadienyl cation, respectively. The other prominent fragments are given in Figure 2. The two doublets of ^{1}H-NMR signals at δ 7.68 (d, J = 8.8 Hz, 2H, H-2" & H-6") and 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5") were assigned to the 4-chlorophenylsulfonyl moiety; five signals at δ 3.65-3.62 (m, 2H, H_e-2' & H_e-6'), 2.87-2.82 (m, 1H, H-4'), 2.62 (dt, J = 12.4, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.12-2.08 (m, 2H, H_e-3' & H_e-5') and 2.00-1.91 (m, 2H, H_a-3' & H_a-5') were assigned to the piperidine moiety; and the signals at δ 7.53 (dd, J = 7.2, 1.2 Hz, 1H, H-6"'), 7.38 (dd, J = 8.0, 0.8 Hz, 1H, H-3"'), 7.23-7.19 (m, 2H, H-4"' & H-5"') and 4.51 (s, 2H, H-7"') were assigned to the 2-chlorobenzyl group. ^{13}C-NMR also corroborated the carbon skeleton of the molecule by exhibiting the following signals: six at δ (ppm) 168.7 (C-5), 164.1 (C-2), 139.5 (C-1"), 134.8 (C-4"), 134.4 (C-1"') and 133.6 (C-2"') for six quaternary carbons; seven at δ (ppm) 131.4 (C-4"'), 129.7 (C-5"'), 129.6 (C-3"'), 129.5 (C-3" & C-5"), 128.9 (C-2" & C-6"), 127.0 (C-6"') and 32.2 (C-4') for nine methine carbons; and three at δ (ppm) 44.9 (C-2' & C-6'), 34.5 (C-7"') and 28.2 (C-3' & C-5') for five methylene carbons. On the basis of these spectral data, the structure of **7n** was determined to be 5-{1-[(4-chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-chlorobenzyl)thio]-1,3,4-oxadiazole. Likewise, the structures of other synthesized compounds were corroborated through spectral data.

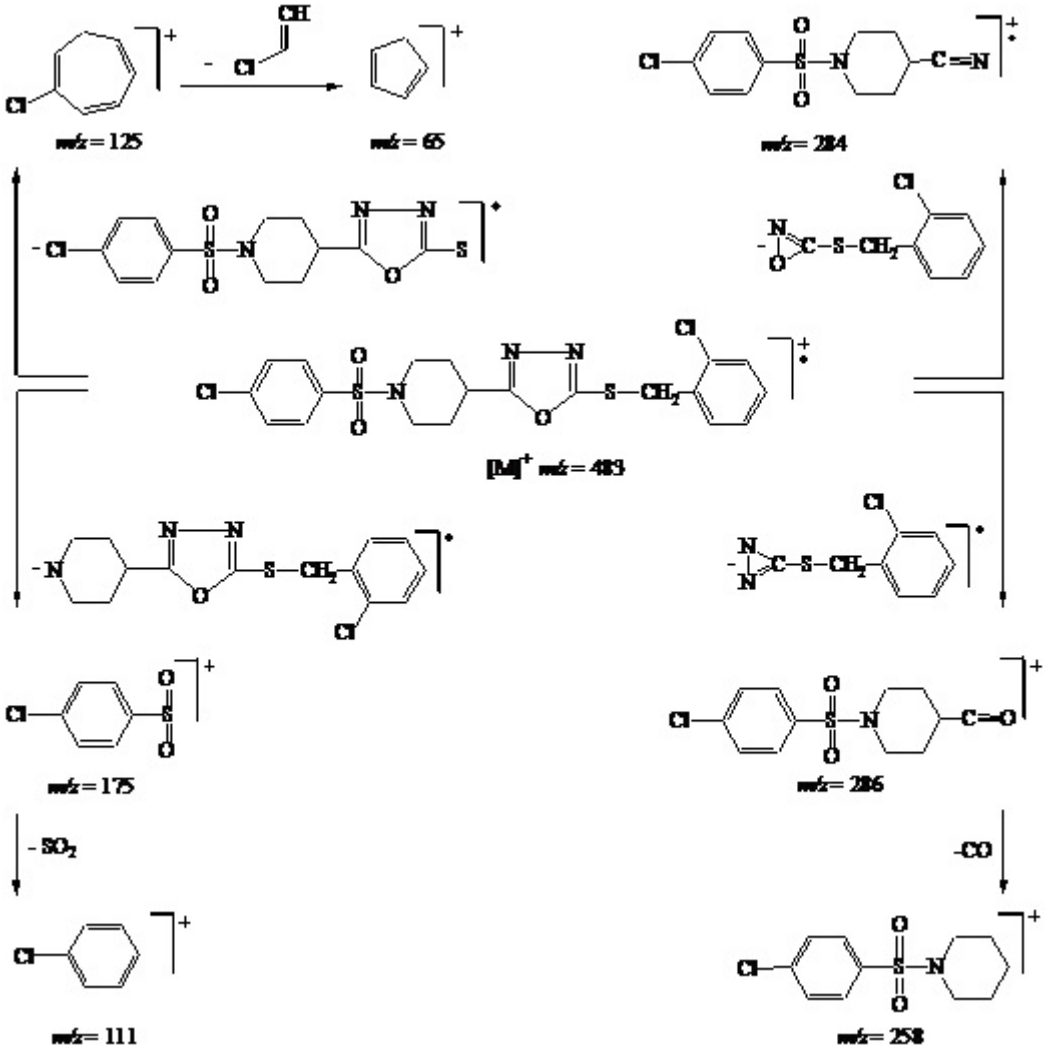


FIGURE 2 - Proposed mass fragmentation pattern of 5-{1-[(4-chlorophenyl)sulfonyl]piperidin-4-yl}-2-[(2-chlorobenzyl)thio]-1,3,4-oxadiazole (**7n**).

Antibacterial activity (in vitro)

Antibacterial activity was evaluated for all the compounds and the results are interpreted as % inhibition and MIC in Table II and Table III, respectively. The reference drug was ciprofloxacin. The results demonstrated slightly moderate to strong antibacterial potential of the synthesized compounds against *S. typhi* and *B. subtilis* and weakly moderate against the other three bacterial strains. Against *S. typhi*, **7q**, **7t**, **7u**, **7w** and **7z** were the most active compounds with MIC values of 9.23±1.34, 9.22±3.28, 9.51±4.91, 8.78±2.50 and 8.23±3.00 µM, respectively. The high activity of these compounds was attributed to the presence of the 2-bromobenzyl, 3-phenylpropyl, 3-nitrobenzyl, 2-iodoethyl and 1,3-dioxalan-2-ylmethyl groups, respectively. The reference drug, ciprofloxacin, showed a MIC value of 7.15±1.29 µM. The most active compound was **7z**, bearing three heterocyclic rings in its structure. The other molecules were also found to be moderately effective growth inhibitors of this bacterial strain. The growth of the bacterial strain *E. coli* was inhibited by almost all the compounds but to a very low extent, although some were moderate inhibitors. The most active compound against this strain was **7b**, bearing an ethyl group, and exhibited a MIC value of 10.32±3.65 µM compared to the reference at 7.90±1.87 µM. Two compounds, **7p** and **7v**, were inactive against this strain. Likewise, *P. aeruginosa* and *S. aureus* were inhibited by all the compounds but to a weak to moderate level. The most active compound against both of the strains was **7u**, bearing a 3-nitrobenzyl group, and showed a MIC value of 10.40±1.67 and 10.58±1.82 µM compared to the reference at 8.21±1.21 and 8.00±2.98 µM for *P. aeruginosa* and *S. aureus*, respectively. Compound **7p** was inactive against both strains, while **7v** and **7y** were also inactive against *S. aureus*. The compounds displayed moderate to strong activity against *B. subtilis*. The compounds **7m**, **7n**, **7s** and **7t** were the most active ones with MIC values of 9.28±4.43, 8.95±1.93, 9.70±2.21 and 9.25±1.43 µM compared to that of the reference drug, i.e., 7.12±2.11 µM. The better activities of these compounds might have been due to the presence of the 4-fluorobenzyl, 2-chlorobenzyl, 2-phenylethyl and 3-phenylpropyl groups, respectively. Overall, the halogenated groups attached to sulfur of 1,3,4-oxadiazole, boosted considerably bioactive potential. No doubt, the antibacterial potential was already increased by the 4-chlorophenyl group present in all the synthesized molecules. The size and position of halogens in the molecule have a considerable effect on biological activities (Liu *et al*., 2012).

TABLE II - The % inhibition for antibacterial activity of synthesized compounds

Compounds	% INHIBITION				
	***S. typhi* (-)**	***E. coli* (-)**	***P. aeruginosa* (-)**	***S. aureus* (+)**	***B. subtilis* (+)**
7a	75.59±0.32	60.79±0.42	62.60±1.99	59.05±1.95	76.92±1.57
7b	73.23±1.86	61.25±0.42	58.06±0.92	57.05±4.05	78.43±1.06
7c	71.00±0.91	67.78±5.00	68.37±0.05	64.85±0.55	77.17±0.40
7d	76.18±1.18	65.23±1.62	68.98±1.02	60.40±2.80	79.80±1.92
7e	63.95±2.77	62.08±2.18	58.88±0.71	56.85±1.65	63.69±4.70
7f	72.77±0.59	64.58±0.88	66.68±0.87	56.65±2.75	72.78±2.37
7g	67.64±1.73	63.06±1.30	63.06±1.22	60.90±1.60	65.30±4.49
7h	70.05±2.14	60.09±1.57	61.07±2.70	55.35±2.85	73.23±0.30

(continuing)

TABLE II - The % inhibition for antibacterial activity of synthesized compounds

Compounds	% INHIBITION				
	***S. typhi* (-)**	***E. coli* (-)**	***P. aeruginosa* (-)**	***S. aureus* (+)**	***B. subtilis* (+)**
7i	63.95±1.23	55.69±0.42	55.87±0.50	51.45±1.45	65.56±4.49
7j	62.68±0.77	54.58±0.79	55.56±3.32	53.05±1.05	61.01±1.62
7k	70.68±0.32	62.18±0.14	63.72±0.36	55.75±1.15	72.17±2.07
7l	76.27±1.00	66.90±1.25	66.58±1.28	64.30±1.50	62.47±2.88
7m	63.55±2.45	55.56±2.87	51.53±4.69	50.45±2.75	67.58±5.00
7n	73.59±0.50	61.62±0.97	65.26±1.28	56.75±4.75	75.20±1.57
7o	66.64±0.18	57.96±1.57	61.28±1.79	60.70±2.70	66.52±5.00
7p	58.50±2.05	39.68±5.00	47.14±3.47	35.75±1.85	56.62±3.99
7q	74.59±0.41	61.39±1.02	63.57±0.41	58.90±2.70	76.57±0.61
7r	63.50±1.77	60.23±1.81	62.14±0.41	57.95±0.95	65.71±2.17
7s	68.91±1.36	62.64±1.69	67.81±1.07	61.95±0.75	68.69±0.20
7t	72.05±2.23	66.30±0.56	69.29±0.20	62.80±1.00	74.19±2.27
7u	72.95±1.05	63.61±1.11	66.07±1.17	62.90±0.60	68.94±4.80
7v	52.86±0.68	47.78±1.94	50.56±3.93	43.45±1.25	57.88±4.75
7w	75.36±0.91	66.62±1.06	68.21±0.05	64.90±0.50	73.74±0.61
7x	61.32±1.14	54.49±0.05	58.27±2.35	56.25±3.25	66.01±3.79
7y	54.91±1.82	58.15±4.44	51.38±2.70	49.40±4.70	57.32±0.15
7z	79.14±0.32	57.31±5.00	60.82±4.69	51.45±4.05	75.05±0.40
Ciprofloxacin	**91.79±1.45**	**90.87±0.56**	**92.13±0.97**	**90.45±2.98**	**91.18±1.22**

TABLE III - The MIC of antibacterial activity of synthesized compounds

Compounds	MIC (µM)				
	S. typhi (-)	***E. coli (-)***	***P. aeruginosa (-)***	***S. aureus (+)***	***B. subtilis (+)***
7a	12.51±2.41	13.77±1.73	14.90±4.50	13.85±1.22	11.92±2.14
7b	10.46±4.74	10.32±3.65	15.91±5.00	12.67±3.11	11.72±3.07
7c	13.07±5.00	12.60±4.23	12.84±1.42	11.12±3.78	10.66±2.93
7d	10.44±4.48	12.24±4.71	12.40±1.17	11.04±4.46	11.96±4.57
7e	15.67±5.00	14.55±4.52	15.31±1.42	13.17±2.50	12.99±1.14
7f	10.22±3.62	13.82±3.27	13.62±2.67	14.69±3.85	12.18±1.36
7g	12.75±2.59	15.37±3.65	12.86±3.08	11.86±5.00	13.26±4.71
7h	13.40±1.03	15.41±0.77	15.00±1.08	17.14±5.00	16.02±3.21
7i	12.53±1.98	17.29±0.58	16.16±2.92	17.75±1.21	12.18±2.64
7j	14.49±2.24	18.31±0.10	17.95±2.17	16.50±1.22	12.41±1.17
7k	11.78±5.00	13.14±2.31	13.55±4.25	11.84±1.95	11.52±4.57
7l	10.36±5.00	10.38±1.83	12.22±1.75	10.65±1.99	12.65±1.36
7m	12.48±3.97	17.10±0.19	19.03±1.50	17.83±5.00	9.28±4.43
7n	10.44±2.33	12.09±3.85	14.20±2.75	12.00±3.18	8.95±1.93
7o	11.68±1.95	15.63±4.33	15.56±2.50	14.25±3.72	11.04±3.36
7p	15.53±4.31	-	-	-	18.19±0.71
7q	9.23±1.34	14.86±1.58	14.73±2.08	11.44±1.22	10.79±2.29
7r	11.81±1.95	11.16±1.35	12.32±1.83	12.01±1.20	10.54±4.57
7s	10.86±4.91	11.20±1.96	12.54±1.92	10.91±0.74	9.70±2.21
7t	9.22±3.28	11.41±1.19	11.83±1.17	10.65±2.23	9.25±1.43
7u	9.51±4.91	10.68±1.10	10.40±1.67	10.58±1.82	10.63±2.79
7v	16.78±1.03	-	19.64±4.33	-	12.84±4.14
7w	8.78±2.50	10.38±2.60	10.68±2.50	10.62±1.50	11.39±5.00

(continuing)

TABLE III - The MIC of antibacterial activity of synthesized compounds

Compounds	MIC (µM)				
	S. typhi (-)	*E. coli (-)*	*P. aeruginosa (-)*	*S. aureus (+)*	*B. subtilis (+)*
7x	15.86±1.60	18.60±2.79	16.99±0.58	15.60±1.35	13.10±4.36
7y	17.61±1.52	17.97±0.38	19.49±3.25	-	16.61±2.57
7z	8.23±3.00	11.20±1.35	11.56±2.67	18.83±3.99	10.78±4.71
Ciprofloxacin	**7.15±1.29**	**7.90±1.87**	**8.21±1.21**	**8.00±2.98**	**7.12±2.11**

AChE inhibition studies

All the synthesized compounds were tested for AChE inhibitory activity and the results (% inhibition and IC_{50}) are given in Table IV. This activity nullifies the effects of AChE causing mental disorders. The main stream of the tested compounds was found active in the variable range from slightly active to highly active because of the collective effect of three heterocyclic moieties shown in the general synthetic scheme. Compounds **7m**, **7n**, **7q**, **7s**, **7t**, **7x** and **7y** were the most active members of this series, acting as anti-AChE agents with IC_{50} values of 95.57±0.21, 72.24±0.18, 52.23±0.23, 51.39±0.21, 65.91±0.23, 46.21±0.22 and 83.56±0.17 µM, respectively, as compared to that of eserine used as a reference standard, i.e., 0.04±0.0001 µM. The high activity of the above compounds might have been due to the presence of electron withdrawing-species in the *N*-substituted aromatic ring shown in Table I. The compounds showing moderate potential against AChE were **7i**, **7j**, **7k**, **7l**, **7p**, **7u** and **7v** with IC_{50} values of 133.25±0.15, 179.34±0.12, 185.42±0.17, 139.81±0.22 and 189.64±0.19 µM, respectively. The presence of bulky groups in the *N*-substituted aromatic ring was responsible for moderate activity. The low activity of compounds **7e** and **7f** was due to the presence of electron-rich moieties in the *N*-substituted ring. Among the active members of this series, compounds **7q**, **7s** and **7x** were the most active agents and could be recommended for *in vivo* studies. The highest inhibition potential of these highly active members could have been due to the 2-bromobenzyl, 2-phenylethyl and 2-chloroehyl substituents, respectively.

TABLE IV - Acetyl cholinesterase (AChE) and Urease inhibition studies

Compounds	AChE		Urease	
	Inhibition (%) at 0.5 mM	IC_{50} (µM)	Inhibition (%) at 0.25 mM	IC_{50} (µM)
7a	28.32±0.16	-	28.72±0.12	-
7b	37.95±0.18	-	32.43±0.17	-
7c	25.74±0.14	-	35.56±0.13	
7d	23.45±0.17	-	98.14±0.11	23.58±0.02

(continuing)

TABLE IV - Acetyl cholinesterase (AChE) and Urease inhibition studies

Compounds	AChE		Urease	
	Inhibition (%) at 0.5 mM	IC_{50} (µM)	Inhibition (%) at 0.25 mM	IC_{50} (µM)
7e	67.34±0.26	397.51±0.19	34.83±0.16	-
7f	69.35±0.29	334.72±0.21	32.45±0.15	-
7g	44.52±0.22	-	25.87±0.14	-
7h	18.39±0.25	-	24.65±0.18	-
7i	84.34±0.29	129.25±0.18	24.76±0.12	-
7j	26.82±0.18	-	32.12±0.15	-
7k	82.62±0.26	179.34±0.18	35.24±0.13	-
7l	83.24±0.28	178.52±0.22	96.72±0.04	2.14±0.003
7m	91.52±0.29	95.57±0.16	98.13±0.02	0.63±0.001
7n	91.39±0.22	72.24±0.14	97.54±0.03	2.17±0.006
7o	84.27±0.28	139.81±0.19	97.16±0.05	1.13±0.003
7p	83.53±0.29	187.64±0.23	97.24±0.04	1.21±0.005
7q	90.65±0.21	47.23±0.16	29.75±0.13	-
7r	35.74±0.15	-	96.56±0.02	6.28±0.003
7s	90.73±0.24	51.39±0.17	32.81±0.13	-
7t	90.29±0.21	72.91±0.15	36.75±0.12	-
7u	83.63±0.29	183.72±0.21	97.62±0.04	2.39±0.005
7v	79.52±0.28	215.53±0.23	97.34±0.07	2.15±0.002
7w	18.22±0.14	-	14.11±0.12	-
7x	90.38±0.25	49.21±0.17	96.26±0.06	2.26±0.003
7y	90.52±0.26	83.56±0.16	97.54±0.03	2.14±0.002
7z	16.56±0.12	-	23.85±0.14	-
Eserine	**91.27±1.17**	**0.04±0.0001**	**98.21±0.18**	**21.25±0.15**

Urease inhibition studies

All the synthesized compounds were tested for anti-urease activity with reference to thiourea used as the standard. The results showed that all the compounds had outstanding urease inhibitory activity with a few exceptions (Table IV). The compounds were even much more active compared to the standard used. Compounds **7l**, **7m**, **7n**, **7o**, **7p**, **7r**, **7u**, **7v**, **7x** and **7v** were highly active against urease with IC_{50} values of 2.14±0.003, 0.63±0.001, 2.17±0.006, 1.13±0.003, 1.21±0.005, 6.28±0.003, 2.39±0.005, 2.15±0.002, 2.26±0.003 and 2.14±0.002 µM compared to thiourea with an IC_{50} value of 21.25±0.15 µM. The high level of anti-urease activity might have been attributed to the presence of electron-withdrawing substituents (Table I), such as **7m** with a 4-fluorobenzyl group, the most active compound of the series. All the tested compounds showed exceptional urease inhibitory potential and could be used to design new drugs to control the negative effects of urease after *in vivo* studies.

*Docking study for binding of **7x** with AChE*

The ***7x*** compound was used to evaluate its molecular docking in AChE inhibition studies. **7x** showed the highest inhibitory potential against AChE. It was necessary to identify the active site present in the molecule, responsible for its best activity. To understand the possible factors responsible for its better activity, its docking studies were carried out. The Surflex-Dock module of SybylX-1.3 was used to dock ligand **7x** into the active site of AChE enzyme (Figure 3). The ~~authenticity~~ reliability of our docking procedure was attained by the extraction of donepezil and re-docking into the active cite of AChE. The comparison of docked poses and experimentally determined binding mode was done by donepezil in AChE. Molecular docking results of ligand poses were overlapped with the crystallographic confirmation of donepezil in AChE, which showed the reliability of docking studies. The graphic analysis of docking results (Figure 3) demonstrated that ligand **7x** penetrated deeply into the two main active sites of AChE (CAS and PAS). In the active site of AChE, the ligand acquired such a binding conformation, so that the nitrogen of the oxdiazole ring (B-ring) was able to form a H-bond with the backbone NH_2 of Phe295 (Figure 3). The SO_2 group of 4-chlorobezene sulfonyl was also able to form a H-bond with NH_2 groups of TYR337 and TRP86. The chlorine of the 4-chlorobezene sulfonyl group (A-ring) was shown to penetrate deeply into the CAS region where

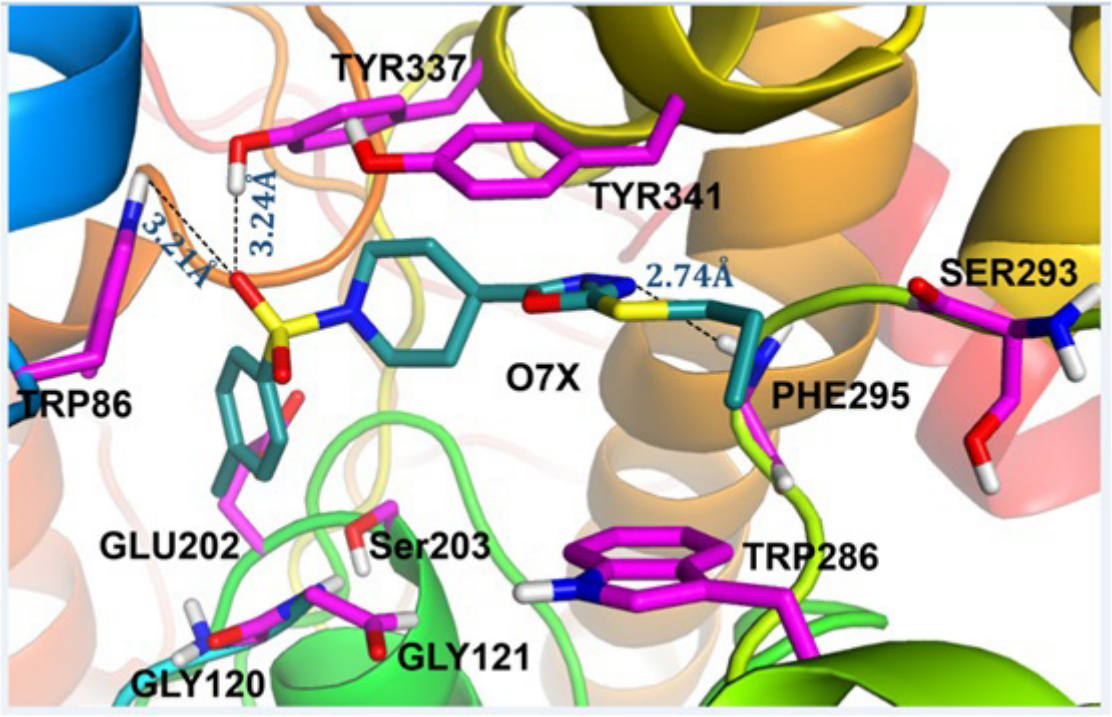


FIGURE 3 - Docked conformation of AChE inhibitors **7x** (Cyan), **7x** (Magenta) superimposed over experimental conformation of donepezil (Magenta) in AChE main binding site.

its Cl at C4 of ring-A could establish a hydrophobic or van der Waals interaction with surrounding residues Glu202, Ser203 and Gly120. The chlorine of the ethyl group substituted at the *S* position extended well near the solvent-exposed region where it could establish H-bonds with the backbone and side chain of the surrounding residue Phe295. The following variations in ligand-receptor interaction pattern could account for the higher AChE inhibitory potency of compound **7x**.

BSA binding studies using fluorescence measurements

Nitrogen-containing compounds are well known for their broad spectrum of biological applications. Oxadiazole and its derivatives are the class of compounds having such functionalities with various biological applications such as antibacterial, antispasmodic, anti-inflammatory, antiplatelet aggregation, tumor cell growth inhibition and differentiation, tumor cell apoptosis, etc. (Holt, Chaires, Trent, 2008). The current study was designed to determine the binding of the synthesized compounds to BSA to better understand their pharmacological effects. The selected synthesized compounds were used to evaluate the effects of different substituents on BSA binding capacity. The binding of these compounds was determined by fluorometric titrations of BSA with varying concentration of the derivatives (0 - 5.57 x 10^{-4} M). Their binding abilities and binding sites were compared to that of the fluorescence markers warfarin and ibuprofen.

BSA shows a maximum emission at λ_{em} = 336 nm. Addition of the synthesized compounds was found to quench the fluorescence intensity of BSA, showing that these derivatives were able to bind to BSA, causing some modification in the microenvironment around the amino acid residues situated in the sub-domain of BSA, leading to a hypochromic shift in the fluorescence spectrum. Fluorescence quenching can be classified as either a static or dynamic process and is caused by different mechanisms including excited state reactions, molecular rearrangements, energy transfer, formation of ground state complex and collisional quenching (Wang *et al*., 2013). The type of quenching mechanism is usually interpreted by the Stern-Volmer equation:

$$F_o / F = 1 + K_{SV} [Q] = K_q \tau_o [Q] + 1 \qquad \text{Equation 1}$$

where F_o and F are the fluorescence intensities of BSA respectively before and after addition of quencher, K_{sv} is the Stern-Volmer quenching constant, [Q] is the concentration of the quencher, K_q is the apparent bimolecular quenching rate constant, τ_o is the average lifetime of the biomolecule without the quencher and its value is 10^{-8} s (Zhang *et al*., 2012). The emission spectra of the test compounds and their respective Stern-Volmer plots are given in Figure 4.

In general, the calculated K_q values (Table V) were found to be larger than the maximum scattering collision quenching constant (2×10^{10} M^{-1} s^{-1}) in dynamic quenching, which indicated that the fluorescence quenching process of BSA with the synthesized compounds, as well as warfarin and ibuprofen, were primarily governed by a static quenching mechanism (Wang *et al*., 2013), rather than a dynamic one. The binding constant K_a and the number of binding sites n of the compounds were determined using the double logarithm Equation 2.

$$\text{Log } [(F_0 - F) / F] = \log K_a + n \log[Q] \qquad \text{Equation 2}$$

The double logarithm plots of the different compounds are shown in Figure 5 and their binding constant Ka is given in Table V. The presence of halogen atoms in (**7o** and **7m**) was found to decrease the binding constant. However, compound **7u** showed the strongest binding with BSA, which was due to the presence of a nitro group at the meta position, enhancing the binding of **7u** to BSA. The strong binding of compound **7u** to BSA may account for its high antibacterial potential.

CONCLUSION

The synthesized compounds with notable yields were structurally identified through spectral data. The interactions with amino acid residues as well as with specific proteins responsible for the pharmacological activity of the synthesized compounds were availed through docking and BSA binding interactions, respectively. The antibacterial activity results demonstrated moderate to very high activity against *S. typhi* and *B. subtilis* but weak to moderate activity against the other three bacterial strains tested. The presence of halogenated groups in the molecules had a great effect on the antibacterial potential of the synthesized molecules. Similarly, all the compounds proved to be very active against AChE. But above all, the urease inhibition potential of these compounds was very impressive compared to the standard used. Thus, the molecules with

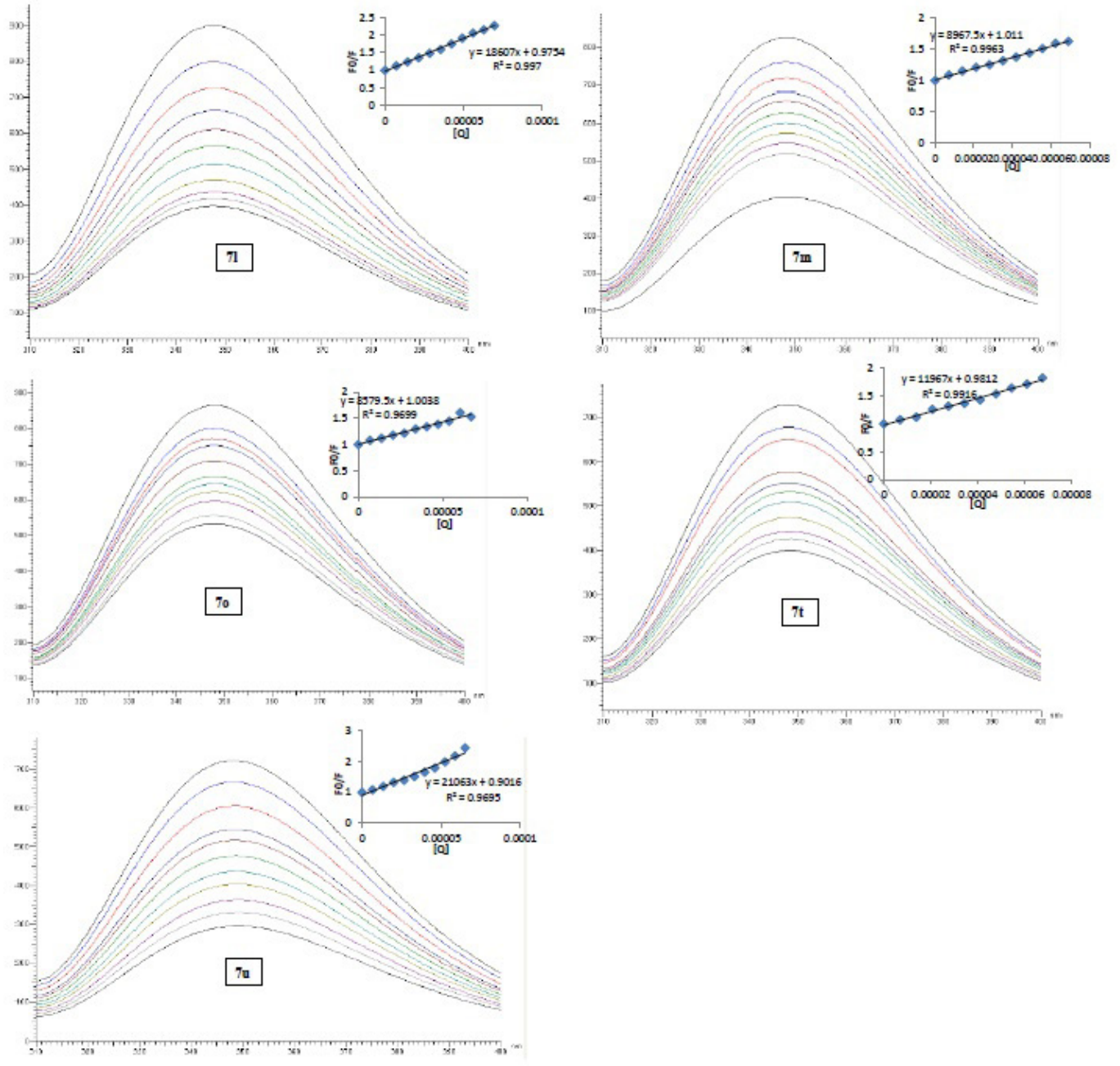


FIGURE 4 - Fluorescence emission spectra of BSA and different amounts of selected compounds (**7l**, **7m**, **7o**, **7t**, **7u**) at 295 nm and 298 K (inset graph corresponding to the Stern-Volmer plot).

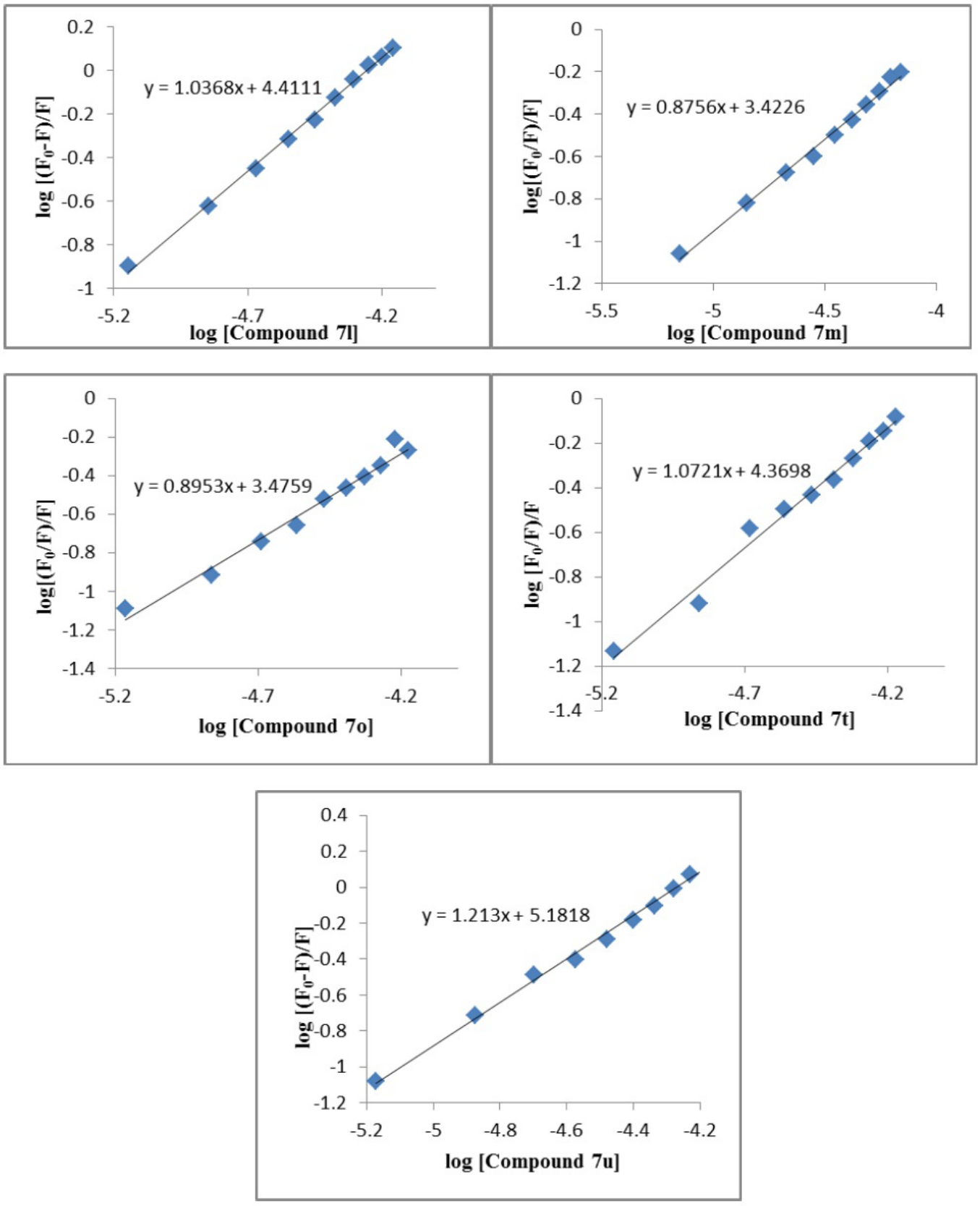


FIGURE 5 - Double logarithm plots of compounds **7l**, **7m**, **7o**, **7t** and **7u**.

TABLE V - Binding constant and number of binding site of compounds warfarin and ibuprofen with BSA at 298K

Compounds	Ksv(L/mol) x 10^4	Kq(L/mols^{-1}) x 10^{12}	Kq(L/mols^{-1}) x 10^{12}	Ka (L/mol)	n
7l	1.8607	1.8607	1.8607	2.58 x 104	1.04
7m	0.8967	0.8967	0.8967	2.65 x 103	0.88
7o	0.8579	0.8579	0.8579	2.99 x 103	0.90
7t	1.1967	1.1967	1.1967	2.34 x 104	1.07
7u	2.1063	2.1063	2.1063	1.52 x 105	1.21

high bioactive potential could be considered for further *in vivo* studies and as candidates in drug discovery programs in the pharmaceutical industry.

ACKNOWLEDGEMENT

The authors acknowledge the Higher Education Commission (HEC) of Pakistan for the financial assistance for the completion of this project. Dr. A. Leyva (USA) helped with English editing of the manuscript.

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Received for publication on 4th February 2018
Accepted for publication on 2nd January 2019