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Synthesis and Pharmacological Screening: Sulfa Derivatives of 2-Pipecoline-Bearing 1,3,4-Oxadiazole Core[1]

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Abstract—An electrophile, 1-(4-(bromomethylbenzenesulfonyl)-2-methylpiperidine, was synthesized by the reaction of 2-methylpiperidine (2-pipecoline) and 4-bromomethylbenzenesulfonyl chloride in a weak basic medium under pH control. A series of nucleophiles, 5-aryl/aralkyl-1,3,4-oxadiazol-2-thiols, were synthesized from corresponding carboxylic acids in three steps. The title molecules were synthesized by coupling the electrophile to nucleophiles in an aprotic medium using LiH as an activator. The structures of all synthesized compounds were corroborated through IR, ^{1}H NMR, and EI-MS techniques. All the compounds were screened for their pharmacological behavior, particularly, antibacterial and enzyme inhibitory activities. Notably efficient results were obtained against both gram-positive and gram-negative bacterial strains. Regarding enzyme inhibition, compounds were efficient against acetylcholinesterase and butyrylcholinesterase.

Keywords: 1,3,4-oxadiazole, 2-methylpiperidine, antimicrobial activity, anti-enzymatic activity, sulfonamide

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INTRODUCTION

Substituted 1,3,4-oxadiazoles are efficacious pharmacological intermediates in drug development. Many non-natural antitumor compounds possess oxadiazole ring [1]. Drug molecules containing such moiety were also found to have various other biological activities including analgesic, anti-inflammatory, anti-proliferative [2], antibacterial, herbicidal, and antifungal [3]. Epilepsy, a neurological disorder, is the third wide spread disorder all over the world. Epileptic patients suffer from severe fits. The anticonvulsants are to stop the firing of neurons that starts seizures and also function as mood stabilizers. Semicarbazones based on 2,5-disubstituted oxadiazole moiety are extraordinary anticonvulsant drug for epileptic patients [4]. Owing to their biological activities, 1,3,4-oxadiazoles are also utilized in agricultural field as insecticides, fungicides, and herbicides. In material sciences, electronic properties of oxadiazoles make them potentially advantageous target molecules. Many are used as organic light emitting diodes (OLEDs) and laser dyes. 1,3,4-Oxadiazole also exhibits electroluminescent properties when it is fused with electron deficient ring system, such as pyridine, furan, thiophene, and naphthalene [5].

Sulfonamides are synthetic antibiotics that are clinically used to control bacterial infection in humans and animals. In animal husbandry, sulfonamides are fed to poultry animals to fight against gastrointestinal and respiratory disease. These have some adverse effects in humans like accumulation in tissues, hypertension, and allergy. Prolonged exposure of these medicines may be carcinogenic; also, the organism becomes bacteria-resistant [6]. Cyclic sulfonamides, like sultams, are the structural part of drugs such as HIV integrase inhibitors, MMP-2 inhibitors, and carbonic anhydrase inhibitors and anti-AIDS agents [7].

Piperidine is an abundant structural moiety, which is present in several biologically active natural and synthetic compounds [8]. Piperidine has various commercial, pharmacological, and curative applications. It is used as solvent, curing agent for rubber, food additive, and intermediate for inorganic synthesis [9].

Enzymes belonging to cholinesterase family constitute operational enzyme system in our body. Acetylcholine (ACh), a serine protease neurotransmitter, is responsible for serine hydrolysis. AChE stops transmission of signal through hydrolysis of acetylcholine [10–12]. BChE is known as "pseudo" or "non-neuro-

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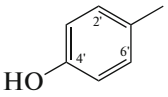
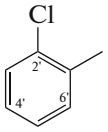
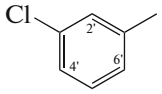
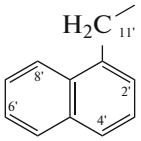
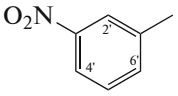
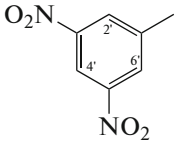
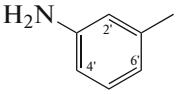
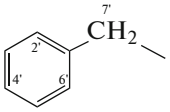
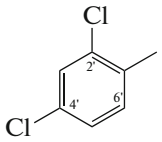
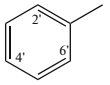
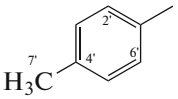
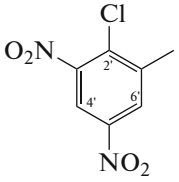
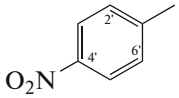
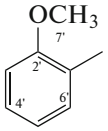
Table 1. Different aralkyl/aryl groups

Compd.	R	Compd.	R	Compd.	R
(VIa)		**(VIf)**		**(VIk)**	
(VIb)		**(VIg)**		**(VIl)**	
(VIc)		**(VIh)**		**(VIm)**	
(VId)		**(VIi)**		**(VIn)**	
(VIe)		**(VIj)**			

nal cholinesterase". BChE has been reported a valuable agent as an investigative marker for Alzheimer disease [13, 14]. Urease catalyzes the hydrolysis of urea and it protects the bacteria in the acidic environment of the stomach [15]. α-Glucosidase causes type-2 diabetes mellitus [16].

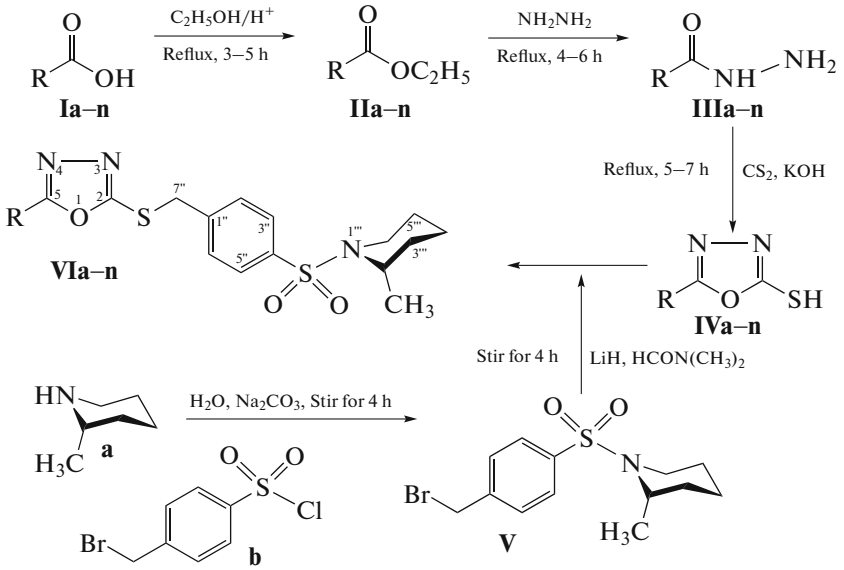
In search for new drug candidates, a large number of organic compounds are being synthesized and evaluated for pharmacological behavior. In continuation of our previous work [12–14], this research project was a successful effort to fuse three bioactive moieties within one core. The synthesized molecules were explored for their pharmacological behavior regarding antibacterial effects against gram-negative and gram-positive bacteria and enzyme inhibitory effects against cholinesterase, urease, and α-glucosidase enzymes. The size, nature, and position of substituents in a molecule greatly affect the biological activities [17]. So groups of different nature, like electron donating or withdrawing, along with variation in their position, were employed in the synthesis to evaluate their antibacterial and enzyme-inhibitory activities.

RESULTS AND DISCUSSION

A series of compounds bearing different functionalities was developed in order to evaluate the role of the functional groups in biological activity and to get more bioactive compounds. The general protocol of synthesis is sketched in Scheme and the varying aralkyl/aryl groups are listed in Table 1. The synthesized molecules were subjected to structural and pharmacological analysis. The results of structural analysis are given under spectral characterization and that of pharmacology are given in Tables 2 and 3.

Table 2. MIC values for antibacterial inhibition study of synthesized compounds

Compounds	MIC, mg/mL				
	S. typhi (–)	*E. coli* (–)	*P. aeruginosa* (–)	*B. subtilis* (+)	*S. aureus* (+)
(VIa)	–	10.92 ± 0.18	10.64 ± 0.90	14.69 ± 0.77	10.24 ± 0.65
(VIb)	18.78 ± 0.46	15.86 ± 0.52	–	18.79 ± 0.10	9.76 ± 0.65
(VIc)	–	–	9.79 ± 0.18	–	12.71 ± 0.65
(VId)	11.76 ± 0.54	10.76 ± 0.61	9.80 ± 0.70	10.87 ± 0.64	10.54 ± 0.32
(VIe)	–	12.78 ± 0.90	–	–	–
(VIf)	–	–	–	–	–
(VIg)	16.75 ± 0.59	9.64 ± 0.12	14.25 ± 0.85	12.65 ± 0.58	16.98 ± 0.10
(VIh)	9.36 ± 0.64	12.53 ± 0.55	9.72 ± 0.51	11.43 ± 0.64	14.28 ± 0.90
(VIi)	9.58 ± 0.41	11.32 ± 0.88	9.93 ± 0.38	13.87 ± 0.59	10.27 ± 0.78
(VIj)	–	–	–	–	–
(VIk)	10.87 ± 0.87	9.85 ± 0.90	14.58 ± 0.56	19.79 ± 0.41	10.59 ± 0.41
(VIl)	15.24 ± 0.73	10.53 ± 0.49	9.11 ± 0.12	12.33 ± 0.62	16.90 ± 0.52
(VIm)	12.54 ± 0.37	8.83 ± 0.57	9.17 ± 0.42	14.90 ± 0.46	9.65 ± 0.15
(VIn)	9.75 ± 0.50	9.42 ± 0.90	12.87 ± 0.53	17.86 ± 0.20	12.83 ± 0.26
Ciprofloxacin	7.83 ± 0.78	8.01 ± 0.12	7.98 ± 0.89	7.22 ± 0.67	7.00 ± 1.54



Scheme. Outline for the synthesis of 5-substituted-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (**VIa–n**).

Chemistry

Compound (**V**), an electrophile, was synthesized from 2-methylpiperidine and 4-bromomethylbenzenesulfonyl chloride in the presence of aqueous weak basic medium. The product was filtered out at pH 5–6. Dilute HCl was employed for this purpose but too low pH has negative effect. A number of carboxylic acids are converted into heterocyclic 1,3,4-oxadiazoles, the nucleophiles. These nucleophiles were mixed with compound (**V**) in dimethyl formamide (DMF) using LiH as activator to get final compounds. The compound (**VIk**) was separated as sticky solid. The IR spectrum of 5-(3-chlorophenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole in KBr solid showed absorption bands at frequencies3035 (Ar C–H), 2534 (S–H), 1659 (C=N), 1526 (Ar C=C), 1432 (SO_2), 1227, 1059 (C–O–C), and 618 (C–S). The molecular formula was affirmed by EI-

Table 3. Enzyme inhibition activity of synthesized compounds

Compounds	AChE		BChE		Urease		α-Glucosidase	
	inhibition at 0.5 mM, %	IC_{50}, μM	inhibition at 0.5 M, %	IC_{50}, μM	inhibition at 0.5 mM, %	IC_{50}, μM	inhibition at 0.5 mM, %	IC_{50}, μM
(VIa)	86.34 ± 0.22	27.4 ± 0.03	76.36 ± 0.33	40.5 ± 0.05	66.65 ± 0.45	175.62 ± 0.19	73.28 ± 0.36	229.34 ± 0.25
(VIb)	19.18 ± 0.51	–	75.39 ± 0.27	51.3 ± 0.03	62.76 ± 0.32	167.98 ± 0.08	–	–
(VIc)	44.91 ± 0.35	–	93.27 ± 0.08	8.51 ± 0.004	47.97 ± 0.64	–	23.45 ± 0.17	–
(VId)	53.27 ± 0.29	443.2 ± 0.06	83.22 ± 0.31	45.3 ± 0.05	58.61 ± 0.71	161.76 ± 0.17	14.83 ± 0.13	–
(VIe)	19.64 ± 0.47	–	85.42 ± 0.27	40.2 ± 0.09	53.78 ± 0.82	425.76 ± 0.22	43.51 ± 0.19	–
(VIf)	46.91 ± 0.55	–	76.33 ± 0.32	58.2 ± 0.04	61.92 ± 0.36	218.87 ± 0.11	19.52 ± 0.15	–
(VIg)	86.48 ± 0.23	17.2 ± 0.01	93.77 ± 0.11	9.72 ± 0.01	42.12 ± 0.42	–	87.46 ± 0.27	157.45 ± 0.18
(VIh)	–	–	–	–	–	–	–	–
(VIi)	55.42 ± 0.26	378.7 ± 0.09	84.37 ± 0.18	33.2 ± 0.03	64.89 ± 0.63	187.54 ± 0.27	13.74 ± 0.12	–
(VIj)	54.32 ± 0.37	443.7 ± 0.09	87.34 ± 0.22	24.1 ± 0.05	49.47 ± 0.31	–	14.52 ± 0.14	–
(VIk)	–	–	–	–	–	–	–	–
(VIl)	81.35 ± 0.21	39.2 ± 0.07	86.45 ± 0.21	31.2 ± 0.02	67.92 ± 0.45	161.43 ± 0.19	68.53 ± 0.34	148.52 ± 0.19
(VIm)	30.64 ± 0.44	–	79.45 ± 0.26	43.3 ± 0.04	57.85 ± 0.98	201.93 ± 0.07	19.45 ± 0.15	–
(VIn)	–	–	–	–	–	–	–	–
Control	Eserine		Eserine		Thiourea		Acarbose	
	82.82 ± 1.09	0.85 ± 0.0001	91.29 ± 1.17	0.04 ± 0.0001	98.45 ± 0.87	21.25 ± 0.15	92.23 ± 0.14	38.25 ± 0.12

IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ-Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA). AChE, acetylcholinesterase, BChE, butyrylcholinesterase

MS indicating molecular ion peak at m/z 464 and characteristic peaks at (m/z) 98 and 111 showing the presence of 2-methylpiperidinyl and 3-chlorophenyl group in the molecule, respectively. The EI-MS data of (**VIk**) is elucidated in Figure as mass fragmentation pattern, which is helpful to study mass spectrum of all targeted molecules. In the ^{1}H NMR spectrum, signals of aromatic protons appearing at δ (ppm) 7.76 (d, J = 8.0 Hz, 2H, H-3", H-5") and 7.54 (d, J = 7.6 Hz, 2H, H-2", H-6") justified the benzene sulfonyl ring. The signals of aromatic protons appearing at δ (ppm) 7.98 (s, 1H, H-2') and 7.44–7.39 (m, 3H, H-4', H-5', H-6') confirmed the presence of 3-chlorophenyl ring. Also, aliphatic protons resonated at δ (ppm) 4.23–4.12 (m, 1H, H_e-6'"), 3.67 (br.s, 1H, H_a-6'"), 3.23 (br.s, 1H, H-2'"), 3.02–2.86 (m, 2H, H_e-4', H_e-5'"), and 1.54–1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'") for piperidine ring. The aliphatic protons for methylene and methyl carbons resonated at δ (ppm) 4.77 (s, 2H, H-7") and 1.02 (d, J = 7.2 Hz, 3H, CH_3-7'"). On the basis of described affirmations, the compound (**VIk**) was named as 2-(3-chlorophenyl)-5-(4-(2-methylpiperidin-1-ylsulphonyl)benzylthio)-1,3,4-oxadiazole. On the basis of IR, ^{1}H NMR, and EI-MS data, the synthesized compounds (**VIa**–**n**) were elucidated as described in the Experimental section.

Antibacterial Activity

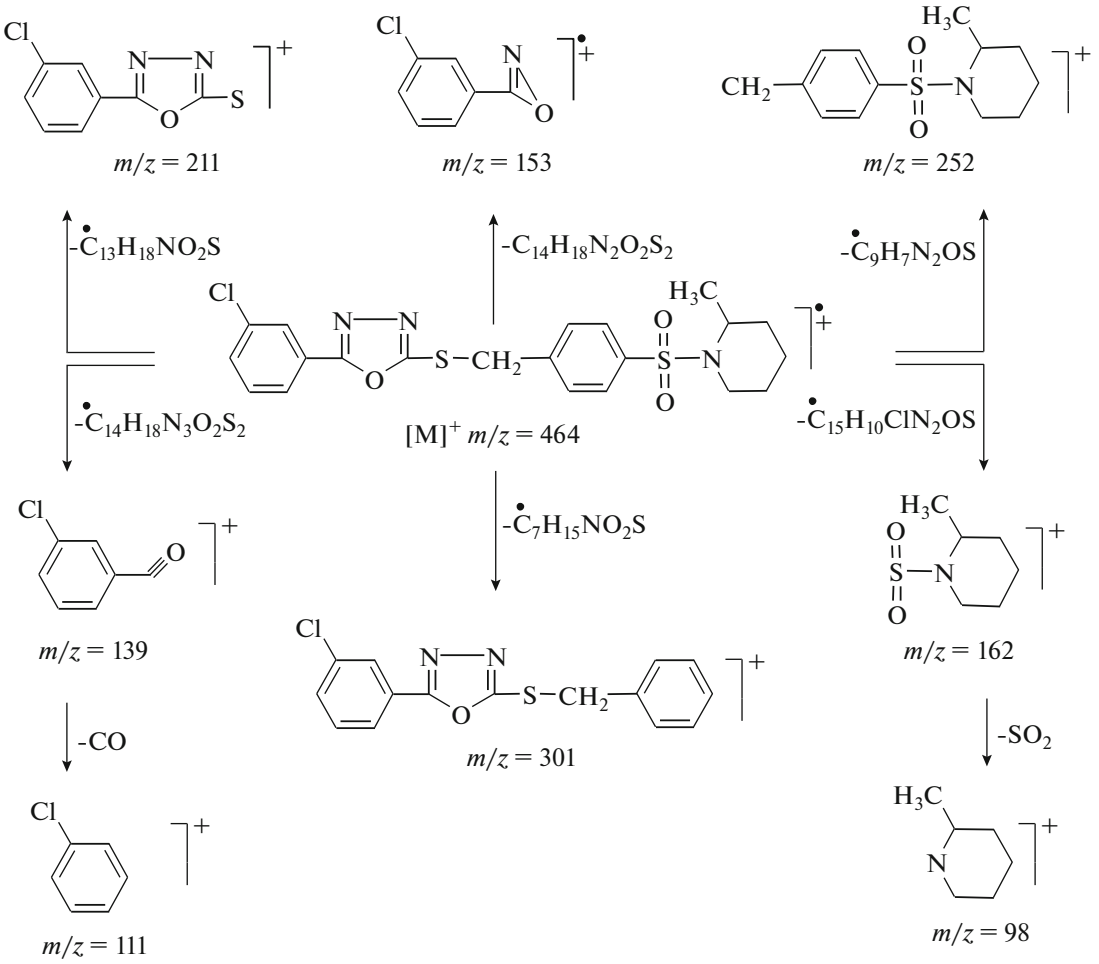
All the target compounds were evaluated against gram-positive (*B. subtilis* and *S. aureus)* and gram-negative bacterial strains (*S. typhi, E. coli,* and *P. aeruginosa)* using ciprofloxacin as a reference drug. Percentage inhibition and minimum inhibitory concentration (MIC) values of all synthesized compounds are given in Table 2.

Against *S. typhi*, (**VIh**), (**VIi**), and (**VIn**) showed very good inhibition potential as indicated by their MIC values of 9.36 ± 0.64, 9.58 ± 0.41, and 9.75 ± 0.50 mg/mL, respectively. The other more efficient compounds against this strain were (**VIk**), (**VId**), and (**VIm**). Compounds (**VIb**), (**VIg**), and (**VIl**) showed moderate activity against this bacterial strain. The compounds (**VIa**), (**VIc**), (**VIe**), (**VIf**), and (**VIj**) were potentially inactive. Against *E. coli*, the most active compound with excellent inhibitory potential was (**VIm**) with MIC of 8.83 ± 0.57 mg/mL as compared to that of ciprofloxacin, 8.01 ± 0.12 mg/mL. Compounds (**VIc**), (**VIf**), and (**VIj**) were inactive. Compounds (**VIg**), (**VIk**), (**VIn**), and (**VIl**) possessed best inhibitory potential against this gram-negative bacteria, probably because of 3-nitrophenyl, 3-clorophenyl, 2-cloro-3,5-dinitrophenyl, and 3,5-dinitrophenyl substituents attached within the core of these target compounds, respectively. The compounds (**VIa**) and (**VId**) also showed good inhibitory potential. The compounds (**VIi**), (**VIh**), (**VIe**), and (**VIb**) showed moderate inhibition potential as depicted by their MIC values, which were 11.32 ± 0.88, 12.53 ± 0.55, 12.78 ± 0.90, and 15.86 ± 0.52 mg/mL, respectively. Against *P. aeruginosa*, (**VIl**) showed best inhibition with MIC value of 9.11 ± 0.12 mg/mL. Compounds (**VIm**), (**VIh**), (**VIc**), (**VId**), and (**VIi**) also proved as promising inhibitors with MIC values of 9.17 ± 0.42 < 9.72 ± 0.51 < 9.79 ± 0.18 < 9.80 ± 0.70 < 9.93 ± 0.38 mg/mL, respectively. Compounds (**VIa**), (**VIn**), and (**VIk**) showed good inhibition potential depicted by their MIC values of 10.64 ± 0.90 < 12.87 ± 0.53 < 14.58 ± 0.56 mg/mL.

Against *S. aureus*, compounds (**VIb**) and (**VIm**) were the best inhibitors having MIC values of 9.65 ± 0.15 and 9.76 ± 0.65 mg/mL, respectively. Compounds (**VIa**) and (**VIi**) showed good inhibition potential against this bacterium compared to reference, ciprofloxacin with MIC of 7.00 ± 1.54 mg/mL. Their MICs were rather close, that is, 10.24 ± 0.65 and 10.54 ± 0.32 mg/mL. The varying groups in these two compounds were *p*-hydroxy and *p*-methyl having electron donating nature. Compounds (**VId**) and (**VIk**) had MIC values of 10.54 ± 0.32 and 10.59 ± 0.41 mg/mL, respectively. Compounds (**VIc**) and (**VIh**) had very close inhibition potential against *S. aureus*. Compounds (**VIg**), (**VIl**), and (**VIn**) also showed good inhibition potential. Compounds (**VIe**), (**VIf**), and (**VIj**) were inactive against this bacterial strain. Against *B. subtilus*, (**VIc**), (**VIe**), (**VIf**), and (**VIj**) were inactive. Compound (**VId**) showed good inhibition against this bacterium. Compounds (**VIh**), (**VIl**), (**VIg**), (**VIi**), (**VIa**), (**VIb**), (**VIn**), and (**VIk**), all exhibited moderate activity as shown by their MIC values given in Table 2.

Enzyme Inhibition Studies

All the synthesized compounds were screened against AChE and BChE against eserine used as a standard. Their inhibitory potential values are expressed as IC_{50} values in Table 3. Amongst all derivatives, 2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-5-(3-aminophenyl)-1,3,4-oxadiazole (**VIc**) showed very good inhibition with IC_{50} value of 8.51 ± 0.004 μM against BChE. The enhanced potential of this derivative may be because of the 3-aminophenyl ring as substituent, which has electron donating character. On the other hand, it was inactive against AChE. Target molecule 2-(4-(2-methylpiperidin-1-ylsulphonyl)benzylthio)-5-(3-nitrophenyl)-1,3,4-oxadiazole (**VIg**) possessing IC_{50} of 9.72 ± 0.01 μM also showed good inhibitory potential against BChE. Compounds (**VIj**) < (**VIl**) < (**VIi**) with IC_{50} values (μM) 24.1 ± 0.05 < 31.2 ± 0.02 < 33.2 ± 0.03 were also found moderate inhibitors against BChE when compared with reference eserine with IC_{50} value of 0.04 ± 0.0001 μM. All other compounds showed moderate inhibition activity against this class of enzymes while (**VIh**), (**VIk**), and (**VIn**) remained inactive.



Mass fragmentation pattern of 2-(3-chlorophenyl)-5-(4-(2-methylpiperidin-1-ylsulfonyl)benzylthio)-1,3,4-oxadiazole (**VIk**).

Compound (**VIg**) showed very good inhibition against AChE with IC_{50} of 17.2 ± 0.01 μM, which is best among the whole series against AChE. Compounds (**VIa**) and (**VIl**) showed moderate potential against AChE as indicated by their IC_{50} values of 27.4 ± 0.03 < 39.2 ± 0.07 μM, respectively with reference to standard eserine, 0.85 ± 0.0001 μM. The somewhat higher activity of these compounds was probably because of *p*-hydroxy, 3-nitrophenyl, and 3,5-dinitrophenyl moieties present within the cores of synthesized molecules. Compounds (**VIi**), (**VId**), and (**VIj**) exhibited weak inhibitory potential against AChE with IC_{50} 378.7 ± 0.09 < 443.2 ± 0.06 < 443.7 ± 0.09 μM; while (**VIb**), (**VIc**), (**VIe**), (**VIf**), (**VIh**), (**VIk**), (**VIm**), and (**VIn**) remained inactive.

All target compounds were evaluated against yeast α-glucosidase. Reference used was acarbose. The inhibition potential values are given in Table 3. Only compounds (**VIl**), (**VIg**), and (**VIa**) showed inhibition having IC_{50} values of 148.52 ± 0.19, 157.45 ± 0.18, and 229.34 ± 0.25, respectively. All other derivatives were inactive. Synthesized target molecules were also evaluated for anti-urease enzyme inhibition activity. Standard used for this was thiourea. All derivatives showed weak inhibition potential with reference of thiourea. Compounds (**VIc**), (**VIg**), and (**VIj**) remained inactive against the urease enzyme.

EXPERIMENTAL

All the chemicals were purchased from Alfa Aesar, Sigma Aldrich, and Merck through local suppliers. The solvents used were of analytical grade and were processed without further purification. Reactions were monitored by TLC (thin layer chromatography) on pre-coated silica gel G-25-UV_{254} plates using ethyl acetate and *n*-hexane as solvent system. Melting points were checked on Gallonkamp melting point apparatus by open capillary tube and were uncorrected. IR spectra (KBr, υ_{max}, cm^{-1}) were recorded on a MIDAC M 2000 spectrometer. ^1H NMR spectra (δ, ppm; *J*, Hz) were recorded in $CDCl_3$ on a Bruker spectrometer operating at 400 (^1H NMR) or 100 MHz (^{13}C NMR) at 25°C. Mass spectra (EI-MS) were mea-

sured on a JEOL JMS-600H instrument along with the data system.

*General Procedure for Synthesis of Ethyl Esters (**IIa–n**)*

Aryl/aralkyl carboxylic acids (**Ia–n**) (0.032 mol) were homogenized in ethanol (99%, 30 mL) in a 250 mL round bottom flask. Concentrated sulfuric acid (2.5 mL) was added in the mixture and set to reflux for 3–5 h. TLC was developed for monitoring reaction completion. At maximum completion, the 10% aqueous sodium carbonate solution was poured to neutralize the mixture up to pH of 9–10 after addition of 150 mL distilled water. This step converted untreated organic acid and sulfuric acid into salts washed away by aqueous layer. The esters were filtered or extracted by solvent extraction technique using 50 mL chloroform or diethyl ether from a separating funnel. Chloroform was distilled off to collect esters (**IIa–n**) [18, 19].

*General Procedures for Synthesis of Aryl/Aralkyl Carbohydrazides (**IIIa–n**)*

The synthesized aryl/aralkyl esters (**IIa–n**) (3.5 mL) were diluted in 250 mL ethanol followed by addition of 4.8 mL 80% hydrazine. Refluxing was continued for 4–6 h. After monitoring by TLC, ice cold distilled H_2O was poured to acquire the precipitates of (**IIIa–n**), which were filtered and washed off with cold distilled H_2O [18, 19].

*General Procedures for Synthesis of 5-Substituted-1,3,4-Oxadiazol-2-Thiols (**IVa–n**)*

Aryl/aralkyl carbohydrazides (**IIIa–n**) (0.012 mol) were shaken well with absolute ethanol (20 mL) and set to reflux after the addition of carbon disulfide (0.024 mol) and solid potassium hydroxide (0.012 mol) for 5–7 h. After the final TLC, distilled water would be added along with diluted hydrochloric acid to make pH of 3 to acquire the precipitates. The addition of acid is crucial to transform the salt form of 5-substituted-1,3,4-oxadiazol-2-thiol into acidic one, but limited amount, because the excess reduces the amount of product. The precipitates were further filtered, washed with distilled water, and re-crystallized from methanol [18, 19].

Procedure for Synthesis of 1-(4-Bromomethylbenzenesulfonyl)-2-Methylpiperidine (V)

2-Methylpiperidine (**a**) (0.0127 mol) was taken in a round bottom flask and diluted with 4–5 mL of distilled water. 4-Bromomethylbenzenesulfonyl chloride (**b**) (0.0127 mol) was added pinch by pinch to the mixture during vigorous stirring at room temperature. The 10% aqueous sodium carbonate solution was gradually added to maintain pH around 9. The reaction was stirred for 4 h and monitored with TLC to check reaction completion. At the end of reaction, the mixture was acidified with dilute hydrochloric acid and kept at pH 5–7 to remove excessive base. Precipitates were filtered, washed with cold distilled water, and dried [20, 21]. ^{1}H NMR: 7.73 (d, J = 8.0 Hz, 2H, H-3", H-5"), 7.59 (d, J = 7.6 Hz, 2H, H-2", H-6"), 4.23 (s, 2H, H-7"), 4.46 (br.s, 1H, H_e-6'"), 3.69 (br.s, 1H, H_a-6'"), 3.24 (br.s, 1H, H-2'"), 3.10–2.94 (m, 2H, H_e-4'", H_e-5'"), 1.59–1.26 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.05 (d, J = 8.0 Hz, 3H, CH_3-7'"); ^{13}C NMR: 146.3 (C-4"), 143.6 (C-1"), 132.7 (C-2", C-6"), 127.1 (C-3", C-5"), 56.4 (C-2'"), 43.6 (C-6'"), 33.8 (C-3'"), 31.2 (C-7'), 24.9 (C-5'"), 19.3 (C-4'"), 18.1 (C-7'").

*General Procedure for Synthesis of S-Substituted Derivatives (**VIa–n**)*

The synthesized nuclephiles (**IVa–n**) (0.005 mol) were homogenized in *N,N*-dimethylformamide (10 mL) in a round bottom flask (50 mL) and then solid lithium hydride (0.005 mol) was added along with continuous stirring. The 0.005 mole of electrophile (**V**) was poured into homogeneous solution after 0.25 h and the solution was further stirred for 4–6 h. After a single-spot TLC, ice cold distilled water was introduced into the reaction contents along with aqueous sodium hydroxide to adjust pH to 10–12. The precipitates were filtered, washed with distilled water, and dried [13].

5-(4-Hydroxyphenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIa). Light brown sticky solid; yield 78%; molecular formula: $C_{21}H_{23}N_3O_4S_2$; molecular mass 445.5 g mol^{-1}; IR: 3031 (Ar C-H), 2450 (S–H), 1675 (C=N), 1523 (Ar C=C), 1398 (SO_2), 1231, 1059 (C–O–C), 615 (C-S); ^{1}H NMR: 7.91 (d, J = 7.6 Hz, 2H, H-2', H-6'), 7.84 (d, J = 7.2 Hz, 2H, H-3', H-5'), 7.75 (d, J = 8.4 Hz, 2H, H-3", H-5"), 7.56 (d, J = 7.6 Hz, 2H, H-2", H-6"), 4.48 (br.s, 1H, H_e-6'"), 4.21 (s, 2H, H-7"), 3.68 (br.s, 1H, H_a-6'"), 3.22 (br.s, 1H, H-2'"), 3.05–2.87 (m, 2H, H_e-4'", H_e-5'"), 1.56–1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.09 (d, J = 8.0 Hz, 3H, CH_3-7'"); ^{13}C NMR: 168.4 (C-5), 164.3 (C-2), 159.4 (C-4'), 145.2 (C-4"), 141.8 (C-1"), 131.3 (C-2", C-6"), 125.3 (C-3", C-5"), 123.9 (C-1'), 123.3 (C-2', C-6'), 117.7 (C-3', C-5'), 56.7 (C-2'"), 43.2 (C-6'"), 33.5 (C-3'"), 31.4 (C-7"), 24.2 (C-5'"), 19.4 (C-4'"), 18.3 (C-7'"); EI-MS (m/z): 445 $[M]^+$, 283 $[C_{15}H_{11}N_2O_2S]^+$, 252 $[C_{13}H_{18}NO_2S]^+$, 193 $[C_8H_5N_2O_2S]^+$, 162 $[C_6H_{12}NO_2S]^+$, 135 $[C_7H_5NO_2]^+$, 121 $[C_7H_5O_2]^+$, 98 $[C_6H_{12}N]^+$, 93 $[C_6H_5O]^+$.

5-(Naphthalen-1-ylmethyl)-2-(4-(2-methylpipridin-1-ylsulfonyl)benzylthio)-1,3,4-oxadiazole (VIb). Creamy amorphous solid; yield 79%; mp 82–84°C; molecular formula: $C_{26}H_{27}N_3O_3S_2$; molecular mass 493.6 g mol^{-1}; IR: 3029 (Ar C-H), 2560 (S–H), 1662

(C=N), 1520 (Ar C=C), 1390 (−SO_2), 1233, 1055 (C−O−C), 616 (C−S); ^{1}H NMR: 7.91 (d, J = 8.0 Hz, 1H, H-8'), 7.84 (d, J = 8.0 Hz, 2H, H-3", H-5"), 7.62 (d, J = 8.4 Hz, 1H, H-5'), 7.53 (d, J = 6.4 Hz, 1H, H-4'), 7.50−7.43 (m, H-3', H-6', H-7'), 7.42 (d, J = 7.2 Hz, H-2", H-6"), 7.34 (d, J = 8.0 Hz, 1H, H-2'), 4.73 (s, 2H, H-11'), 4.57 (s, 2H, H-7"), 4.22 (br.s, 1H, H_e-6'"), 3.69−3.60 (m, 1H, H_a-6'"), 3.24 (br.s, 1H, H-2'"), 3.05-2.89 (m, 2H, H_e-4'", H_e-5'"), 1.54-1.23 (m, 4H, H_e-3'", H_a-3'" H_a-4'", H_a-5'"), 1.03 (d, J = 7.0 Hz, 3H, CH_3-7'"); ^{13}C NMR: 165.2 (C-5), 164.6 (C-2), 148.6 (C-1'), 144.4 (C-4"), 140.0 (C-1"), 130.9 (C-5'), 130.4 (C-4'), 129.6 (C-10'), 128.8 (C-2", C-6"), 127.8 (C-7'), 126.7 (C-3", C-5"), 125.8 (C-6'), 125.3 (C-3'), 124.8 (C-2'), 122.9 (C-8'), 120.4 (C-9'), 55.4 (C-2'"), 43.2 (C-6'"), 33.6 (C-3'"), 32.5 (C-11'), 31.2 (C-7"), 25.7 (C-5'"), 18.9 (C-4'"), 17.8 (C-7'"); EI-MS (*m/z*): 493 $[M]^+$, 332 $[C_{20}H_{16}N_2OS]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 241 $[C_{13}H_9N_2OS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 213 $[C_{13}H_9N_2O]^+$, 163 $[C_6H_{12}NO_2S]^+$, 183 $[C_{12}H_9NO]^+$, 169 $[C_{12}H_9O]^+$, 141 $[C_{11}H_9]^+$, 98 $[C_6H_{12}N]^+$.

5-(3-Aminophenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIc). Light brown sticky solid; yield 80%; molecular formula: $C_{21}H_{24}N_4O_3S_2$; molecular mass 444.5 g mol^{-1}; IR: 3031 (Ar C−H), 2432 (S−H), 1688 (C=N), 1522 (Ar C=C), 1420 (−SO_2), 1222, 1056 (C−O−C), 615 (C−S); ^{1}H NMR: 7.95 (d, J = 8.0 Hz, 1H, H-6'), 7.81 (s, 1H, H-2'), 7.75 (d, J = 7.6 Hz, 2H, H-3", H-5"), 7.57 (d, J = 8.0 Hz, 2H, H-2"-H-6"), 7.50−7.46 (m, 2H, H-4', H-5'), 4.52 (s, 2H, H-7"), 4.21 (br.s, 1H, H_e-6'"), 3.66 (br.s, 1H, H_a-6'"), 3.23−3.16 (m, 1H, H-2'"), 3.01−2.91 (m, 2H, H_e-4'", H_e-5'"), 1.54−1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.02 (d, J = 6.4 Hz, 3H, CH_3-7'"); ^{13}C NMR: 171.1 (C-5), 164.7 (C-2), 147.5 (C-3'), 144.7 (C-4"), 140.6 (C-1"), 132.5 (C-5'), 130.2 (C-1'), 128.7 (C-2", C-6"), 126.4 (C-3", C-5"), 122.9 (C-6'), 118.3 (C-4'), 116.3 (C-2'), 55.6 (C-2'"), 43.7 (C-6'"), 33.9 (C-3'"), 31.3 (C-7"), 25.2 (C-5'"), 18.7 (C-4'"), 17.5 (C-7'"); EI-MS (*m/z*): 444 $[M]^+$, 282 $[C_{15}H_{12}N_3OS]^+$, 252 $[C_{13}H_{18}NO_2S]^+$, 192 $[C_8H_6N_3OS]^+$, 162 $[C_6H_{12}NO_2S]^+$, 134 $[C_7H_6N_2O]^+$, 120 $[C_7H_6NO]^+$, 98 $[C_6H_{12}N]^+$, 92 $[C_6H_6N]^+$.

5-Phenyl-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VId). Light brown sticky solid; yield 77%; molecular formula: $C_{21}H_{23}N_3O_3S_2$; molecular mass 429.5 g mol^{-1}; IR: 3027 (Ar C-H), 2589 (S-H), 1671 (C=N), 1521 (Ar C=C), 1431 (SO_2), 1231, 1059 (C−O−C), 610 (C−S bond str.); ^{1}H NMR: 7.95 (d, J = 7.2 Hz, 1H, H-2', H-6'), 7.75 (d, J = 7.6 Hz, 2H, H-3", H-5"), 7.57 (d, J = 8.0 Hz, 2H, H-2", H-6"), 7.52−7.45 (m, 3H, H-3', H-4', H-5'), 4.52 (s, 2H, H-7"), 4.20 (br.s, 1H, H_e-6'"), 3.64 (br.s, 1H, H_a-6'"), 3.23−3.16 (m, 1H, H-2'"), 3.02−2.91 (m, 2H, H_e-4'", H_e-5'"), 1.54−1.22 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.02 (d, J = 6.4 Hz, 3H, CH_3-7'"); ^{13}C NMR: 167.7 (C-5), 164.6 (C-2), 145.7 (C-4"), 142.5 (C-1"), 133.2 (C-3', C-5'), 131.5 (C-2', C-6'), 129.7 (C-4'), 129.0 (C-1'), 127.6 (C-2", C-6"), 125.2 (C-3", C-5"), 56.4 (C-2'"), 43.3 (C-6'"), 33.4 (C-3'"), 31.9 (C-7"), 23.7 (C-5'"), 18.9 (C-4'"), 18.1 (C-7'"); EI-MS (*m/z*): 429 $[M]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 163 $[C_6H_{12}NO_2S]^+$, 119 $[C_7H_5NO]^+$, 105 $[C_7H_5O]^+$, 77 $[C_6H_5]^+$, 98 $[C_6H_{12}N]^+$.

5-(4-Nitrophenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIe). Red amorphous solid; Yield 76%; mp 132−134°C; molecular formula: $C_{21}H_{22}N_4O_5S_2$; molecular mass 474.5 g mol^{-1}; IR: 3037 (Ar C−H), 2535 (S−H), 1668 (C=N), 1529 (Ar C=C), 1430 (SO_2), 1232, 1064 (C−O−C), 613 (C−S); ^{1}H NMR: 8.34 (d, J = 8.4 Hz, 2H, H-3', H-5'), 8.15 (d, J = 8.4 Hz, 2H, H-2', H-6'), 7.92 (d, J = 7.2 Hz, 2H, H-3", H-5"), 7.77 (d, J = 8.0 Hz, 2H, H-2", H-6"), 4.52 (s, 2H, H-7"), 4.24 (br.s, 1H, H_e-6'"), 3.69 (br.s, 1H, H_a-6'"), 3.22 (m, 1H, H-2'"), 3.02−2.96 (m, 2H, H_e-4'", H_e-5'"), 1.70−1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.05 (d, J = 7.2 Hz, 3H, CH_3-7'"); ^{13}C NMR: 165.3 (C-5), 164.2 (C-2), 149.1 (C-4'), 144.6 (C-4"), 141.8 (C-1"), 138.5 (C-1'), 128.6 (C-2", C-6"), 125.8 (C-3", C-5"), 124.0 (C-2', C-6'), 123.5 (C-3', C-5'), 55.8 (C-2'"), 43.9 (C-6'"), 34.7 (C-3'"), 32.3 (C-7"), 24.2 (C-5'"), 19.1 (C-4'"), 18.3 (C-7'"); EI-MS (*m/z*): 474 $[M]^+$, 312 $[C_{15}H_{10}N_3O_3S]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 222 $[C_8H_4N_3O_3S]^+$, 163 $[C_6H_{12}NO_2S]$, 164 $[C_7H_4N_2O_3]^+$, 122 $[C_6H_4NO_2]^+$,150 $[C_7H_4NO_3]^+$, 98 $[C_6H_{12}N]^+$

5-(2-Chlorophenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIf). Light yellow amorphous solid; yield 73%; molecular formula: $C_{21}H_{22}ClN_3O_3S_2$; molecular mass 464.0 g mol^{-1}; IR: 3031 (Ar C−H), 2530 (S−H), 1665 (C=N), 1523 (Ar C=C), 1399 (SO_2), 1224, 1059 (C−O−C), 615 (C−S); ^{1}H NMR: 7.89 (d, J = 8.0 Hz, 1H, H-6'), 7.76 (d, J = 8.4 Hz, 2H, H-3", H-5"), 7.57 (d, J = 8.0 Hz, 2H, H-2", H-6"), 7.52 (d, J = 8.0 Hz, 1H, H-3'), 7.44 (t, J = 7.6 Hz, 1H, H-5'), 7.37 (t, J = 7.6 Hz, 1H, H-4'), 4.53 (s, 2H, H-7"), 4.21 (br.s, 1H, H_e-6'"), 3.72−3.65 (m, 1H, H_a-6'"), 3.24−3.13 (m, 1H, H-2'"), 3.02−2.99 (m, 2H, H_e-4'", H_e-5'"), 1.55−1.23 (m, 4H, H_e-3'", H_a-3'" H_a-4'", H_a-5'"), 1.03 (d, J = 6.4 Hz, 3H, CH_3-7'"); ^{13}C NMR: 165.2 (C-5), 161.9 (C-2), 144.3 (C-4"), 140.5 (C-1"), 138.3 (C-4'), 136.3 (C-2'), 135.1 (C-3'), 128.8 (C-2", C-6"), 127.2 (C-6'), 126.4 (C-5'), 126.0 (C-3", C-5"), 124.1 (C-1'), 55.1 (C-2'"), 43.9 (C-6'"), 33.4 (C-3'"), 31.7 (C-7"), 25.6 (C-5'"), 18.8 (C-4'"), 17.9 (C-7'"); EI- MS (*m/z*): 466 $[M+2]^+$, 464 $[M]^+$, 301 $[C_{15}H_{10}ClN_2O_3S_2]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 211 $[C_8H_4ClN_2OS]^+$, 162 $[C_6H_{12}$-

$NO_2S]^+$, 153 $[C_7H_4ClNO]^+$, 139 $[C_7H_4ClN]^+$, 111 $[C_6H_4Cl]^+$, 98$[C_6H_{12}N]^+$.

5-(3-Nitrophenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIg). Light yellow amorphous solid; yield 83%; mp 110–112°C; molecular formula: $C_{21}H_{22}N_4O_5S_2$; molecular mass 474.5 g mol^{-1}; IR: 3032 (Ar C–H), 2532 (S–H), 1651 (C=N), 1520 (Ar C=C), 1427 (SO_2), 1230, 1057 (C–O–C), 613 (C–S); ^{1}H NMR: 7.89 (s, 1H, H-2'), 7.76 (d, J = 8.4 Hz, 2H, H-3", H-5"), 7.65 (d, J = 8.0 Hz, 1H, H-6'), 7.57 (d, J = 8.0, 2H, H-2", H-6"), 7.47 (t, J = 7.6 Hz, 1H, H-4'), 7.35 (t, J = 7.6 Hz, 1H, H-5'), 4.53 (s, 2H, H-7"), 4.21 (br.s, 1H, H_e-6'"), 3.72–3.65 (m, 1H, H_a-6'"), 3.24-3.13 (m, H-2'"), 3.02–2.99 (m, 2H, H_e-4'", H_e-5'"), 1.55–1.23 (m, 4H, H_e-3'", H_a-3'" H_a-4'", H_a-5'"), 1.03 (d, J = 6.4 Hz, 3H, CH_3-7'"); ^{13}C NMR: 165.9 (C-5), 163.7 (C-2), 145.9 (C-3'), 144.1 (C-4"), 141.4 (C-1"), 134.4 (C-6'), 132.3 (C-1'), 130.5 (C-5'), 128.6 (C-2", C-6"), 127.1 (C-3", C-5"), 125.3 (C-4'), 120.7 (C-2'), 57.4 (C-2'"), 44.5 (C-6'"), 34.1 (C-3'"), 32.3 (C-7"), 25.8 (C-5'"), 19.2 (C-4'"), 18.0 (C-7'"); EI-MS (m/z): 474 $[M]^+$, 312 $[C_{15}H_{10}N_3O_3S]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 222 $[C_8H_4N_3O_3S]^+$, 162 $[C_6H_{12}NO_2S]^+$, 164 $[C_7H_4N_2O_3]^+$, 122 $[C_6H_4NO_2]^+$, 150 $[C_7H_4NO_3]^+$, 98 $[C_6H_{12}N]^+$.

5-Benzyl-2-(4-(2-methylpiperidin-1-yl-sulfonyl)-benzylthio)-1,3,4-oxadiazole (VIh). Light green amorphous solid; yield 76%; molecular formula: $C_{22}H_{25}N_3O_3S_2$; molecular mass: 443.5 g mol^{-1}; IR: 3035 (Ar C–H), 2536 (S–H), 1659 (C=N), 1526 (Ar C=C), 1432 (SO_2), 1229, 1059 (C–O–C), 618 (C–S); ^{1}H NMR: 7.75 (d, J = 8.0 Hz, 2H, H-3", H-5"), 7.57 (d, J = 7.6 Hz, 2H, H-2", H-6"), 7.33 (d, J = 7.6 Hz, 2H, H-3', H-5'), 7.26 (t, J = 8.0 Hz, 1H, H-4'), 7.23 (d, J = 7.6 Hz, 2H, H-2', H-6'), 4.77 (s, 2H, H-7'), 4.53 (s, 2H, H-7"), 4.23-4.12 (m, 1H, H_e-6'"), 3.67 (br.s, 1H, H_a-6'"), 3.23 (br.s, 1H, H-2'"), 3.02–2.86 (m, 2H, H_e-4'"-H_e-5'"), 1.54–1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.02 (d, J = 7.2 Hz, 3H, CH_3-7'"); ^{13}C NMR: 165.6 (C-5), 164.7 (C-2), 144.3 (C-4"), 141.0 (C-1"), 136.2 (C-1'), 128.8 (C-2", C-6"), 128.5 (C-3', C-5'), 128.3 (C-2', C-6'), 127.2 (C-3", C-5"), 125.9 (C-4'), 55.2 (C-2'"), 43.4 (C-6'"), 34.2 (C-3'"), 33.1 (C-7'), 32.7 (C-7"), 24.5 (C-5'"), 19.4 (C-4'"), 18.6 (C-7'"); EI-MS (m/z): 443 $[M]^+$, 281 $[C_{16}H_{13}N_2OS_2]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 193 $[C_9H_9N_2OS]^+$, 162 $[C_6H_{12}NO_2S]^+$, 133 $[C_8H_7NO]^+$, 91 $[C_7H_7]^+$, 119 $[C_8H_7O]^+$, 98 $[C_6H_{12}N]^+$.

5-(4-Methylphenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIi). Light yellow amorphous solid; yield 81%; molecular formula: $C_{22}H_{25}N_3O_3S_2$; molecular mass 443.5 g mol^{-1}; IR: 3039 (Ar C–H), 2537 (S-H), 1641 (C=N), 1533 (Ar C=C), 1439 (SO_2), 1223, 1079 (C–O–C), 620 (C–S); ^{1}H NMR: 7.85 (d, J = 7.2 Hz, 2H, H-2', H-6'), 7.75 (d, J = 8.0 Hz, 2H, H-3", H-5"), 7.56 (d, J = 7.6, 2H, H-2", H-6"), 7.27 (d, J = 8.0 Hz, 2H, H-3', H-5'), 4.52 (s, 2H, H-7"), 4.21 (br.s, 1H, H_e-6'"), 3.66-3.58 (m, 1H, H_a-6'"), 3.24 (br.s, 1H, H-2'"), 3.02–2.83 (m, 2H, H_e-4'", H_e-5'"), 1.54–1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 2.39 (s, 3H, H-7'), 1.03 (d, J = 6.4 Hz, 3H, CH_3-7'"); ^{13}C NMR: 164.9 (C-5), 164.1 (C-2), 144.9 (C-4"), 142.7 (C-4'), 141.3 (C-1"), 130.5 (C-2', C-6'), 129.4 (C-1'), 128.4 (C-2", C-6"), 127.6 (C-3', C-5'), 125.4 (C-3", C-5"), 56.2 (C-2'"), 43.3 (C-6'"), 34.5 (C-3'"), 32.1 (C-7"), 24.7 (C-5'"), 20.4 (C-7'), 19.3 (C-4'"), 18.5 (C-7'"); EI-MS (m/z): 443 $[M]^+$, 281 $[C_{16}H_{13}N_2OS]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 191 $[C_9H_8N_2OS]^+$, 163 $[C_6H_{12}NO_2S]^+$, 133 $[C_8H_7NO]^+$, 119 $[C_8H_7O]^+$, 98 $[C_6H_{12}N]^+$, 91 $[C_6H_4CH_3]^+$.

5-(2-Methoxyphenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIj). Brown sticky solid; yield 75%; molecular formula: $C_{22}H_{25}N_3O_4S_2$; molecular mass: 459.5 g mol^{-1}; IR: 3041 (Ar C–H), 2540 (S–H), 1645 (C=N), 1533 (Ar C=C), 1439 (SO_2), 1225, 1069 (C–O–C), 625 (C–S); ^{1}H NMR: 7.84 (d, J = 7.6 Hz, 1H, H-6'), 7.79 (t, J = 8.8 Hz, 1H, H-4'), 7.75 (d, J = 8.0 Hz, 2H, H-3", H-5"), 7.57 (d, J = 7.6, 2H, H-2", H-6"), 7.47 (t, J = 8.0 Hz, 1H, H-5'), 4.50 (s, 2H, H-7"), 4.21 (br.s, 1H, H_e-6'"), 3.96 (s, 3H, H-7'), 3.68 (br.s, 1H, H_a-6'"), 3.23 (br.s, 1H, H-2'"), 3.02–2.92 (m, 2H, H_e-4'", H_e-5'"), 1.53–1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.03 (d, J = 6.4 Hz, 3H, CH_3-7'"); ^{13}C NMR: 169.9 (C-5), 164.5 (C-2), 154.6 (C-2'), 143.8 (C-4"), 141.8 (C-1"), 130.7 (C-4'), 128.2 (C-2", C-6"), 127.6 (C-3", C-5"), 124.4 (C-6'), 122.3 (C-5'), 113.5 (C-3'), 108.7 (C-1'), 57.1 (C-2'"), 56.4 (C-7'), 44.8 (C-6'"), 34.2 (C-3'"), 31.9 (C-7"), 25.4 (C-5'"), 19.4 (C-4'"), 18.6 (C-7'"); EI-MS (m/z): 495 $[M]^+$, 207 $[C_9H_7N_2O_2S]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 297 $[C_{16}H_{13}N_2O_2S]^+$, 163 $[C_6H_{12}NO_2S]^+$, 149 $[C_8H_{7-}NO2]^+$, 135 $[C_8H_7O_2]^+$, 107 $[C_7H_7O]^+$, 98 $[C_6H_{12}N]^+$.

5-(3-Chlorophenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIk). Light brown sticky solid; yield 79%; molecular formula: $C_{21}H_{22}ClN_3O_3S_2$; molecular mass: 464.0 g mol^{-1}; IR: 3035 (Ar C–H), 2534 (S–H), 1659 (C=N), 1526 (Ar C=C), 1432 (SO_2), 1227, 1059 (C–O–C), 618 (C–S); ^{1}H NMR: 7.98 (s, 1H, H-2'), 7.76 (d, J = 8.0 Hz, 2H, H-3", H-5"), 7.54 (d, J = 7.6 Hz, 2H, H-2", H-6"), 7.44–7.39 (m, 3H, H-4', H-5', H-6'), 4.77 (s, 2H, H-7"), 4.23–4.12 (m, 1H, H_e-6'"), 3.67 (br.s, 1H, H_a-6'"), 3.23 (br.s, 1H, H-2'"), 3.02–2.86 (m, 2H, H_e-4'", H_e-5'"), 1.54–1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.02 (d, J = 7.2 Hz, 3H, CH_3-7'"); ^{13}C NMR: 165.7 (C-5), 164.1 (C-2), 143.5 (C-4"), 141.3 (C-1"), 133.1 (C-3'), 130.9 (C-2'), 130.1 (C-1'), 128.9 (C-2", C-6"), 128.3 (C-4'), 128.0 (C-6'), 127.7 (C-5'), 127.1

(C-3", C-5"), 57.3 (C-2'"), 44.5 (C-6'"), 34.6 (C-3'"), 32.4 (C-7"), 25.7 (C-5'"), 19.2 (C-4'"), 18.4 (C-7'"); EI-MS (*m/z*): 466 $[M+2]^+$, 464 $[M]^+$, 301 $[C_{15}H_{10}ClN_2OS]^+$, 252 $[C_{13}H_{18}NO_2S]^+$, 211 $[C_8H_4ClN_2OS]^+$, 162 $[C_6H_{12}NO_2S]^+$, 153 $[C_7H_4ClNO]^+$, 139 $[C_7H_4ClO]^+$, 111 $[C_6H_4Cl]^+$, 98 $[C_6H_{12}N]^+$.

5-(3,5-Dinitrophenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIl). Green amorphous solid; Yield 80%; mp 112–114°C; molecular formula: $C_{21}H_{21}N_5O_7S_2$; molecular mass 519.5 g mol^{-1}; IR: 3035 (Ar C–H), 2534 (S–H), 1669 (C=N), 1526 (Ar C=C), 1432 (SO_2), 1234, 1059 (C–O–C), 618" (C–S); ^{1}H NMR: 8.85 (s, 2H, H-2', H-6'), 8.52 (s, 1H, H-4'), 7.75 (d, J = 8.0 Hz, 2H, H-3", H-5"), 7.57 (d, J = 7.6 Hz, 2H, H-2", H-6"), 4.77 (s, 2H, H-7"), 4.23–4.12 (m, 1H, H_e-6'"), 3.67 (br.s, 1H, H_a-6'"), 3.23 (br.s, 1H, H-2'"), 3.02–2.86 (m, 2H, H_e-4'", H_e-5'"), 1.54–1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.02 (d, J = 7.2 Hz, 3H, CH_3-7'"); ^{13}C NMR: 169.7 (C-5), 164.5 (C-2), 147.3 (C-3', C-5'), 144.6 (C-4"), 140.1 (C-1"), 135.4 (C-1'), 128.7 (C-2", C-6"), 128.1 (C-2', C-6'), 126.8 (C-3", C-5"), 114.7 (C-4'), 56.6 (C-2'"), 43.8 (C-6'""), 34.3 (C-3'"), 32.6 (C-7"), 24.2 (C-5'"), 19.5 (C-4'"), 18.8 (C-7'"); EI-MS (*m/z*): 519 $[M]^+$, 357 $[C_{15}H_9N_4O_5S]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 267 $[C_8H_4N_4O_5S]^+$, 163 $[C_6H_{12}NO_2S]^+$, 209 $[C_7H_3N_3O_5]^+$, 195 $[C_7H_3N_2O_5]^+$, 167 $[C_6H_3N_2O_4]^+$, 98 $[C_6H_{12}N]^+$.

5-(2,4-Dichlorophenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIm). Off white amorphous solid; yield 80%; mp 86–88°C; molecular formula: $C_{21}H_{21}Cl_2N_3O_3S_2$; molecular mass 498.4 g mol^{-1}; IR: 3035 (Ar C–H), 2534 (S–H), 1689 (C=N), 1526 (Ar C=C), 1432 (SO_2), 1227, 1060 (C–O–C), 618 (C–S); ^{1}H NMR: 7.93 (s, 1H, H-3'), 7.85 (d, J = 7.6 Hz, 1H, H-6'), 7.75 (d, J = 8.0 Hz, 2H, H-3", H-5"), 7.48 (d, J = 7.6 Hz, 2H, H-2", H-6"), 7.44 (d, J = 8.0 Hz, 1H, H-5'), 4.77 (s, 2H, H-7"), 4.23-4.12 (m, 1H, H_e-6'"), 3.67 (br.s, 1H, H_a-6'"), 3.23 (br.s, 1H, H-2'"), 3.02–2.86 (m, 2H, H_e-4'", H_e-5'"), 1.54–1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.02 (d, J = 7.2 Hz, 3H, CH_3-7'"); ^{13}C NMR: 167.2 (C-5), 164.6 (C-2), 143.7 (C-4"), 139.3 (C-1"), 135.3 (C-4'), 133.8 (C-2'), 131.4 (C-6'), 130.7 (C-3'), 128.6 (C-2", C-6"), 127.4 (C-5'), 127.0 (C-3", C-5"), 125.8 (C-1'), 57.6 (C-2'"), 44.2 (C-6'"), 34.5 (C-3'"), 32.7 (C-7"), 25.1 (C-5'"), 19.4 (C-4'"), 18.8 (C-7'"); EI-MS (*m/z*): 501 $[M+4]^+$, 499 $[M+2]^+$, 497 $[M]^+$, 336 $[C_{15}H_9Cl_2N_2OS]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 246 $[C_8H_3Cl_2N_2OS]^+$, 162 $[C_6H_{12}NO_2S]^+$, 186 $[C_7H_3Cl_2NO]^+$, 145 $[C_6H_3Cl_2]^+$, 172 $[C_7H_3Cl_2O]^+$, 98 $[C_6H_{12}N]^+$.

5-(2-Chloro-3,5-dinitrophenyl)-2-(4-(2-methylpiperidin-1-yl-sulfonyl)benzylthio)-1,3,4-oxadiazole (VIn). Light green amorphous solid; yield 80%; mp 150–152°C; molecular formula: $C_{21}H_{20}ClN_5O_7S_2$; molecular mass 554 g mol^{-1}; IR: 3035 (Ar C–H), 2534 (S–H), 1679 (C=N), 1526 (Ar C=C), 1432 (SO_2), 1224, 1059 (C–O–C), 618 (C–S); ^{1}H NMR: 7.98 (s, 1H, H-6'), 7.83 (s, 1H, H-4'), 7.75 (d, J = 8.0 Hz, 2H, H-3", H-5"), 7.57 (d, J = 7.6 Hz, 2H, H-2", H-6"), 4.77 (s, 2H, H-7"), 4.23–4.12 (m, 1H, H_e-6'"), 3.67 (br.s, 1H, H_a-6'"), 3.23 (br.s, 1H, H-2'"), 3.02–2.86 (m, 2H, H_e-4'", H_e-5'"), 1.54–1.23 (m, 4H, H_e-3'", H_a-3'", H_a-4'", H_a-5'"), 1.02 (d, J = 7.2 Hz, 3H, CH_3-7'"); ^{13}C NMR: 170.6 (C-5), 164.2 (C-2), 153.6 (C-3'), 147.4 (C-5'), 143.9 (C-4"), 139.1 (C-1"), 135.8 (C-1'), 133.2 (C-2'), 128.8 (C-2", C-6"), 127.3 (C-3", C-5"), 126.3 (C-6'), 123.4 (C-4'), 57.1 (C-2'"), 44.3 (C-6'"), 34.7 (C-3'"), 32.2 (C-7"), 25.4 (C-5'"), 19.2 (C-4'"), 18.6 (C-7'"); EI-MS (*m/z*): 555 $[M+2]^+$, 553 $[M]^+$, 392 $[C_{15}H_8ClN_4O_5S]^+$, 301 $[C_8H_2ClN_4O_5S]^+$, 251 $[C_{13}H_{17}O_2NS]^+$, 238 $[C_{12}H_{16}NO_2S]^+$, 162 $[C_6H_{12}NO_2S]^+$, 243 $[C_7H_2ClN_3O_5]^+$, 229 $[C_7H_2ClN_2O_5]^+$, 201 $[C_6H_2ClN_2O_4]^+$, 98 $[C_6H_{12}N]^+$.

Antibacterial Assay

The activity was measured in sterile 96-well micro plates under aseptic conditions. The method is based on the principle that microbial cell number increases as the microbial growth proceeds in a log phase of growth, which results in increased absorbance of broth medium [22]. Three gram-negative (*Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella typhi*) and two gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) were included in the study. The organisms were maintained on stock culture agar medium. The test samples with suitable solvents and dilutions were pipetted into wells (20 μg/well) and maintained overnight. After suitable dilution with fresh nutrient broth, thefresh overnight bacterial culture was poured into wells (180 μL). The initial absorbance of the culture was strictly maintained between 0.12–0.19 at 540 nm. The total volume in each well was kept to 200 μL. The incubation was done at 37°C for 16–24 h with lid on the micro plate. The absorbance was measured at 540 nm using micro plate reader, before and after incubation, and difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = [(X - Y)/X] \times 100,$$

where X is absorbance in control with bacterial culture and Y is absorbance in test sample. Results are mean of triplicate (n = 3) ± SEM. Ciprofloxacin was taken as standard.

Cholinesterase Enzyme Inhibition Assay

The acetylcholinesterase and butyrylcholinesterase activity was measured according to the method

described [23] with minor modification in ration of quantities taken. Total volume of reaction mixture was taken as 100 μL; it contained 60 μL of Na_2HPO_4 as buffer (50 mM) and pH was maintained at 7.7. Test sample (10 μL/well, 0.5 mM) was added followed by addition of AChE or BChE (10 μL, 0.5 units/well; Sigma Inc.). The contents were homogenized, pre-read at 405 nm, pre-incubated at 37°C for 10 min. The reaction was initiated by the addition of 10 μL of 0.5 mM acetylthiocholine chloride (for AChE) or butyrylthiocholine chloride (for BChE) as substrate. Then, 10 μL of DTNB was added, which was 0.5 mM per well. After 15 min of incubation at 37°C, absorbance was measured at 405 nm (using 96-well plate reader Synergy HT, Biotek, USA). All experiments were performed as triplicates with reference to their suitable standard. Eserine 0.5 mM per well was taken as control. The percent inhibition was calculated by using the formula:

$$\text{Inhibition } (\%) = [(\text{Control} - \text{test})/\text{control}] \times 100.$$

IC_{50} values were calculated by using EZ Fit "enzyme kinetics software", Parrella Scientific Inc., Amherst, USA.

α-*Glucosidase Enzyme Inhibition Assay*

The enzyme inhibition activity against α-glucosidase was measured with small modification in the reported procedure [24]. α-Glucosidase assay was made with addition of 70 μL phosphate buffer at pH 6.8 with 50 mM concentration to 10μL (0.5 mM) of test compound, which was further mixed with 10 μL (0.057 units) enzyme to make the total volume of the reaction mixture 100 μL. This blank sample was mixed and the absorbance was measured at 400 nm, which was followed by incubation at 37°C for 10 min. Subsequently, the enzymatic activity was preceded by the addition of 10 μL of *p*-nitrophenylglucopyranoside (0.5 mM) substrate. Acarbose was utilized as positive control for this enzymatic activity. Again, absorbance was taken at 400 nm followed by incubation for 30 min at 37°C. All tests were performed in twice. The percentage inhibition and IC_{50} were calculated similarly to cholinesterase assay.

Urease Enzyme Inhibition Assay

The enzyme assay is the modified form of the commonly known Berthelot assay [25]. A total volume of 85 μL assay mixture was prepared; it contained 10 μL of phosphate buffer of pH 7.0 in each well in the 96-well plate. It was followed by addition of 10 μL of sample solution and 25 μL of enzyme solution (0.135 units). Contents were pre-incubated at 37°C for 5 min. Then, 40 μL of urea stock solution (20 mM) was added to each well and incubation continued at 37°C for further 10 min. After given time, 115 μL phenol hypochlorite reagents were added in each well (freshly prepared by mixing 45 μL phenol reagent with 70 μL of alkali reagent). For color development, incubation was performed at 37°C for another 10 min. Absorbance was measured at 625 nm using the 96-well plate reader Synergy HT BioTek, USA. The percentage inhibition and IC_{50} values were calculated similarly to cholinesterase assay.

CONCLUSION

All the molecules were synthesized in excellent yields given in experimental section. The structures of all target molecules were well corroborated by their spectral data of IR, ^1H and ^{13}C NMR, and EI-MS. All the compounds were subjected to antibacterial and enzyme inhibition analysis. The different bacterial strains taken into account included gram-positive (*B. subtilis* and *S. aureus)* and gram-negative bacterial strains (*S. typhi, E. coli*, and *P. aeruginosa)*. The enzyme inhibition study involved cholinesterases (AChE, BChE), α-glucosidase, and urease enzyme. The compounds remained more efficient against gram-negative bacterial strains as compared to gram-positive ones. The BChE enzyme is more efficiently inhibited by these compounds as compared to others. In this way, the most active compounds could be potential target in the drug invention and drug development program.

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