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In vitro biological assessment of 1,3,4-oxadiazole sandwiched by azinane and acetamides supported by molecular docking and BSA binding studies

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The 1,3,4-Oxadiazole is an aromatic heterocyclic moiety recognized in drug research for its low lipophilicity. The multiple functionalities, heterocyclic azinane, sulfonamide, 1,3,4-oxadiazole and acetamide, are combined collectively to enhance the bioactivity potential of synthesized molecules. All the compounds were acquired by following microwave assisted and conventional techniques in a comparative way. The synthesized derivatives were screened for their antibacterial and enzyme inhibition potential. Furthermore, BSA binding analysis was executed to infer about the interaction with serum albumin. The spectral data of IR, EI-MS, ^{1}H-NMR and ^{13}C-NMR were used to elucidate the final structures of compounds. The synthesized compounds had a modest antibacterial potential. Compound **8f** bearing 2-methyl-4,5-dinitrophenyl group was the most active one against all the bacterial strains taken into account and α-glucosidase enzyme. Compound **8d** bearing 4-nitrophenyl group was the best acetyl cholinesterase inhibitor and **8i** bearing phenylethyl group was the best urease inhibitor.

1. Introduction

The microbial resistance against current drugs and increasing health related issue because of enzymatic activities within the human body has much influenced the drug discovery program. [1] In the field of pharmacology, the heterocyclic cores have gained much importance owing to their high bioactivity. [2] A number of bioactive compounds known to possess high bioactive potential including synthetic and natural are based on nitrogen containing heterocyclic core. [3] These bioactive compounds are known to be employed as solvent, food additive, curing agent in rubber industry and intermediate in inorganic synthesis. [4] Among the heterocyclic cores, oxadiazole and especially 1,3,4-oxadiazole has presented a range of biological activities. [2,5] The notable activities of this heterocyclic core include anti-tuberculosis, antidepressant, analgesic, anti-mycobacterial, anti-inflammatory, anti-tumor, anticancer, anticonvulsant, anti-HIV, antimicrobial and anti-malarial ones. [6-9] Because of stability, better ability to transport charge and fluorescent properties that are provided by the aryl groups, condensed multi-aryl heterocyclic compounds have attracted particular interest in the fields of electrochemistry, photochemistry, biochemistry, and advanced functional materials. [10] The synthesis of heterocyclic compounds is a vast field as evident from a lot of literature review. We have given some highlights regarding synthetic strategies. Cycloaddition reaction is employed as a universal method for synthesis of heterocylic compounds. [11-14] Many C-C and C-X (X = N, O, S) bonds may be produced in a one-pot approach with regio-selective and stereo-selective properties through cycloaddition

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protocol which has become the most significant method for the synthesis of fused polyheterocycles. Particular focus has been placed on the production of four-, five-, six-, and seven-membered rings by [2 + 2], [3 + 2], [4 + 2], and [5 + 2] cycloaddition reactions. Recent developments have enabled the environment friendly and economically appealing synthesis of fused polycyclic heteroarenes such as indoles, isoquinolines, benzothiazoles, and pyridines. This is particularly true of the oxidative cycloaddition of alkynes through C-H bond cleavage. [15]

Kula et al. have worked a lot for the synthesis of heterocyclic compounds through [3+2] cycloaddition. They have presented new aldimine *N*-oxides precursors for cycloaddition, [16] cycloaddition of diaryldiazomethanes and (E)-3,3,3-trichloro-1-nitroprop-1-ene, [17] study on thermal decomposition of 3,3-diphenyl-4-(trichloromethyl)-5-nitropyrazoline, [18] and cycloaddition of benzonitrile *N*-oxide and nitroalkenes. [19] Kula K. as a co-worker employed this cycloaddition methodology for the synthesis of different analogs of pyrazolines, [20] nicotine, [21] isoxazolines, [22,23] and pyrrolidines. [24] Here, we synthesized certain compounds with improved bioactive potential that have multiple heterocyclic moieties which are naturally bioactive. Utilizing both conventional and microwave assisted synthetic techniques; the entire library of compounds was synthesized. Additionally, to increase the biological potential, the diverse active heterocyclic cores sulfonamide, thioether, and acetamide have been merged into a single unit as given in **Fig. 1**.

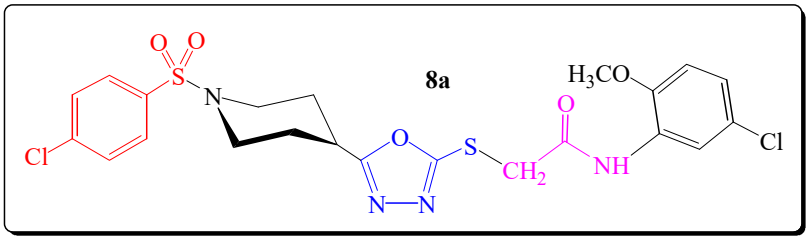
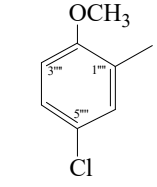
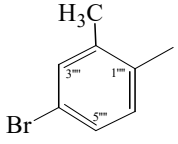
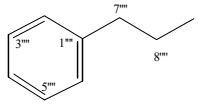
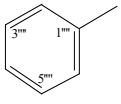
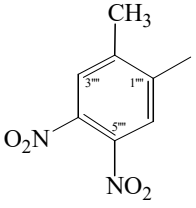
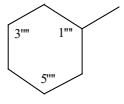
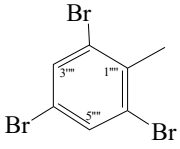
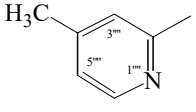
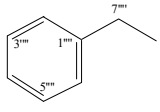
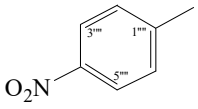
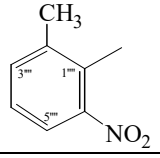


Fig. 1. Heterocyclic cores bearing sulfamoyl, thioether and acetamide functionality

2. Materials and methods

Microwave assisted and conventional techniques on comparative grounds were employed to avail the library of compounds presented in **Scheme 1** and **Table 1**. It is well justified by the results given in **Table 2** and **Table 3**, that the microwave assisted synthetic protocol is outstanding in terms of better yield in minimum time. All synthesized compounds were evaluated for their antibacterial and anti-enzymatic potential.

Table 1. Different varying substituents

Comp	R	Comp	R	Comp	R
8a		**8e**		**8i**	
8b		**8f**		**8j**	
8c		**8g**		**8k**	
8d		**8h**		**8l**	

Finally, the structure activity relationship (SAR) was established with the assistance of BSA binding interaction and docking studies. Chemical reagents were Sigma Aldrich and Alfa Aesar branded and purchased from local suppliers. Analytical grade solvents were employed for synthetic work. The purification of compounds was checked by TLC, prepared on aluminum plates by coating silica gel. Mobile phase was made from *n*-hexane and EtOAc; and UV_{254} lamp was used for visualization of spots. Gallonkemp apparatus was applied to get the melting points and were uncorrected. JMS-HX-110 spectrometer, Bruker spectrometer and Jasco-320-A spectrophotometer were utilized to record EIMS with data system, ^{1}H-NMR (400 MHz) & ^{13}C-NMR (100 MHz) in $CDCl_3$ and IR spectra (by KBr pellet method) respectively.

Table 2. Comparison of conventional and microwave assisted methods

Compounds	Reaction Yield (%)	
	Microwave	Conventional
8a	94	67
8b	76	58
8c	81	63
8d	90	78
8e	93	74
8f	69	48
8g	87	65
8h	93	66
8i	92	72
8j	92	80
8k	83	71
8l	91	68

Table 3. Comparison of conventional and microwave assisted methods

Compounds	Reaction time	
	Microwave (seconds)	Conventional (hours)
8a	54	12
8b	56	15
8c	53	17
8d	76	16
8e	55	12
8f	69	14
8g	73	15
8h	60	13
8i	78	16
8j	87	18
8k	59	17
8l	59	15

2.1 Synthesis

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

Ethyl piperidin-4-carboxylate (0.062 mol; **2**) and 4-chlorobenzenesulfonyl chloride (0.062 mol; **1**) were set to stir for 4 hours in water. During the reaction, pH was sustained to 9-10 through addition of 20 % aqueous Na_2CO_3 solution. After supervision by TLC, precipitates were acquired by pouring excess cold distilled water. Product was filtered, washed and dried.

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

Hydrazine hydrate (0.039 mol) and compound **3** (0.039 mol) were refluxed for 3.5 hours in EtOH. After single spot by TLC, product was attained as precipitates on addition of excess distilled water and then filtered, washed and dried.

2.1.3 Synthesis of 5-[1-(4-chlorophenylsulfonyl)-4-piperidinyl]-1,3,4-oxadiazol-2-thiol (5)

Carbon disulphide (0.070 mol) and compound **4** (0.035 mol) were refluxed for 6 hours in EtOH containing pre-dissolved potassium hydroxide (0.035 mol). After final supervision by TLC, the mixture was diluted by excess cold distilled water and acidified to pH of 4-5 by dilute HCl. The precipitates of product were filtered, washed, dried and re-crystallized by EtOH.

2.1.4 General procedure for synthesis of N-aralkyl/phenyl/aryl-2-bromoacetamides (7a-l)

2-Bromoacetyl bromide (0.033 mol) and aralkyl/phenyl/aryl amines (0.033 mol; **6a-l**) were set to stir for 2 hour in distilled water. During the reaction, pH was sustained to 9-10 through addition of 20% aqueous Na_2CO_3 solution. Reaction was supervised by TLC. The precipitates of product were filtered, washed and dried.

2.1.5 General synthesis of 5-{1-[(4-chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(aralkyl/phenyl/aryl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8a-l)

Conventional method: Compound **5** (0.0044 mol) was activated in DMF with NaH by stirring for 0.75 hour. Then, during stirring, *N*- aralkyl/phenyl/aryl-2-bromoacetamides (0.0044 mol; **7a-l**) were added and stirred for 660-1080 minutes (12-18 hours). TLC was used to observe the reaction. After adding excess cold distilled water, the final products were filtered, rinsed and dried.

Microwave method: Stirring compound **5** (0.0044 mol) in DMF with NaH, activated it. Then, while stirring, *N*-aralkyl/phenyl/aryl-2-bromoacetamides (0.0044 mol; **7a-l**) were added and stirred for half an hour. To synthesis our desired series of compounds **8a-l**, the reaction mixture was microwave irradiated for 53-87 seconds. TLC was used to examine the reaction's progress. After adding excess cold distilled water, the final products were filtered, rinsed and dried. The products were retained for structural analysis and biological evaluation. In comparison to conventional synthesis, the purity and yield of compounds using microwave aided synthesis were outstanding in a very short time.

2.2 Antibacterial activity assay

Antibacterial activity was evaluated by using sterilized 96-wells microplates kept under aseptic conditions following the reported method. [9,25] Method was modified a little for the size of volume made along with that of sample taken.

2.3 Acetyl cholinesterase assay

Following the published methodology [26] with slight modifications, acetyl cholinesterase inhibitory activity was measured. It was chosen to use a mixture of Na_2HPO_4 buffer (pH 7.7), 10 µL test compound (0.5 mM well^{-1}) and 10 µL acetyl cholinesterase (0.005 mM well^{-1}). Before incubation, the mixture's absorption was measured at 405 nm, and the entire combination was incubated for 15 minutes at 35 °C. The reaction was begun by adding 10 µL of 0.5 mM well^{-1} acetyl thiocholine iodide as substrate and 10 µL of DTNB (0.5 mM well^{-1}). The absorption was measured at 405 nm using a 96-well plate reader Synergy HT, Biotek, USA, after 15 minutes of incubation. For the positive control research, Eserine (0.5 mM well^{-1}) was employed as a standard. The equation given below assisted us to calculate the percent inhibition.

$$\text{Inhibition (\%)} = \frac{\text{Control - Test}}{\text{Control}} \text{ x } 100$$

The activity in the presence of the relevant substances is referred as test, while the activity in the absence of the inhibitor is referred as control. The average value of IC_{50} was computed using the EZ-Fit enzyme kinetics program (Perrella Scientific Inc., Amherst, USA).

2.4 α-Glucosidase inhibition activity assay

The α-glucosidase inhibitory activity was estimated with minor adjustments using the reported method. [27] A homogenous mixture of 100 µL containing 70 µL of 50 mM phosphate buffer saline (pH 6.8), 10 µL (0.5 mM) test chemical, and 10 µL (0.057 units) *α*-glucosidase was mixed and absorbance was measured before incubation at 400 nm at 37 ºC. The reaction was started by adding 10 µL of 0.5 mM substrate (*p*-nitrophenylglucopyranoside) to the reaction mixture. Acarbose was used as a positive control in the study. For half an hour, the cells were incubated at 37 ºC. Absorption was measured at 400 nm using a Synergy HT microplate reader after incubation. All calculations were done in triplicate. The % inhibition and IC_{50} was tested by using the previous equation as mentioned for acetyl cholinesterase.

2.5 Urease inhibition assay

A volume of 985 microliter mixture having test compound (0 to 100 µL), urea (850 µL) and phosphate buffer (100 mM, pH 7.4) was made to access the anti-urease activity by the reported method. [28] The reaction was initiated by adding 15 µL of urease enzyme. After 60 minutes at 37 °C for 30 minutes, the extent of reaction was measured using 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 solution B (containing 250 mg sodium hydroxide and 820 µL of sodium hypochlorite 5 percent in 50 mL of distilled water). The inhibition of urease activity was formerly regarded as a 100 percent control activity. The percent inhibition and IC_{50} was calculated using the same formula as for the acetyl cholinesterase enzyme.

2.6 Molecular docking

The co-crystal 3D-structure recombinant human AChE in association with donepezil (PDB entry: 4EY7) was retrieved from the RCSB Protein Data Bank in order to examine the interaction of ligands in the active site of AChE utilising chemo informatics (PDB). Prior to docking investigations, all protein structures were further processed using the structure preparation tools contained in the SYBYL-X 1.3 biopolymer module. Following the energy minimization using the Powell

algorithm with a convergence gradient of 0.5 kcal mol^{-1} for 1000 cycles, missing hydrogens were added, charges were applied, and atom types were assigned according to the AMBER 7 FF99 force field. Furthermore, the examined inhibitors' 3D structures were created using Sybyl-X 1.3's SKETCH module, followed by energy reduction utilizing the Tripos force field with GasteigereHückel atomic charge. The Surflex-Dock module of SybylX-1.3 was used to perform the molecular docking studies. [29] By protomol (an idealised active site) creation, the empirically determined active conformation of donepezil in the AChE active site was employed as a starting conformation to identify the possible binding pocket. [30] The identified active site can be used to generate a variety of ligand positions. [31] The Hammerhead scoring method was used to rate these potential ligand poses. [29,32] All factors controlling protomol extent were left at their default values (threshold = 0.50 and bloat = 0). Finally, the synthesised compounds were docked in the idealised active site of AChE using a "whole" molecular alignment algorithm, and the best twenty docked postures for each inhibitor were preserved.

2.7 BSA protein binding interactions using fluorescence measurements

Fluorometric titration of a solution of BSA (3 mL, 3 M) in phosphate buffer (20 mM, pH 7.4) with the produced compounds solutions (1 mg/mL in DMSO solvent) was used to determine the BSA quenching constant. At 298 K, the BSA solutions with and without test compounds were stimulated at 295 nm, and the intensity of the BSA solutions with and without test compounds was recorded at 336 nm. The aforesaid experiment was performed at two different temperatures for thermodynamic investigations of BSA and produced derivatives, 303 K and 308 K for each synthesized compound, respectively. At three temperatures of 298, 303, and 308 K, three sets of fluorescence spectra were obtained. Fluorometric titration of BSA solution (3 mL, 3 M) with and without site markers (Ibuprofen or warfarin) was carried out with synthesized compound solutions (1 mg/mL in DMSO solvent) for site selective binding investigations. The solutions were scanned at 298 K with a 295 nm excitation wavelength. [32]

2.8 Characterization of the synthesized compounds

2.8.1 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(5-chloro-2-methoxyphenyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8a)

Off white amorphous solid; Yield: 79 %; m.p. 153-154 °C; Molecular formula: $C_{22}H_{22}Cl_2N_4O_5S_2$; Molecular Mass: 557 $gmol^{-1}$; IR (KBr, υ_{max}, cm^{-1}): 3321 (N-H), 3029 (Ar C-H), 1649 (C=O), 1675 (C=N), 1607 (Ar C=C), 1393 (S=O), 1174, 1032 (C-O-C), 1059 (C-N), 678 (C-Cl), 614 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.17 (br.s, 1H, -NH), 8.34 (d, J = 2.0 Hz, 1H, H-6""), 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 6.99 (dd, J = 8.8, 2.8 Hz, 1H, H-4""), 6.73 (d, J = 8.4 Hz, 1H, H-3""), 3.99 (s, 2H, H-2"'), 3.81 (s, 3H, CH_3O-2""), 3.70-3.67 (m, 2H, H_e-2' & H_e-6'), 2.90-2.85 (m, 1H, H-4'), 2.61 (dt, J = 12.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.15-2.11 (m, 2H, H_e-3' & H_e-5'), 2.01-1.95 (m, 2H, H_a-3' & H_a-5'); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ (ppm) 169.3 (C-5), 165.0 (C-2), 164.3 (C-1""), 146.8 (C-2""), 139.6 (C-1"), 134.8 (C-4"), 129.5 (C-3" & C-5"), 129.0 (C-2" & C-6"), 128.2 (C-1""), 126.0 (C-5""), 123.8 (C-4""), 119.9 (C-6""), 110.9 (C-3""), 56.0 (CH_3O-2""), 45.0 (C-2' & C-6'), 36.3 (C-2"'), 32.4 (C-4'), 28.3 (C-3' & C-5'); EIMS (*m/z*): 559 $[M+2]^+$, 557 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 184 $[C_8H_7ClNO_2]^+$, 175 $[C_6H_4ClO_2S]^+$, 156 $[C_7H_7ClNO]^+$, 111 $[C_6H_4Cl]^+$.

2.8.2 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(phenyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8b)

White amorphous solid; Yield: 83 %; m.p. 183-184 °C; Molecular formula: $C_{21}H_{21}ClN_4O_4S_2$; Molecular Mass: 493 $gmol^{-1}$; IR (KBr, υ_{max}, cm^{-1}): 3348 (N-H), 3039 (Ar C-H), 1649 (C=O), 1688 (C=N), 1619 (Ar C=C), 1374 (S=O), 1156, 1022 (C-O-C), 1046 (C-N), 659 (C-Cl), 642 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.06 (br.s, 1H, -NH), 7.70 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.51 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.48 (d, J = 8.8 Hz, 2H, H-2"" & H-6""), 7.29 (t, J = 7.6 Hz, 2H, H-3"" & H-5""), 7.09 (t, J = 7.6 Hz, 1H, H-4""), 3.92 (s, 2H, H-2"'), 3.73-3.70 (m, 2H, H_e-2' & H_e-6'), 2.90-2.84 (m, 1H, H-4'), 2.58 (dt, J = 12.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.16-2.12 (m, 2H, H_e-3' & H_e-5'), 2.02-1.92 (m, 2H, H_a-3' & H_a-5'); EIMS (*m/z*): 495 $[M+2]^+$, 493 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 120 $[C_7H_6NO]^+$, 111 $[C_6H_4Cl]^+$, 92 $[C_6H_6N]^+$.

2.8.3 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(2,4,6-tribromophenyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8c)

White amorphous solid; Yield: 81 %; m.p. 190-191 °C; Molecular formula: $C_{21}H_{18}Br_3ClN_4O_4S_2$; Molecular Mass: 729 $gmol^{-1}$; IR (KBr, υ_{max}, cm^{-1}): 3353 (N-H), 3032 (Ar C-H), 1653 (C=O), 1683 (C=N), 1612 (Ar C=C), 1375 (S=O), 1159, 1020 (C-O-C), 1049 (C-N), 656 (C-Cl), 640 (C-S), 627 (C-Br); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.22 (br.s, 1H, -NH), 7.69 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.40 (s, 2H, H-3"" & H-5""), 3.89 (s, 2H, H-2"'), 3.75-3.71 (m, 2H, H_e-2' & H_e-6'), 2.90-2.84 (m, 1H, H-4'), 2.58 (dt, J = 12.0, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.16-2.12 (m, 2H, H_e-3' & H_e-5'), 2.01-1.95 (m, 2H, H_a-3' & H_a-5'); EIMS (*m/z*): 737 $[M+8]^+$, 735 $[M+6]^+$, 733 $[M+4]^+$, 731 $[M+2]^+$, 729 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 356 $[C_7H_3Br_3NO]^+$, 328 $[C_6H_3Br_3N]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 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 $[C_6H_4Cl]^+$.

2.8.4 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(4-nitrophenyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8d)

Dark brown amorphous solid; Yield: 76 %; m.p. 101-102 °C; Molecular formula: $C_{21}H_{20}ClN_5O_6S_2$; Molecular Mass: 538 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3357 (N-H), 3019 (Ar C-H), 1638 (C=O), 1678 (C=N), 1609 (Ar C=C), 1536 (N=O), 1364 (S=O), 1166, 1012 (C-O-C), 1056 (C-N), 669 (C-Cl), 632 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.83 (br.s, 1H, -NH), 8.17 (d, J = 9.2 Hz, 2H, H-3"" & H-5""), 7.69 (d, J = 8.4 Hz, 2H, H-2"" & H-6""), 7.68 (d, J = 8.8 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 4.16 (s, 2H, H-2'"), 3.77-3.74 (m, 2H, H_e-2' & H_e-6'), 2.90-2.87 (m, 1H, H-4'), 2.56 (dt, J = 12.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.17-2.13 (m, 2H, H_e-3' & H_e-5'), 2.03-1.93 (m, 2H, H_a-3' & H_a-5'); EIMS (*m/z*): 540 $[M+2]^+$, 538 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 165 $[C_7H_5N_2O_3]^+$, 137 $[C_6H_5N_2O_2]^+$, 111 $[C_6H_4Cl]^+$.

2.8.5 5-{1-[(4-Chlorophenyl) sulfonyl]piperidin-4-yl}-2-{[N-(4-bromo-2-methylphenyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8e)

Off white amorphous solid; Yield: 74 %; m.p. 160-161 °C; Molecular formula: $C_{22}H_{22}BrClN_4O_4S_2$; Molecular Mass: 585 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3347 (N-H), 3038 (Ar C-H), 1651 (C=O), 1689 (C=N), 1610 (Ar C=C), 1379 (S=O), 1165, 1023 (C-O-C), 1049 (C-N), 654 (C-Cl), 649 (C-S), 623 (C-Br); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.83 (br.s, 1H, -NH), 7.83 (d, J = 9.2 Hz, 1H, H-6""), 7.69 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.31-7.27 (m, 2H, H-3"" & H-5""), 3.95 (s, 2H, H-2'"), 3.71-3.68 (m, 2H, H_e-2' & H_e-6'), 2.89-2.86 (m, 1H, H-4'), 2.61 (dt, J = 10.0, 2.4 Hz, 2H, H_a-2' & H_a-6'), 2.20 (s, 3H, CH_3-2""), 2.15-2.12 (m, 2H, H_e-3' & H_e-5'), 2.01-1.96 (m, 2H, H_a-3' & H_a-5'); EIMS (*m/z*): 589 $[M+4]^+$, 587 $[M+2]^+$, 585 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 213 $[C_8H_7BrNO]^+$, 185 $[C_7H_7BrN]^+$, 111 $[C_6H_4Cl]^+$.

2.8.6 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(2-methyl-4,5-dinitrophenyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8f)

Dark brown amorphous solid; Yield: 77 %; m.p. 126-127 °C; Molecular formula: $C_{22}H_{21}ClN_6O_8S_2$; Molecular Mass: 597 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3332 (N-H), 3041 (Ar C-H), 1634 (C=O), 1676 (C=N), 1601 (Ar C=C), 1533 (N=O), 1364 (S=O), 1168, 1017 (C-O-C), 1042 (C-N), 656 (C-Cl), 636 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.82 (br.s, 1H, -NH), 8.86 (s, 1H, H-6""), 7.79 (s, 1H, H-3""), 7.69 (d, J = 8.8 Hz, 2H, H-2" & H-6"), 7.51 (d, J = 8.8 Hz, 2H, H-3" & H-5"), 3.93 (s, 2H, H-2'"), 3.76-3.73 (m, 2H, H_e-2' & H_e-6'), 2.93-2.86 (m, 1H, H-4'), 2.57 (dt, J = 12.0, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.42 (s, 3H, CH_3-2""), 2.16-2.12 (m, 2H, H_e-3' & H_e-5'), 1.99-1.95 (m, 2H, H_a-3' & H_a-5'); EIMS (*m/z*): 599 $[M+2]^+$, 597 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 224 $[C_8H_6N_3O_5]^+$, 196 $[C_7H_6N_3O_4]^+$, 175 $[C_6H_4ClO_2S]^+$, 150 $[C_7H_6N_2O_2]^+$, 111 $[C_6H_4Cl]^+$.

2.8.7 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(4-methyl-2-pyridinyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8g)

Light brown sticky solid; Yield: 75 %; m.p. 129-130 °C; Molecular formula: $C_{21}H_{22}ClN_5O_4S_2$; Molecular Mass: 508 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3327 (N-H), 3028 (Ar C-H), 1648 (C=O), 1679 (C=N), 1608 (Ar C=C), 1395 (S=O), 1175, 1039 (C-O-C), 1058 (C-N), 672 (C-Cl), 615 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.58 (br.s, 1H, -NH), 8.02 (d, J = 8.0 Hz, 1H, H-6""), 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.51 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.15 (s, 1H, H-3""), 7.08 (d, J = 8.0 Hz, 1H, H-5""), 3.94 (s, 2H, H-2'"), 3.69-3.66 (m, 2H, H_e-2' & H_e-6'), 2.99-2.94 (m, 1H, H-4'), 2.59 (dt, J = 12.8, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.40 (s, 3H, CH_3-4""), 2.21-2.17 (m, 2H, H_e-3' & H_e-5'), 1.98-1.94 (m, 2H, H_a-3' & H_a-5'); EIMS (*m/z*): 510 $[M+2]^+$, 508 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 135 $[C_7H_7N_2O]^+$, 111 $[C_6H_4Cl]^+$, 107 $[C_6H_7N_2]^+$.

2.8.8 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(2-methoxycarbonylphenyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8h)

Light bluish amorphous solid; Yield: 82 %; m.p. 114-115 °C; Molecular formula: $C_{23}H_{23}ClN_4O_6S_2$; Molecular Mass: 550 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3329 (N-H), 3027 (Ar C-H), 1716 (C=O), 1636 (C=O), 1677 (C=N), 1609 (Ar C=C), 1397 (S=O), 1178, 1034 (C-O-C), 1055 (C-N), 675 (C-Cl), 618 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.89 (br.s, 1H, -NH), 8.72 (d, J = 8.4 Hz, 1H, H-6""), 8.12 (d, J = 8.0 Hz, 1H, H-3""), 7.69 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.58 (t, J = 7.6 Hz, 1H, H-5""), 7.53 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.13 (t, J = 7.6 Hz, 1H, H-4""), 3.95 (s, 2H, H-2'"), 3.87 (s, 3H, CH_3OOC-2""), 3.71-3.66 (m, 2H, H_e-2' & H_e-6'), 2.98-2.94 (m, 1H, H-4'), 2.61 (dt, J = 12.8, 2.8 Hz, 2H, H_a-2' & H_a-6'), 2.20-2.17 (m, 2H, H_e-3' & H_e-5'), 1.99-1.94 (m, 2H, H_a-3' & H_a-5'); EIMS (*m/z*): 552 $[M+2]^+$, 550 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 178 $[C_9H_8NO_3]^+$, 150 $[C_8H_8NO_2]^+$, 111 $[C_6H_4Cl]^+$.

2.8.9 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(phenylethyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8i)

Pink amorphous solid; Yield: 82 %; m.p. 189-190 °C; Molecular formula: $C_{23}H_{25}ClN_4O_4S_2$; Molecular Mass: 521 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3328 (N-H), 3024 (Ar C-H), 1655 (C=O), 1676 (C=N), 1605 (Ar C=C), 1392 (S=O), 1171, 1036 (C-O-C), 1054 (C-N), 673 (C-Cl), 611 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.53 (br.s, 1H, -NH), 7.67 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.54 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 7.17-7.12 (m, 5H, H-2"" to H-6""), 3.94 (s, 2H, H-2"'), 3.71-3.67 (m, 2H, H$_e$-2' & H$_e$-6'), 3.21 (t,J = 7.6 Hz,2H, H-8""), 2.99-2.95 (m, 1H, H-4'), 2.62 (dt, J = 12.8, 2.8 Hz, 2H, H$_a$-2' & H$_a$-6'), 2.27 (t,J = 2.4 Hz,2H, H-7""), 2.21-2.17 (m, 2H, H$_e$-3' & H$_e$-5'), 1.97-1.94 (m, 2H, H$_a$-3' & H$_a$-5'); EIMS (*m/z*): 523 $[M+2]^+$, 521 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 148 $[C_9H_{10}NO]^+$, 120 $[C_8H_{10}N]^+$, 111 $[C_6H_4Cl]^+$.

2.8.10 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(cyclohexyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8j)

White amorphous solid; Yield: 88 %; m.p. 147-148 °C; Molecular formula: $C_{21}H_{27}ClN_4O_4S_2$; Molecular Mass: 499 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3331 (N-H), 3029 (Ar C-H), 1659 (C=O), 1652 (C=N), 1583 (Ar C=C), 1459 (S=O), 1232, 1021 (C-O-C), 1153 (C-N), 713 (C-Cl), 639 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.69 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 3.78-3.74 (m, 2H, H$_e$-2' & H$_e$-6'), 3.73 (s, 2H, H-2"'), 3.70-3.67 (m, 1H, H-1""), 2.89-2.84 (m, 1H, H-4'), 2.61 (dt, J = 10.4, 2.0 Hz, 2H, H$_a$-2' & H$_a$-6'), 2.16-2.12 (m, 2H, H$_e$-3' & H$_e$-5'), 1.99-1.86 (m, 2H, H$_a$-3' & H$_a$-5'), 1.78-1.76 (m, 2H, H-4""), 1.71-1.66 (m, 2H, H$_e$-3"" & H$_e$-5""), 1.61-1.58 (m, 2H, H$_a$-3"" & H$_a$-5""), 1.38-1.32 (m, 2H, H$_e$-2"" & H$_e$-6""), 1.19-1.15 (m, 2H, H$_a$-2"" & H$_a$-6""); EIMS (*m/z*): 501 $[M+2]^+$, 499 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 126 $[C_7H_{12}NO]^+$, 111 $[C_6H_4Cl]^+$, 98 $[C_6H_{12}N]^+$.

2.8.11 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(phenylmethyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8k)

White amorphous solid; Yield: 87 %; m.p. 160-161 °C; Molecular formula: $C_{22}H_{23}ClN_4O_4S_2$; Molecular Mass: 507 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3324 (N-H), 3143 (Ar C-H), 1651 (C=O), 1664 (C=N), 1556 (Ar C=C), 1534 (N=O), 1436 (S=O), 1222, 1032 (C-O-C), 1164 (C-N), 715 (C-Cl), 623 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.58 (br.s, 1H, -NH), 7.66 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.51 (d, J = 8.0 Hz, 2H, H-3" & H-5"), 7.29-7.21 (m, 5H, H-2"" to H-6""), 4.43 (s, 2H, H-7""), 3.94 (s, 2H, H-2"'), 3.72-3.67 (m, 2H, H$_e$-2' & H$_e$-6'), 2.98-2.95 (m, 1H, H-4'), 2.63 (dt, J = 12.8, 2.8 Hz, 2H, H$_a$-2' & H$_a$-6'), 2.21-2.16 (m, 2H, H$_e$-3' & H$_e$-5'), 1.98-1.94 (m, 2H, H$_a$-3' & H$_a$-5'); EIMS (*m/z*): 509 $[M+2]^+$, 507 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 134 $[C_8H_8NO]^+$, 106 $[C_7H_8N]^+$, 111 $[C_6H_4Cl]^+$.

2.8.12 5-{1-[(4-Chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[N-(2-methyl-6-nitrophenyl)-2-acetamoyl]thio}-1,3,4-oxadiazole (8l)

Brown sticky solid; Yield: 85 %; m.p. 136-137 °C; Molecular formula: $C_{22}H_{22}ClN_5O_6S_2$; Molecular Mass: 552 gmol^{-1}; IR (KBr, υ_{max}, cm^{-1}): 3321 (N-H), 3042 (Ar C-H), 1653 (C=O), 1661 (C=N), 1552 (Ar C=C), 1533 (N=O), 1431 (S=O), 1220, 1033 (C-O-C), 1162 (C-N), 711 (C-Cl), 620 (C-S); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.65 (br.s, 1H, -NH), 7.95 (d, J = 8.4 Hz, 1H, H-5""), 7.71 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.57 (d, J = 8.0 Hz, 2H, H-3" & H-5"), 7.31 (d, J = 8.8 Hz, 1H, H-3""), 6.55 (t, J = 8.8 Hz, 1H, H-4""), 3.96 (s, 2H, H-2"'), 3.75-3.71 (m, 2H, H$_e$-2' & H$_e$-6'), 2.99-2.95 (m, 1H, H-4'), 2.64 (dt, J = 12.8, 2.8 Hz, 2H, H$_a$-2' & H$_a$-6'), 2.24-2.19 (m, 2H, H$_e$-3' & H$_e$-5'), 2.26 (s, 3H, CH_3-2""), 1.98-1.93 (m, 2H, H$_a$-3' & H$_a$-5'); EIMS (*m/z*): 554 $[M+2]^+$, 552 $[M]^+$, 359 $[C_{13}H_{13}ClN_3O_3S_2]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 179 $[C_8H_7N_2O_3]^+$, 151 $[C_7H_7N_2O_2]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

2.9 Statistical analysis

Three independent experiments were conducted, and the results are presented as mean ± SEM after statistical analysis by Microsoft Excel 2010.

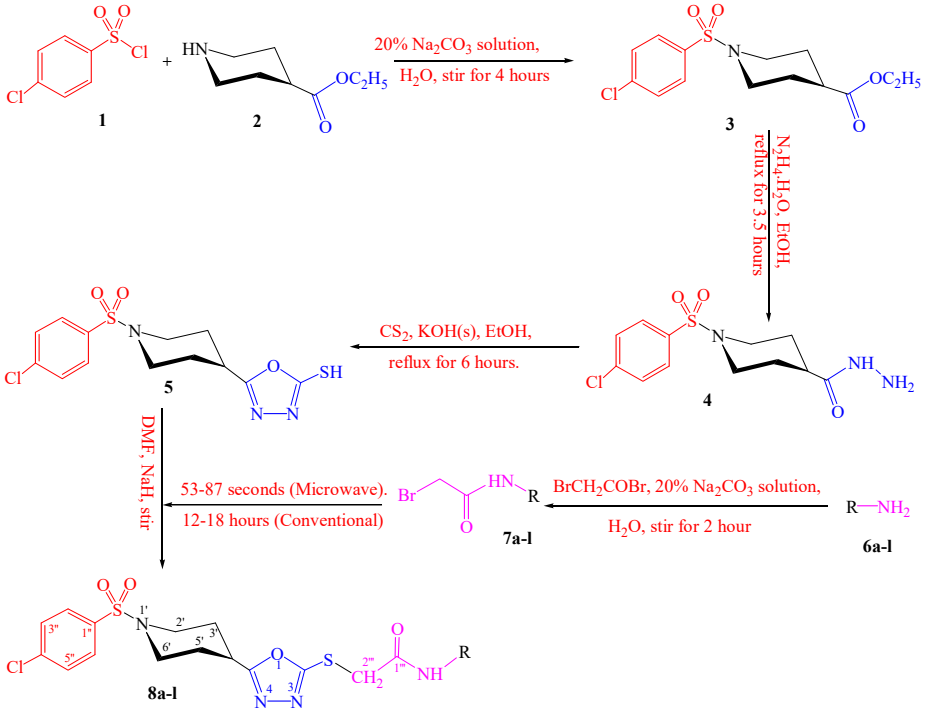
3. Result and discussion

3.1 Discussion

Some heterocyclic acetamide derivatives of azinane were synthesized to evaluate their bioactivity potential. Azinane and 1,3,4-oxadiazole were employed as heterocyclic cores, while sulfonamide, thioether, and acetamide were used to link these cores. The sequence of steps of synthesis is sketched in **Scheme 1**. All the derivatives varied at the nitrogen of acetamide functionality through different aralkyl/phenyl/aryl groups, given in **Table 1**. Substituted azinane, ethyl piperidin-4-carboxylate (**2**), was protected at its nitrogen by stirring with 4-chlorobenzenesulfonyl chloride (**1**) in basic aqueous medium and ethyl 1-[(4-chlorophenyl)sulfonyl]piperidin-4-carboxylate (**3**) was synthesized. To avoid stickiness, sulfonyl chloride was added in small patches. The ester **3** was converted to corresponding hydrazide, 1-[(4-

chlorophenyl)sulfonyl]piperidin-4-carbohydrazide (**4**), by refluxing with hydrated hydrazine in EtOH. The synthesized hydrazide was miscible with water and so was collected by evaporating the solvent. The intermolecular cyclization of **4** through refluxing with CS_2 in the presence of KOH and EtOH, resulted into 5-[1-(4-chlorophenylsulfonyl)-4-piperidinyl]-1,3,4-oxadiazol-2-thiol (**5**). Addition of dilute HCl is necessary to get precipitates but high acidity should be obviated. The aralkyl/phenyl/aryl amines (**6a-l**) were stirred with 2-bromoethanoyl bromide in basic aqueous medium to get different electrophiles, *N*-aralkyl/phenyl/aryl-2-bromoacetamides (**7a-l**).

Finally the synthesized electrophiles, **7a-l**, were reacted with the nucleophile, **5**, in a polar aprotic solvent to get the target molecules, 5-{1-[(4-chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[*N*-(substituted)-2-acetamoyl] thio]}-1,3,4-oxadiazole (**8a-l**) by following microwave assisted and conventional techniques. Synthesis of target compounds, **8a-l**, by microwave assisted method resulted into high yield (**Table 2**) within 53-87 seconds as compared to conventional method which took 12-18 hours (**Table 3**). All the structures were corroborated through IR, EIMS, ^{1}H-NMR and ^{13}C-NMR spectral data. The molecules were screened for their antibacterial (**Table 4**, **Table 5**), anti-enzymatic (**Table 6**, **Table 7**) and BSA binding potential (**Table 8**).



Scheme 1. Synthesis of some heterocyclic acetamide derivatives of azinane.

3.1.1 Chemistry

Compound **8a**, as single compound discussion, was acquired as a pure amorphous solid with melting point of 153-154 °C, appearance of 'off white' and yield of 79 %. The molecular ion peak at *m/z* 557 & an isotopic peak at *m/z* 559; and integration for protons helped to deduce its molecular formula as $C_{22}H_{22}Cl_2N_4O_5S_2$. The different fragments further confirmed the structure of the molecule. The stretching absorptions (υ_{max}, cm^{-1}) confirmed the prominent bonds and functionalities of the molecule. The different absorptions are 3321 (N-H), 3029 (Ar C-H), 1649 (C=O), 1675 (C=N), 1607 (Ar C=C), 1393 (S=O), 1174, 1032 (C-O-C), 1059 (C-N), 678 (C-Cl) and 614 (C-S). In ^{1}H-NMR spectrum (**Fig. 2** to **Fig. 4**), the 5-chloro-2-methoxyphenyl moiety was well confirmed by two doublets & one doublet of doublet in aromatic region and one singlet in aliphatic region at δ 8.34 (d, J = 2.0 Hz, 1H, H-6""), 6.99 (dd, J = 8.8, 2.8 Hz, 1H, H-4""), 6.73 (d, J = 8.4 Hz, 1H, H-3"") and 3.81 (s, 3H, CH_3O-2""). The azinane moiety was confirmed by five signals in aliphatic region at δ 3.70-3.67 (m, 2H, H_{eq}-2' & H_{eq}-6'), 2.90-2.85 (m, 1H, H-4'), 2.61 (dt, J = 12.0, 2.4 Hz, 2H, H_{ax}-2' & H_{ax}-6'), 2.15-2.11 (m, 2H, H_{eq}-3' & H_{eq}-5') and 2.01-1.95 (m, 2H, H_{ax}-3' & H_{ax}-5'). Two *ortho* coupled doublets in aromatic region of ^{1}H-NMR spectrum at δ 7.68 (d, J = 8.4 Hz, 2H, H-2" & H-6") and 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5") supported 4-chlorobenzenesulfonyl group. The acetamide functionality presented two singlets at δ 9.17 (br.s, 1H, -NH) and 3.99 (s, 2H, H-2'"). The Broad Band (BB) and Distorsionless Enhancement by Polarization Transfer (DEPT) modes of ^{13}C-NMR (**Fig. 5** to **Fig. 7**) confirmed twenty two carbons (one methyl, five methylene, eight methine and eight quaternary) through eighteen signals as δ 169.3 (C-5), 165.0 (C-2), 164.3 (C-1'"), 146.8 (C-2""), 139.6 (C-1"), 134.8 (C-4"), 129.5 (C-3" & C-5"), 129.0 (C-2" & C-6"), 128.2 (C-1""), 126.0 (C-5""), 123.8 (C-4""), 119.9 (C-6""), 110.9 (C-3""), 56.0 (CH_3O-2""), 45.0 (C-2' & C-6'), 36.3 (C-2'"), 32.4 (C-4') and 28.3 (C-3' & C-5'). Thus the compound **8a** was confirmed and named as 5-{1-[(4-chlorophenyl)sulfonyl]piperidin-4-yl}-2-{[*N*-(5-chloro-2-methoxyphenyl)-2-acetamoyl]thio}-1,3,4-oxadiazole.
Similar pathway was adopted for the confirmation of other molecules.

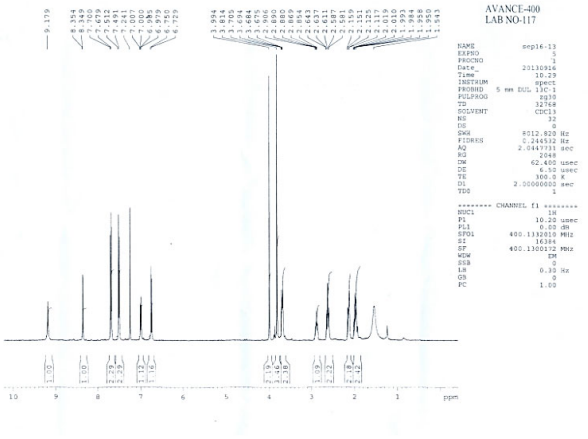


Fig. 2. [1]H-NMR spectrum of compound **8a (**main**)**

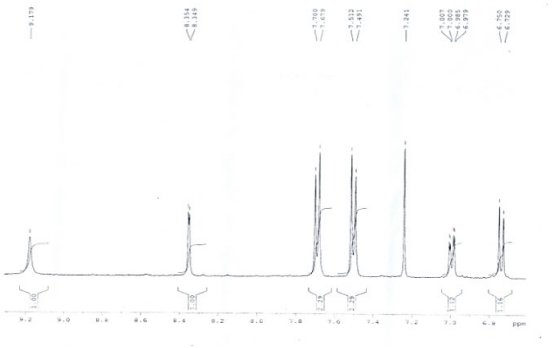


Fig. 3. [1]H-NMR spectrum of compound **8a (**aromatic**)**

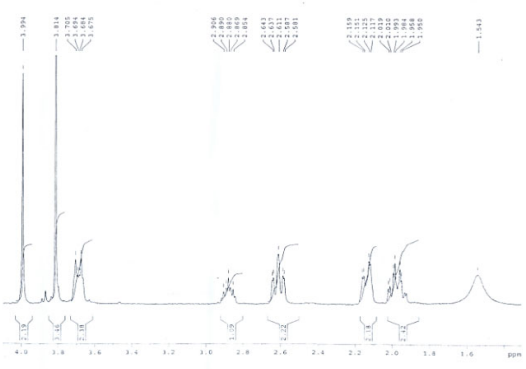


Fig. 4. [1]H-NMR spectrum of compound **8a (**aliphatic**)**

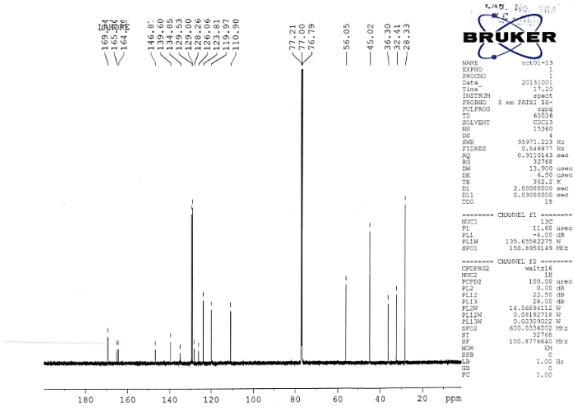


Fig. 5. [13]C-NMR spectrum of compound **8a (**main**)**

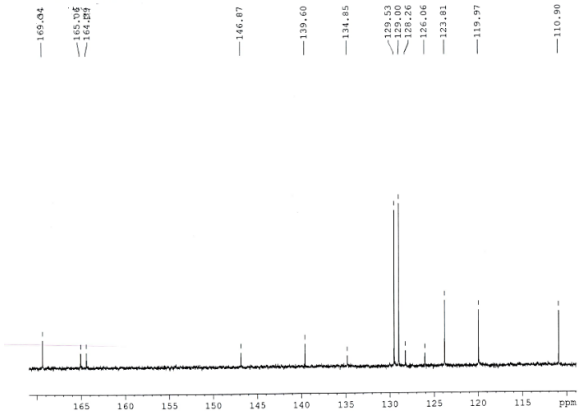


Fig. 6. [13]C-NMR spectrum of compound **8a (**Quaternary & methine**)**

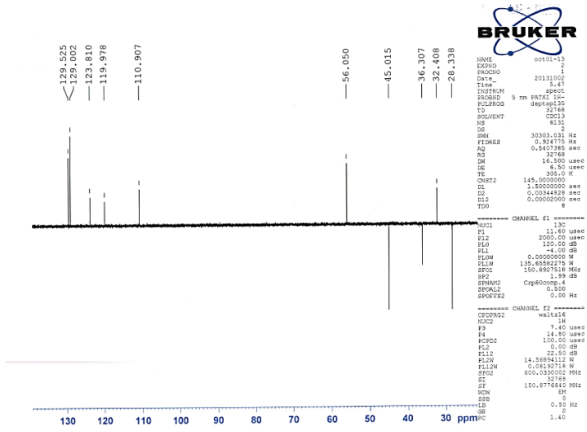


Fig. 7. [13]C-NMR spectrum of compound **8a (**methyl, methylene & methine**)**

3.2 Biological studies

Antibacterial (in vitro) activity

Gram-positive and Gram-negative bacterial strains have been selected for antibacterial potential evaluation. The results for antibacterial screening are presented as % inhibition and MIC (minimum inhibitory concentration) in **Table 4** and **Table 5**, respectively. The results in the form of mean ± SEM were obtained after performing three independent experiments. The synthesized compounds exhibited moderate activity against the selected bacterial strains. The most of compounds remained active against *S. typhi* and was moderately inhibited by the most of compounds. Four compounds **8a**, **8f**, **8i** and **8l** remained the most active ones with MIC values of 10.91±3.71, 10.23±1.76, 9.00±4.84 and 9.60±1.09 µg/mL, respectively, relative to that of ciprofloxacin, 7.15±1.29 µg/mL. These four compounds bear phenyl, 2-methyl-4,5-dinitrophenyl, phenylethyl and 2-methyl-6-nitrophenyl groups respectively. *E. coli* was also actively inhibited by the same four compounds with MIC values of 11.16±1.22, 10.00±2.76, 9.05±2.18 and 9.00±4.03 µg/mL, respectively, as compared to that of ciprofloxacin, 7.90±1.87 µg/mL. All the other compounds remained inactive at all against this strain. *P. aeruginosa* was also inhibited by only four compounds but the most active ones were **8f** and **8l** with MIC values of 9.90±1.25 and 11.76±1.63 µg/mL, respectively, in comparison of that of reference, 8.21±1.21 µg/mL. *S. aureus* was moderately inhibited by **8a**, **8b**, **8d** and **8e** but the most proficiently by **8f** with MIC value of 11.80±1.74 µg/mL relative to that of reference, 8.00±2.98 µg/mL. *B. subtilis* was moderately inhibited by **8f**, **8h**, **8i**, **8j** and **8l** but the most efficiently by **8f** with MIC value of 11.96±5.00 µg/mL relative to that of reference, 7.12±2.11µg/mL. The compound **8f** bearing 2-methyl-4,5-dinitrophenyl group was the most active against all the Gram-negative and Gram-positive bacterial strains.

Table 4. The % age Inhibition of antibacterial activity

Compound	Percentage Inhibition (%)				
	Gram negative bacteria			Gram positive bacteria	
	S.typhi	***E.coli***	***P.aeruginosa***	***S.aureus***	***B.subtilis***
8a	57.73±1.93	61.02±4.35	51.38±3.32	50.59±1.81	40.00±5.00
8b	53.91±1.11	37.96±1.19	39.03±1.28	50.59±2.00	36.77±2.53
8c	48.55±3.14	45.23±1.32	35.82±3.16	36.96±2.45	44.34±1.21
8d	36.09±3.86	40.60±2.73	52.19±5.00	51.76±2.33	24.85±2.42
8e	53.73±2.45	42.50±1.83	46.94±1.41	52.06±1.47	42.78±0.76
8f	71.09±1.43	65.46±4.35	65.97±1.28	58.53±1.31	67.58±4.95
8g	-	-	-	-	-
8h	32.09±2.33	38.38±2.82	20.10±2.51	18.33±2.14	54.09±1.36
8i	80.91±3.97	64.40±2.36	35.66±5.00	32.94±2.32	63.94±1.31
8j	54.09±1.25	23.56±1.42	47.14±2.24	43.14±2.97	56.72±1.16
8k	59.45±1.04	49.81±1.11	46.63±1.31	44.12±3.15	47.22±2.07
8l	69.55±2.00	72.18±3.66	59.34±1.17	49.71±2.96	58.23±2.07
Ciprofloxacin	**91.79±1.45**	**90.87±0.56**	**92.13±0.97**	**90.45±2.98**	**91.18±1.22**

Table 5. MIC of antibacterial activity

Compound	MIC(µg/mL)				
	Gram negative bacteria			Gram positive bacteria	
	S.typhi	***E.coli***	***P.aeruginosa***	***S.aureus***	***B.subtilis***
8a	10.91±3.71	11.16±1.22	17.09±2.38	17.53±0.54	-
8b	18.83±0.78	-	-	19.65±2.70	-
8c	-	-	-	-	-
8d	-	-	13.13±1.25	18.83±0.20	-
8e	13.05±1.32	-	-	16.39±1.54	-
8f	10.23±1.76	10.00±2.76	9.90±1.25	11.80±1.74	11.96±5.00
8g	-	-	-	-	-
8h	-	-	-	-	17.56±1.43
8i	9.00±4.84	9.05±2.18	-	-	15.50±4.24
8j	18.50±1.00	-	-	-	17.38±3.92
8k	15.90±4.12	-	-	-	-
8l	9.60±1.09	9.00±4.03	11.76±1.63	-	13.71±2.29
Ciprofloxacin	**7.15±1.29**	**7.90±1.87**	**8.21±1.21**	**8.00±2.98**	**7.12±2.11**

AChE inhibition activity

All the synthesized compounds, **8a-l**, were evaluated for their anti-acetyl cholinesterase activity (**Table 6** and **Table 7**). The whole series of compounds was found active against this particular enzyme except **8h**, **8i** and **8j**. An array of compounds, **8b**, **8d**, **8f** and **8l** were found the most active members of the current series to act as anti-acetyl cholinesterase agents. The mentioned active compounds possessed IC_{50} values of 94.31±0.13, 43.52±0.12, 63.25±0.12, and 91.42±0.13 µM as compared to the Eserine used as reference with IC_{50} value of 0.04±0.0001 µM. A few compounds showed moderate activity against this enzyme represented as **8a, 8e, 8g** and **8k** having the IC_{50} values of 127.23±0.15, 125.34±0.12, 117.65±0.18, and 157.43±0.13 µM respectively.

Table 6. Percentage inhibition for anti-enzymatic potential of synthesized compounds

Compound	Inhibition (%)		
	AChE at 0.5 mM	α-Glucosidase at 0.5 mM	Urease at 0.25 mM
8a	87.21±0.22	79.77±0.26	23.46±0.16
8b	79.17±0.17	78.48±0.25	37.53±0.16
8c	53.48±0.21	78.64±0.23	15.84±0.12
8d	92.59±0.17	90.39±0.18	17.64±0.13
8e	86.23±0.18	86.51±0.19	41.63±0.17
8f	88.97±0.19	91.66±0.17	27.57±0.13
8g	76.67±0.24	54.80±0.25	65.38±0.19
8h	15.48±0.16	68.41±0.24	18.23±0.12
8i	35.64±0.14	24.67±0.14	98.25±0.14
8j	24.53±0.12	29.46±0.14	14.35±0.15
8k	87.73±0.19	81.10±0.25	17.89±0.12
8l	91.17±0.17	91.54±0.18	26.42±0.12
Standard	**91.27±1.17[a]**	**92.89±0.16[b]**	**98.21±0.18[c]**

Note: a = **Eserine**, b = **Acarbose**, c = **Thiourea**

A compound **8c** was found the least active against the mentioned enzyme having IC_{50} values in the range of 457.53±0.17 µM. Their less potential was might be due to the presence of 2,4,6-tribromophenyl groups attached with triazole ring. The reason behind the least activity was might be due to substitution of bulky groups. The highest anti-enzymatic potential showed by **8b, 8d, 8f** and **8l** was due to the presence of following substituents benzyl, 4-nitrophenyl, 2-methyl-4,5-dinitrophenyl and 2-methyl-6-nitro phenyl in the main skeleton of the structure. Among these the most active compound, **8d** was even best one to act as anti-acetyl cholinesterase agent. The reason for the highest activity of **8d** was might be due the presence of 4-nitrophenyl group having highly electron withdrawing ability, providing the best site for interaction of drugs with enzyme to reduce the negative effects of enzyme.

Table. 7. IC_{50} for anti-enzymatic potential of synthesized compounds

Compound	IC_{50} (µM)		
	AChE	α-Glucosidase	Urease
8a	127.23±0.15	253.28±0.19	-
8b	94.31±0.13	316.52±0.19	-
8c	457.53±0.17	314.29±0.18	-
8d	43.52±0.12	96.58±0.14	-
8e	125.34±0.12	152.73±0.15	-
8f	63.52±0.11	63.52±0.11	-
8g	117.65±0.18	427.36±0.19	158.34±0.15
8h	-	289.34±0.17	-
8i	-	-	11.35±0.06
8j	-	-	-
8k	157.43±0.13	262.83±0.19	-
8l	91.42±0.13	72.38±0.14	-
Standard	**0.04±0.0001[a]**	**37.45±0.14[b]**	**21.25±0.15[c]**

Note: a = **Eserine**, b = **Acarbose**, c = **Thiourea**

Anti-α-glucosidase activity

The following enzyme is responsible for the disturb digestion of glucose resulting in the high level of glucose. The compounds, **8a-l**, were screened against α-glucosidase enzyme to check their active potential against this enzyme (**Table 6** and **Table 7**). All the compounds were found active against this enzyme with variable extent of potential except **8i** and **8j**. A few compounds were found very less active like **8b**, **8c** and **8g**. Some of the compounds from the whole series of compounds found to be intermediate against this enzyme such as **8a**, **8e**, **8h** and **8k** having IC_{50} values of 253.28±0.19, 152.73±0.15, 289.34±0.17 and 262.83±0.19 µM respectively, as compared to the Acarbose used as reference standard with IC_{50} value of 37.45±0.14 µM. The compounds, **8d**, **8f** and **8l** were the best with appreciable anti-α-glucosidase potential. The most active member of the series was **8f** showing the IC_{50} value of 63.52±0.11 µM. The best activity of the compound **8f** was due to the presence of 2-methy-4,5-dinitro phenyl group showing the less steric hindrance for interaction between drug and substrate.

Urease inhibition

The whole series of the synthesized compounds was inactive against urease enzyme except two compounds. The active members of this series were **8g** and **8i** presented in **Table 6** and **Table 7**. Compound **8i** bearing phenylethyl group possessed the activity even more than that of the reference standard. The IC_{50} value of compound **8i** was 11.35±0.06 µM as compared to Thiourea with IC_{50} value of 21.25±0.15 µM. The higher IC_{50} value of this compound showed the higher potential of this compound to behave as highly active anti-urease agent.

3.3 Docking studies of 8d and 8c with AChE enzyme

The two ligands **8c** and **8d** showed highly and weakly inhibitory potency against AChE (457.53±0.17 and 43.52±0.12 µM, respectively). Compound **8c** differs from **8d** only by the replacement of the 4-nitrobenzene group (ring-C) with 2,4,6-tribromobenzene. Thus it was interesting to identify the molecular basis responsible for difference in AChE inhibitory potency among the two selected compounds. Surflex-Dock module of SybylX-1.3 was used to dock either ligand **8c** or **8d** into the active site of AChE enzyme (**Fig. 8**). To confirm the authenticity of our docking procedure donepezil was extracted and re-docked into the active cite of AChE. In order to validate the surflex docking procedure, donepezil was extracted from its co-crystal complex 4EY7 and re-docked to the same binding site. The docked poses were compared with the experimentally determined binding mode of donepezil in AChE. Molecular docking results of the top three ligands poses were found to be almost overlapped to the crystallographic conformation of donepezil in AChE. This indicates that our docking procedure is reliable. Moreover, **Fig. 8B** to **Fig. 8D** shows that both inhibitors acquire similar binding modes in the binding pocket as observed in experimentally determined conformation in 4EY7 complex.

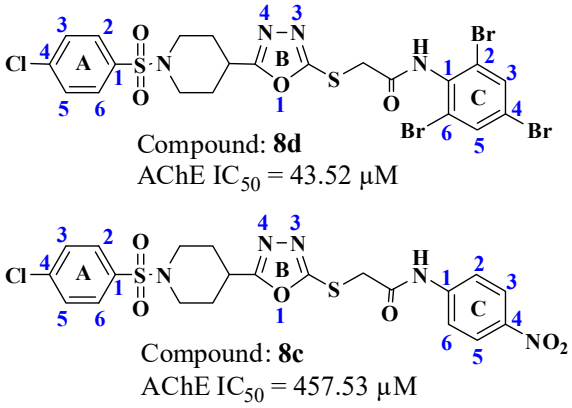


Fig. 8. (A) Potent AChE inhibitors **8d** and **8c** with IC_{50} values

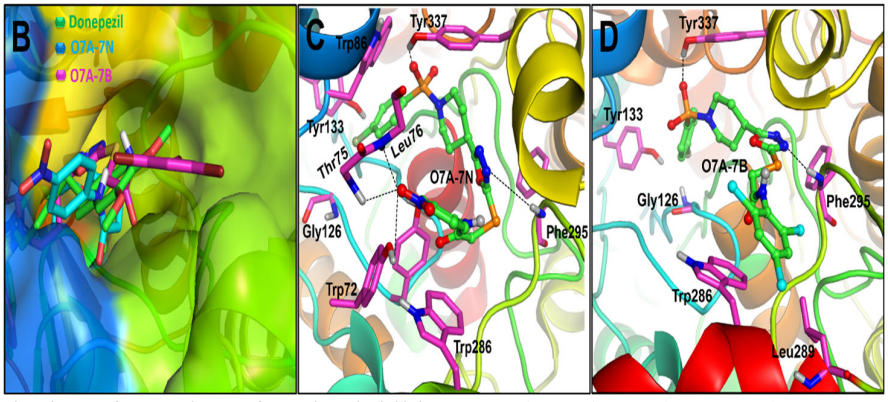
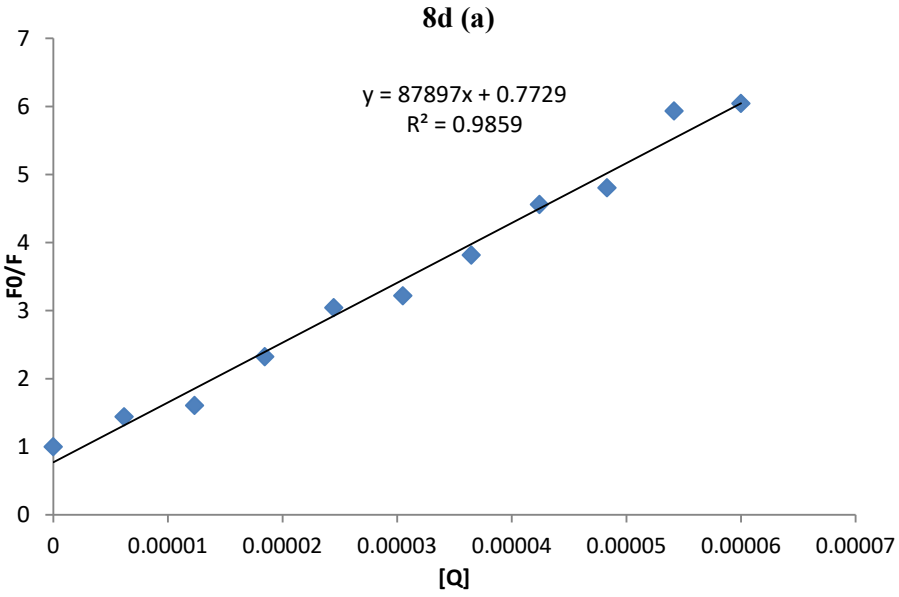


Fig. 8. (B) Docked conformation of AChE inhibitors **8d** (Cyan), **8c** (Magenta) super-positioned over experimental conformation of donepezil (Magenta) in AChE main binding cavity. **(C)** Binding mode of **8d** /AChE complex. **(D) 8c** /AChE complex

The graphic analysis of docking result (**Fig. 8B** to Fig. **8D**) explores that both inhibitors **8c** and **8d** penetrates deeply into the two main active cleft (CAS and PAS) of AChE. In the active site of AChE, both ligands acquire such a binding conformation so that the nitrogen of oxadiazole ring (B-ring) was able to make H-bond with the backbone NH_2 of Phe295. The chlorobenezene group (A-ring) penetrates deeply into the CAS region where its Cl at C4 of A-ring may form hydrophobic or VdW interaction with surrounding residues TRP86, GLY126 and Tyr133. The 4-nitrobenzene ring (C-ring) of **8d** is well accommodated near solvent exposed region where it may establish H-bonds with backbone and side chain of surrounding residues Thr75, Leu76 and Trp72. However, in **8c** the 2,4,6-tribromobenzene moiety (C-ring) was unable to achieve closer contact with surrounding residues. These variations in ligand-receptor interaction pattern could account for the higher AChE inhibitory potency of compound **8d** than **8c**.



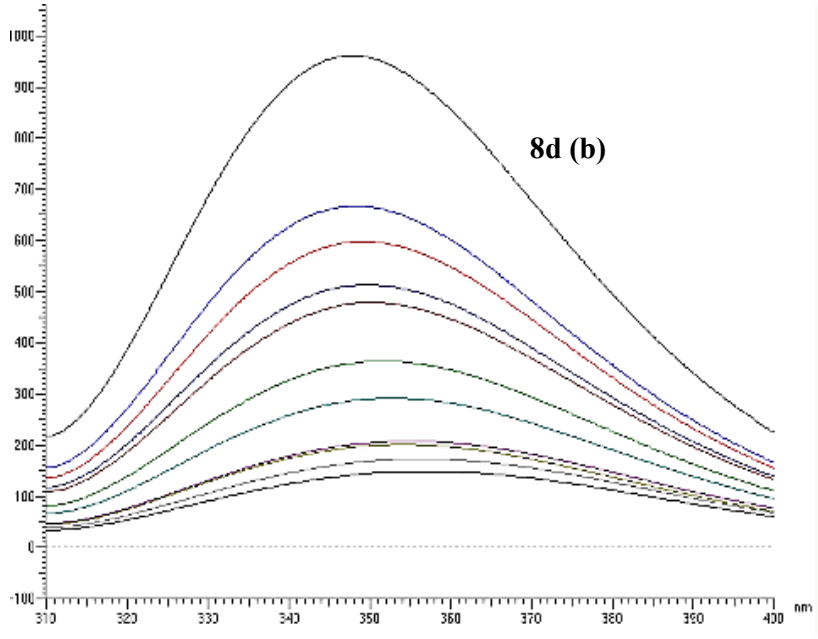


Fig. 9. (a) Stern-Volmer plot of **8d** (**b**) Fluorescence spectra of BSA in presence of different amounts of **8d** at 295 nm and 298 K

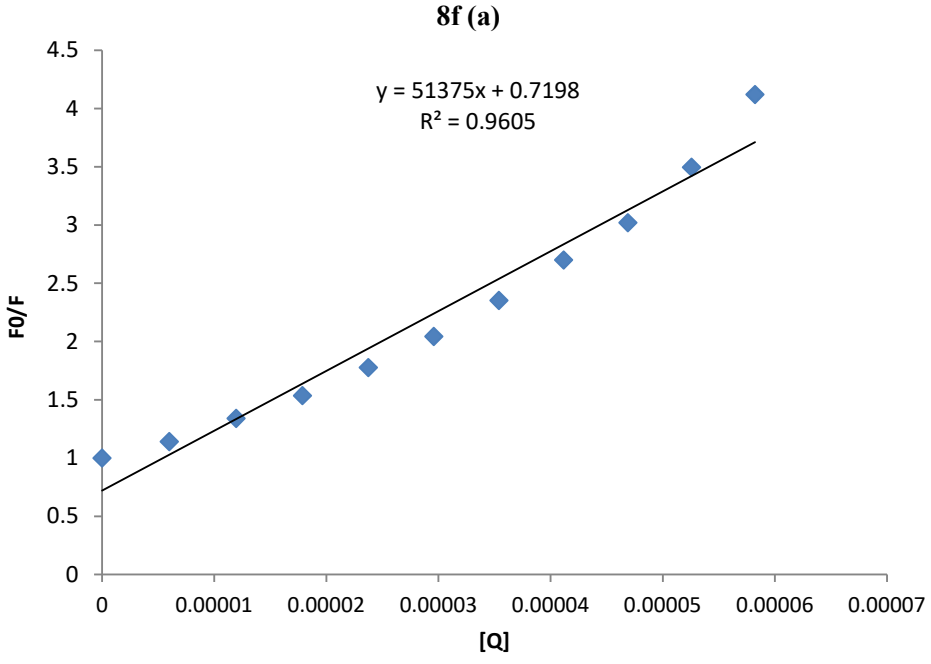
3.4 Bovine serum binding interaction

Oxadiazole and its derivatives were found biologically highly active for antibacterial, anti-spasm, anti-inflammatory, anti-enzymatic potential. Blood plasma protein is responsible for the distribution, metabolism and elimination of the drugs. The current study was carried out to observe the interaction of synthesized molecules with BSA in-order to get the information about the therapeutic effects of these synthesized compounds in pharmacology and pharmacodynamics. The binding studies of various derivatives of oxadiazole with bovine serum albumin were carried out to evaluate the effects of various substituents attached with oxadiazole, at the time of interaction with BSA. The binding study was evaluated through flourometric studies of BSA with different concentrations of synthesized compounds (0 - 5.57 x 10^{-4} M). The binding abilities and binding sites were compared with fluorescent markers warfarin and ibuprofen. Fluorometric titrations of BSA with and without the synthesized compounds or markers were carried out at an emission of 336 nm (Excitation: 295 nm). The emission spectra of BSA in the presence of different compounds of the discussed series are given in **Fig. 9** and **Fig. 10**.

The emission was observed maximum at 336 nm. The addition of 1,3,4-oxadizoles quenched the fluorescence intensity of the compounds showing that the tested compounds have binding ability which in turn cause some modifications in the microenvironment of the amino acid residues situated in the sub-domain of BSA leading to a hypochromic shift in the fluorescence spectra. The subplots in **Fig. 9** and **Fig. 10** shows the Stern-Volmer plot for BSA (**8c**, **8d**, **8f)** binding and the K_{sv} values obtained from the slope are summarized in **Table 8**.

Table. 8. Stern-Volmer quenching constant and quenching rate constant of different compound-BSA system

Compounds	K_{sv} (L/mol) $\times 10^4$	K_q ($Lmol^{-1}s^{-1}$) $\times 10^{12}$	K_a (L/mol)	n
8c	1.0674	1.0674	1.64×10^4	1.04
8d	8.7897	8.7897	1.39×10^6	1.28
8f	5.1375	5.1375	1.21×10^6	1.33



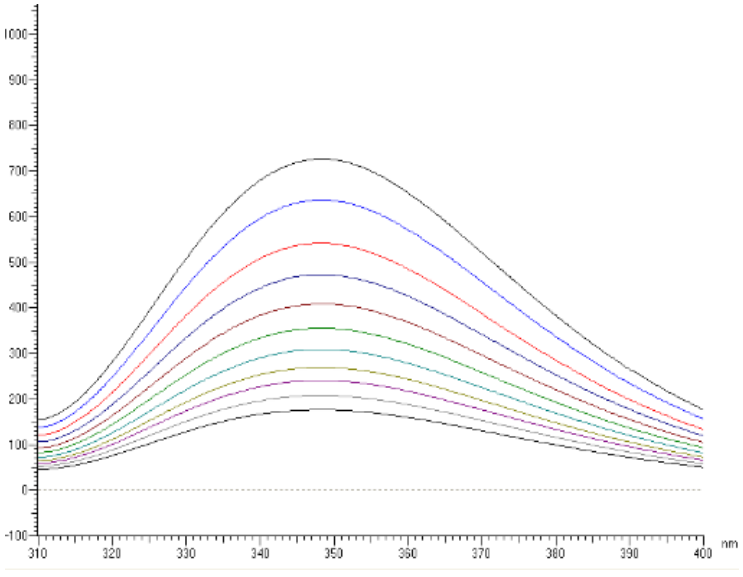


Fig. 10. (a) Stern-Volmer plot of **8f** (**b**) Fluorescence spectra of BSA in presence of different amounts of **8f** at 295 nm and 298 K

Fluorescence quenching can be classified as either static or dynamic process and are caused by different mechanisms including excited state reactions, molecular rearrangements, energy transfer, formation of ground state complex and collisional quenching. The type of quenching mechanism is usually interpreted by the Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = K_q \tau_o[Q] + 1 \quad (1)$$

where F_o and F are the fluorescence intensities of BSA before and after addition of quencher respectively, 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. All the tested compounds were found to develop binding with BSA represented in terms of binding constant. The compounds **8c**, **8d** and **8f** have binding constant such as 1.64 x 10^4, 1.39 x 10^6 and 1.21 x 10^6 respectively. Compounds **8d** and **8f** have higher binding capabilities as represented by binding constant values. The different substituents were found to affect the binding ability of the compounds **8c**, **8d** and **8f** with BSA. Among compounds of current series, presence of nitro group was found to enhance the affinity of the compounds **8d** and **8f** to BSA.

4. Conclusion

The pharmacological importance of heterocyclic compounds prompted us to synthesize some heterocyclic acetamide derivatives of azinane through microwave irradiation and conventional technique. Microwave irradiation technique found very efficient in terms of better yield and within a few minutes. The structural justifications of synthesized compounds have been made through IR, ^{1}H-NMR, ^{13}C-NMR and EIMS spectral data. Synthesized compounds were evaluated for their antibacterial and enzyme inhibition potential. Molecular docking and BSA binding studies further elaborated the binding modes of the most active compounds against enzymes and protein. Compound **8f** bearing 2-methyl-4,5-dinitrophenyl group was the most active against all the bacterial strains. The same compound was also the best anti-α-glucosidase agent. The best anti-acetyl cholinesterase agent was **8d** bearing 4-nitrophenyl group having highly electron withdrawing ability. The best anti-urease agent was **8i** bearing phenylethyl group. The usage of synthesized compounds in drug development programs may result in the development of new anti-bacterial and anti-enzymatic drugs after further analysis.

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