Synthesis of 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated) acetamides as antibacterial and acetyl cholinesterase inhibitors

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Abstract: The synthetic methodology is carried out in multistep which was initiated as phase I by utilizing Fischer esterification methodology of 2-phenylacetic acid (1) to ethyl-2-phenylacetate (2). The ester was reacted with hydrazine hydrate form 2-phenylacetohydrazide (3) which underwent ring closure with carbon disulfide in alcoholic base to achieve 5-benzyl-1,3,4-oxadiazole-2-thiol (4). Phase II, involved the reaction of electrophiles with 2-bromoacetylbromide (5) with arylated/arenylated amines (6a-e) in aqueous alkaline medium under vigorous shaking to generate *N*-substituted-2-bromoacetamides (7a-e). Finally in phase III, the parent oxadiazole reacted with *N*-substituted-2-bromoacetamides and in DMF/LiH to yield 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated) acetamides (8a-e). All the derivatives were screened for their anti-enzymatic potential against acetyl/butyrylcholinesterase and lipoxygenase and for the antibacterial activity. They were found to be weak enzyme inhibitors and also possessed weak antibacterial action with the exception of 8e, which demonstrated prominent anti-enzymatic and antibacterial activity, which may be attributed to the presence of 3,4-dimethoxyphenylacetamide moiety. The LD_{50} data revealed that most of the *N*-substituted derivatives were found to be less cytotoxic.

Keywords: 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated)acetamides, anti-enzymatic analysis, antibacterial action, cytotoxicity, ^{1}H-NMR and EI-MS.

INTRODUCTION

Heterocyclic compounds encompassing five-membered oxadiazole ring possess a variety of valuable biological activities. Substituted 1,3,4-oxadiazoles are of substantial pharmaceutical importance, which can be recognized by progressively increasing number of publications and patents e.g. 2-amino-1,3,4-oxadiazole acts as muscle relaxants (Harry *et al*., 1966). The analogues of 1,3,4-oxadiazole also demonstrate varied bioactivities e.g. anti-microbial, anti-HIV (El-Eman *et al*., 2004), anti-tubercular, anti-malarial (Kuckuguzel *et al*., 2002), analgesic (Preeti *et al*., 1999), anti-inflammatory (Santagati *et al*., 1994), anti-convulsant (Unangast *et al*., 1992), hypoglycemic (Khan *et al*., 2001). Moreover, numerous derivatives of 1,3,4-oxadiazole are recognized as potentially active anti-mycobacterial (Evangelia *et al*., 2005; Macave *et al*., 2005), anti-cancer (Jin *et al*., 2006) agents and are also inhibitors of tyrosinase enzyme (Jakubkiene *et al*., 2003). They also are important intermediates in organic synthesis, because of the presence of nitrogen and exocyclic sulfur atoms which are nucleophilic in nature and are readily attacked by electrophilic reagents (Ravindera *et al*., 2006; Xia-Juan *et al*., 2002; Lokanatha & Lingama, 2000; Farghaly & El-Kashef, 2006; Palaska *et al*., 2002; De Souza *et al*., 2005; Sahin *et al*., 2002, Clapp *et al*., 1984; Sawant *et al*., 2010).

Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) consists of family of enzymes, which includes serine hydrolases. The diverse specificities of these enzymes for substrates and inhibitors are due to the difference in amino acid residues of the active sites. These are responsible for the annihilation of acetylcholine at cholinergic synapses (Cygler *et al*., 1993; Tougu, 2001). The enzymes catalyze the hydrolysis of neurotransmitter; Acetylcholine due to which the nerve impulse is terminated (Bertaccini & Substance, 1982; Gauthier, 2001). Therefore, it is considered essential to search for novel cholinesterase inhibitors to bring in new drug entrant for the treatment of Alzheimer's and other interrelated ailments. Lipoxygenase (LOX, EC 1.13.11.12) oxidizes Fe^{+2} to catalytically active Fe^{+3} by reaction product 15-hydroperoxy-eicosatetraenoic acid & leukotrienes from arachidonic acid and 13-hydroperoxy-octadecadienoic acid from linoleic acid as substrate (Kemal *et al*., 1987). Leukotrienes are biologically dynamic and act as mediators in a range of inflammatory processes e.g. bronchial asthma inflammation (Alitonou, 2006).

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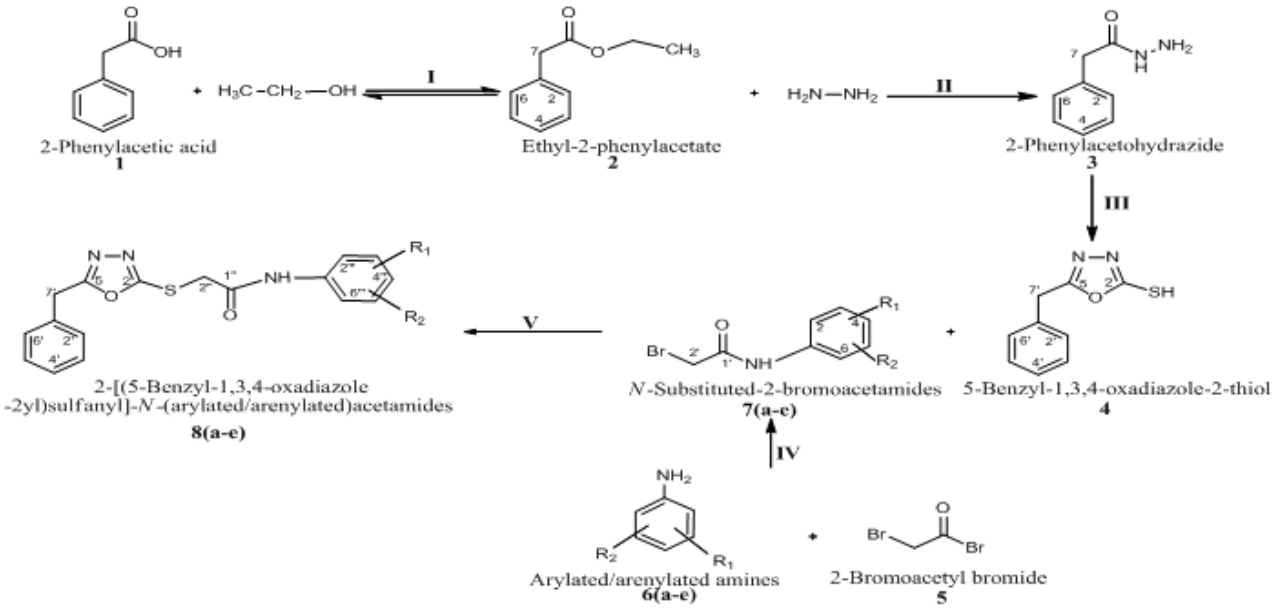

Scheme 1: Outline for the synthesis of 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated)acetamides (8a-e) Reagents & conditions: (I) 2-Phenylacetic acid (1)/H_2SO_4, ethanol/reflux; 6h; (II) Ethyl-2-phenylacetate (2)/$N_2H_4.H_2O$/methanol/stirring; 0.5h at 0°C to RT (III) 2-Phenylacetohydrazide (3)/CS_2/KOH/ethanol/reflux; 6h; (IV) 2-Bromoacetyl bromide (5)/arylated/arenylated amines (6a-e)/10% Na_2CO_3/stirring; 20min, 0°C to RT; (V) 5-Benzyl-1,3,4-oxadiazole-2-thiol (4)/*N*-substituted-2-bromoacetamides (7a-e)/DMF/LiH/stirring; 3h at RT.

The present research work is based on the recent advances on the discovery of correlated bioactive compounds and a continuation of our foregoing research efforts (Aziz-ur-Rehman *et al*., 2012; Siddiqui *et al*., 2013) to synthesize *N*-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2-thiol. Contemporary spectral techniques endorsed the projected structures of the derivatives. Anti-bacterial screening against Gram +ve and Gram -ve bacteria (clinically isolated; Felten *et al*., 2002) revealed that the *N*-substituted derivatives depicted weak to moderate activity and were found to be weak inhibitors of cholinesterases and lipoxygenase enzymes as evident from anti-enzymatic analysis. Moreover, the brine shrimp protocol revealed that most of the compounds are less cytotoxic in comparison to the standard used.

MATERIALS & METHODS

Measurements

The chemicals employed in the current research work were procured from Sigma/Fluka and all were of analytical grade. All solvents consumed herein were distilled prior to use. Melting points were determined in open capillary tubes on electro-thermal Griffin & George m. p. apparatus. The homogeneity of the synthesized compounds and the advancement of the process were examined by ascending thin layer chromatographic technique on pre coated silica gel 60 F_{254} utilizing UV light as visualizing medium at 254 nm. Various percentages of ethyl acetate and *n*-hexane were used as mobile phase. Jasco-320-A spectrophotometer (KBr disc method) was used to record IR spectra and absorption bands are expressed in (wave number; cm^{-1}). Proton NMR was acquired in deuterated chloroform on Bruker spectrometer working at 400 MHz, chemical shifts δ is expressed in ppm and coupling constants (*J*) in Hertz (Hz). JMS-HX-110 spectrometer was used to record Mass spectra (EI-MS).

Synthesis

N-Substituted-2-bromoacetamides (7a-e)

Arylated/arenylated amines were suspended in aqueous sodium carbonate (7mL; 10 %) in an iodine flask which is placed in an ice bath. 2-bromoacetyl bromide was added drop by drop in reaction mixture which was vigorously stirred at RT for 20 minutes till reaction completion. The obtained precipitates were washed with water to obtain pure *N*-substituted-2-bromoacetamides (Siddiqui *et al*., 2013).

***Synthesis of 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-N-(arylated/arenylated)acetamides* (8a-e).**

5-benzyl-1,3,4-oxadiazole-2-thiol (4; 0.001 mol) solubilized in DMF (10mL) was taken in a 25mL round bottomed flask. LiH (0.002 moles) was added in the reaction flask and was stirred at RT for 20 minutes. 2-bromo-*N*-substitutedacetamides (7a-e; 0.001mol) were then added in the reaction flask, which was stirred for 3 hours at room temperature. TLC was performed to check the progress of reaction. 2-[(5-Benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated)acetamides (8a-e) were obtained by quenching the reaction mixture with cold distilled water. Precipitates obtained were filtered, washed with water and air-dried to give product.

Biological assays

Cholinesterase assay

The acetyl and butyrylcholinesterase (AChE/BChE) inhibitory activity was executed according to the process described by (Ellman *et al.*, 1961*)*. The percent inhibition was calculated as under:

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

EZ–Fit Enzyme kinetics software; Perrella Scientific Inc. Amherst, USA was used to calculate IC_{50} values.

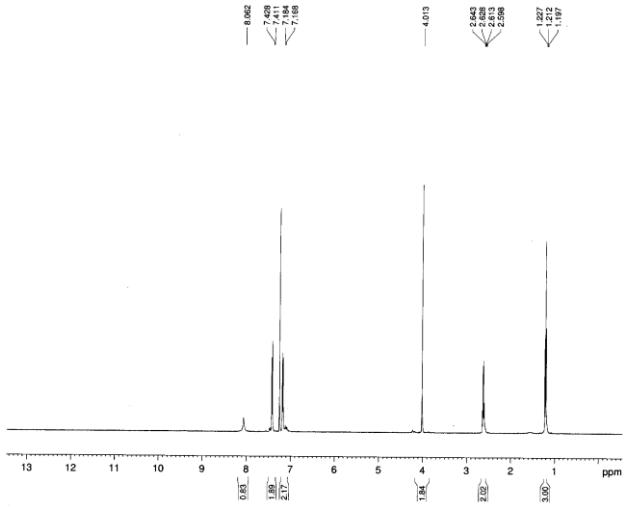

Fig. 1: ^{1}H-NMR spectrum of 2-Bromo-*N*-(4-ethylphenyl) acetamide (7a)

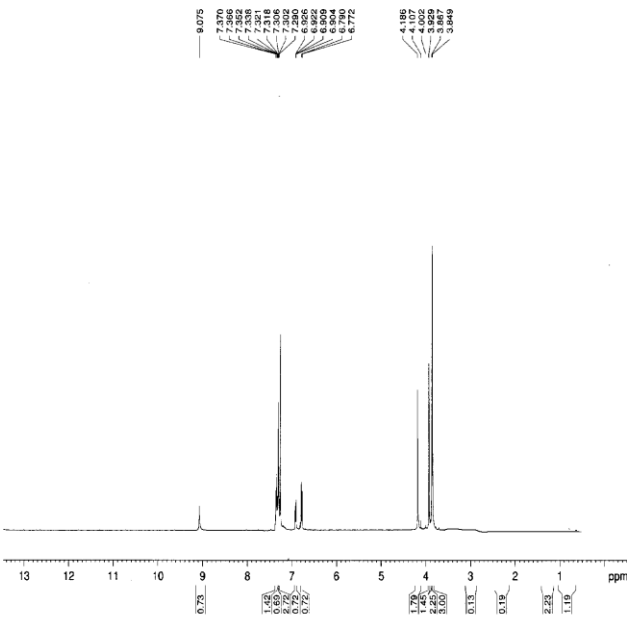

Fig. 2: ^{1}H-NMR spectrum of 2-[(5-Benzyl-1,3,4-oxadiazole-2-yl)sulfanyl]-*N*-(3,4-dimethoxyphenyl) acetamide (8e)

Lipoxygenase assay

Lipoxygenase (LOX) activity was done by already reported method (Baylac and Racine., 2003).

Antibacterial assay

Antibacterial activity of the synthesized derivatives was carried as per method reported by (Kaspady *et al*., 2009 and Jamil *et al*., 2012). Four Gram-negative (*Escherichia coli, Pseudomonas aeruginosa* & *Salmonella typhi*) and two Gram-positive bacteria (*Bacillus subtilis & Staphylococcus aureus*) were included in the study. The absorbance was measured using micro plate reader at 540 nm. Index of bacterial growth was noted as difference between before and after incubation. The percent inhibition was calculated by formula shown below:

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

Where, X = Absorbance of control

Y = Absorbance of test sample

Results were taken in triplicate (n=3, ± SEM). Reference standard was Roxithromycin (1mg/ml). Minimum inhibitory concentration (MIC) was calculated with 5-30 µg/well dilutions and results were calculated using EZ-Fitz Perrella Scientific Inc. Amherst USA software.

Brine shrimp assay

The assay was employed by the method reported by (Ullah *et al*., 2012).

Table 1: Different *N*-substituted-2-bromoacetamides (7a-e)

Codes	7a,8a	7b,8b	7c,8c	7d,8d	7e,8e
R_1	H	H	H	2-C_2H_5	3-OCH_3
R_2	4-C_5H_5	4-OC_2H_5	2-$COCH_3$	6-CH_3	4-OCH_3

STATISTICAL ANALYSIS

All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean ± SEM.

RESULTS

2-[(5-Benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated) acetamides (8a-e) were synthesized from a valuable synthon; 2-phenylacetic acid using method already published (Siddiqui *et al*., 2013).

The first phase of synthetic methodology was started by esterification of 2-phenylacetic acid (1) *via* nucleophilic substitution reaction of ethyl alcohol on electrophilic carbon of the acid in the presence of conc. sulfuric acid to ethyl-2-phenylacetate (2). The ester 2 was reacted with hydrazine hydrate in methanol at 0 ºC to RT under stirring for an hour to form 2-phenylacetohydrazide (3). Cyclization of 3 with carbon disulfide in alcoholic potassium hydroxide yielded 5-benzyl-1,3,4-oxadiazole-2-thiol (4) which was further utilized to achieve different

Table 2: Physical parameters of 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated)acetamides (8a-e).

Code	Appearance	M.P. (°C)	Yield (%)	Mol. For./Wt. ($gmol^{-1}$)	IR KBr, ν_{max} (cm^{-1})
8a	Off-white powder	115	93	$C_{19}H_{19}N_3O_2S$ 353	2923 (C-H str. of aromatic ring), 1641 (C=O amide str.), 1525 (C=C str. of aromatic ring), 1492 (C=N str. of oxadiazole ring)
8b	White pallets	147	96	$C_{19}H_{19}N_3O_3S$ 369	2900 (C-H str. of aromatic ring), 1630 (C=O amide str.), 1572 (C=C str. of aromatic ring), 1451 (C=N str. of oxadiazole ring)
8c	White pallets	189	90	$C_{19}H_{17}N_3O_4S$ 383	2899 (C-H str. of aromatic ring), 1626 (C=O amide str.), 1564 (C=C str. of aromatic ring), 1457 (C=N str. of oxadiazole ring)
8d	Cream pallets	108	93	$C_{20}H_{21}N_3O_2S$ 367	2903 (C-H str. of aromatic ring), 1640 (C=O amide str.),1584 (C=C str. of aromatic ring), 1447 (C=N str. of oxadiazole ring)
8e	White pallets	160	97	$C_{19}H_{19}N_3O_4S$ 385	2893 (C-H str. of aromatic ring), 1624 (C=O amide str.), 1574 (C=C str. of aromatic ring), 1440 (C=N str. of oxadiazole ring)

Table 3: Enzyme Inhibition Analysis of 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated) acetamides (8a-e).

Enzymes	AChE			BChE		LOX	
Compounds	Conc. (mg/ml)	Inhibition (%)	IC_{50} (µM)	Inhibition (%)	IC_{50} (µM)	Inhibition (%)	IC_{50} (µM)
8a	0.5	89.85±0.47	74.71±0.65	35.76±0.09	-	7.020±0.86	-
8b	0.5	85.89±0.24	129.6±0.05	45.46±0.17	-	32.78±0.68	-
8c	0.5	93.25±0.22	107.9±0.04	45.19±0.22	-	19.01±0.45	-
8d	0.5	90.44±0.15	70.84±0.05	74.46±0.26	82.2±0.28	6.38±0.85	-
8e	0.5	92.02±0.06	17.50±0.11	57.77±0.28	72.7±0.25	80.02±0.52	157.2±0.07
Control			Eserine 0.04±0.01		Eserine .85±0.01		Baicalein 22.4±1.03

Note: 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, LOX = Lipoxygenase

N-substituted derivatives. The second phase involved nucleophilic acylation reaction of 2-bromoacetyl bromide (6) with arylated/arenylated amines (7a-e) in alkaline medium at 0 ºC to RT under vigorous shaking to afford electrophiles; *N*-substituted-2-bromoacetamides (7a-e) which were further reacted with 4 in presence of DMF and LiH, which acted as a catalyst under stirring at RT for 3h to yield 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated)acetamides (8a-e). The synthetic pathway is sketched in (Scheme-1; table 1). The physical parameters of the synthesized 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated) acetamides (8a-e) are tabulated in (table 2).The aim was to search for the biologically active molecules bearing 1,3,4-oxadiazole moiety but the biological data revealed that the series of compounds possess moderate to weak anti-bacterial potential. Enzyme inhibition data revealed that the series of synthesized derivatives does not hold potential for anti-enzymatic data against cholinesterases and lipoxygenase as evident from IC_{50} data with the exception of 2-[(5-benzyl-1,3,4-oxadiazole-2-yl)sulfanyl]-*N*-(3,4-dimethoxyphenyl)acetamide (8e).

DISCUSSION

The synthesis of *N*-substituted-2-bromoacetamides 7a-e was substantiated by ^{1}H-NMR analysis which revealed characteristic peaks in the spectra e.g. 7a and 7b displayed A_2B_2 spin system confirming the 1,4-substitutions on the phenyl ring by appearance of *diortho*-coupled doublets at δ 7.40 and δ 7.16 having J=8.4 Hz for H-2 and H-6 and

H-3 and H-5 for 7a similarly, *diortho*-coupled doublets at δ 7.39 (2H, H-2 and H-6) and δ 6.85 (2H, H-3 & H-5) were observed for 7b. Existence of ethyl group in 7a was confirmed by appearance of a quartet at δ 2.61 having integration for 2Hs of -CH_2-1″ group and triplet at δ 1.20 due to -CH_3-2″ group. Appearance of an overlapped multiplet at δ 4.02-3.97 for CH_2-2′ and CH_2-2″ and a triplet at δ 1.38 for CH_3-2″ confirmed the presence of -OC_2H_5 group in 7b. The spectra of 7c showed a multiplet at δ 7.26-7.16, for phenyl protons (3-6) and a singlet at δ 3.84 for CH_3-1″ which appeared deshielded due to the neighborhood of carboxyl group. Structure of 7d was elucidated by appearance of a broad doublet at δ 7.16 for (3 & 5) protons, a quartet resonated at δ 2.51 for CH_2-1′ and a triplet at δ 1.52 for CH_3-2′ confirming the presence of ethyl group and a singlet at δ 1.18 for another CH_3-1″ group. 7e spectra revealed doublet at δ 6.92 having J=1.6 Hz for 1H, a broad doublet at δ 6.82 (J=8.6 Hz, 2H, H-4 & H-5), and two sharp singlets for two methoxy groups at δ 3.88 and δ 3.86 at 1′ and 2′ positions respectively. The characteristic signals of amide and methylene group protons were observed throughout the series.

Structures of 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated)acetamides (8a-e) were supported by NMR data. Compound 8a and 8b displayed A_2B_2 spin system i.e. 2 ortho coupled doublets appeared at δ 7.40 and δ 7.11 having J=8.4 Hz for H-2‴ & H-6‴ and H-3‴ & H-5‴ for 8a and δ 7.38 and δ 6.81 for H-2‴ & H-6‴ and H-3‴ & H-5‴ for 8b. A multiplet appeared at 7.33-7.26 for 5Hs (H-2′ to H-6′) of the phenyl ring of 8a, and a multiplet at 7.33-7.27 for 5Hs (H-2′ to H-6′) of the phenyl ring of 8b was observed. In 8a appearance of a quartet at δ 2.58 for -CH_2-1⁗ and a triplet at δ 1.18 due to -CH_3-2⁗ confirmed the -C_2H_5 group, similarly, a quartet at δ 3.96 for CH_2-1⁗ and a triplet at δ 1.37 for CH_3-2⁗ confirmed the presence of -OC_2H_5 group in 8b. The structure of 8c was interpreted by appearance of a doublet at δ 8.62 for H-3‴, a dt resonated at δ 7.51 for H-4‴, a triplet appeared at 7.10 for H-5‴ and a dd resonated at δ 8.00 for H-6‴ confirmed the presence of benzoate moiety. Moreover, a multiplet at δ 7.26-7.16, for 5 Hs of the phenyl ring (H-2′ to H-6′) and a singlet at δ 3.84 for CH_3-1⁗ due to the neighborhood of carboxyl group. Structure of 8d was explicated by appearance of a doublet at 7.04 for (H-3‴ & H-5‴) and a triplet at 7.12 for H-4‴. Moreover quartet resonated at δ 2.45 for CH_2-1⁗, a triplet at δ 1.02 for CH_3-2⁗ and a singlet at δ 2.12 for CH_3-3⁗ group confirming the attachment of 2-ethyl-6-methylphenyl group. The structure of 8e was established by appearance of a doublet at δ 6.92 having J=1.6 Hz for 1H, a broad doublet at δ 6.78 for H-2″ and a doublet of doublet appeared at δ 6.90 for (H-5‴ & H-6‴). Two sharp singlets for 2 -OCH_3 groups resonated at δ 3.84 and δ 3.86 for 1⁗ and 2⁗ positions respectively. The particular peaks, which appeared throughout the series of the NMR spectrum, i.e. 9.00-11.5, 3.92-4.17 and 3.09-4.18 for -NH, CH_2-2″/7″ and multiplet for phenyl ring (H-2′ to H-6′) respectively were very important in elucidating the structures of all the synthesized derivatives.

Spectral characterization

2-Bromo-N-(4-ethylphenyl)acetamide (7a)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.05 (s, 1H, NH), 7.40 (d, J=8.4 Hz, 2H, H-2 & H-6), 7.16 (d, J=8.4 Hz, 2H, H-3 & H-5), 4.00 (s, 2H, CH_2-2′), 2.61 (q, J=7.6 Hz, 2H, CH_2-1″), 1.20 (t, J=7.6 Hz, 3H, CH_3-2″) (fig. 1). EIMS: *m/z* 241 ($C_{10}H_{12}BrNO$) $[M]^+$, 148 $(C_9H_{10}NO)^+$, 210 $(C_8H_{10}N)^+$, 105 $(C_8H_9)^+$.

2-Bromo-N-(4-ethyoxyphenyl)acetamide (7b)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.01 (s, 1H, NH), 7.39 (d, J=9.0 Hz, 2H, H-2 & H-6), 6.85 (d, J=9.0 Hz, 2H, H-3 & H-5), 4.02-3.97 (m-overlapped, 4H, CH_2-1″ & 2′), 1.38 (t, J=7.0 Hz, 3H, CH_3-2″). EIMS: *m/z* 257 ($C_{10}H_{12}BrNO_2$) $[M]^+$, 164 $(C_9H_{10}NO_2)^+$, 136 $(C_8H_{10}NO)^+$.

Methyl-2-(2-bromoacetamido)benzoate (7c)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 11.5 (s, 1H, NH), 7.26-7.16 (m, 4H, H-3 to H-6), 4.14 (s, 2H, CH_2-2′), 3.84 (s, 3H, CH_3-1″). EIMS: *m/z* 271 ($C_{10}H_{10}BrNO_3$) $[M]^+$, 178 $(C_9H_8NO_3)^+$, 150 $(C_8H_8NO_2)^+$, 59 $(C_2H_3O_2)^+$.

2-Bromo-N-(2-ethyl-6-methylphenyl)acetamide (7d)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.00 (s, 1H, NH), 7.17 (t, J=7.6 Hz, 1H, H-4), 7.10 (br.d, J=7.8 Hz, 2H, H-3 & H-5), 4.06 (s, 2H, CH_2-2′), 2.57 (q, J=7.5 Hz, 2H, CH_2-1″), 1.52 (s, 3H, CH_3-1‴), 1.18 (t, J=7.6 Hz, 3H, CH_3-2″). EIMS: *m/z* 255 ($C_{11}H_{14}BrNO$) $[M]^+$, 162 $(C_{10}H_{12}NO)^+$, 134 $(C_9H_{12}N)^+$, 119 $(C_9H_{11})^+$.

2-Bromo-N-(3,4-dimethoxyphenyl)acetamide (7e)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.04 (s, 1H, NH), 6.92 (d, J=1.6 Hz, 1H, H-2), 6.82 (br.d, J=8.6 Hz, 2H, H-5 & H-6), 4.01 (s, 2H, CH_2-2′), 3.88 (s, 3H, OCH_3-1″), 3.86 (s, 3H, OCH_3-2″). EIMS: *m/z* 273 ($C_{10}H_{12}BrNO_3$) $[M]^+$, 180 $(C_9H_{10}NO_3)^+$, 152 $(C_8H_{10}NO_2)^+$, 137 $(C_8H_9O_2)^+$.

2-[(5-Benzyl-1,3,4-oxadiazole-2-yl)sulfanyl)]-N-(4-ethylphenyl)acetamide (8a)

HR-MS: $[M]^+$ 353.4380 (Calcd. for $C_{19}H_{19}N_3O_2S$; 353.5379); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 9.01 (s, 1H, NH), 7.40 (d, J=8.4 Hz, 2H, H-2‴ & H-6‴), 7.33-7.26 (m, 5H, H-2′ to H-6′), 7.11 (d, J=8.4 Hz, 2H, H-3‴ & H-5‴), 4.16 (s, 2H, CH_2-2″), 3.09 (s, 3H, CH_2-7′), 2.58 (q, J =7.8 Hz, 2H, CH_2-1⁗), 1.18 (t, J=7.6 Hz, 3H, CH_3-2⁗); EIMS: *m/z* 353 ($C_{19}H_{19}N_3O_2S$) $[M]^+$, 162 $(C_{16}H_{12}NO)^+$, 159 $(C_9H_7N_2O)^+$, 148 $(C_9H_{10}NO)^+$, 133 $(C_8H_7NO)^{\cdot+}$, 120 $(C_8H_{10}N)^+$, 119 $(C_8H_7O)^+$, 117 $(C_8H_7N)^+$, 120 $(C_8H_{10}N)^+$, 91 $(C_7H_7)^+$, 65 $(C_5H_5)^+$.

2-[(5-Benzyl-1,3,4-oxadiazole-2-yl)sulfanyl]-N-(4-ethoxyphenyl)acetamide (8b)

HR-MS: $[M]^+$ 369.4374 (Calcd. for $C_{19}H_{19}N_3O_2S$; 369.5375); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 9.00 (s, 1H, NH), 7.38 (br.d, J=8.8 Hz, 2H, H-2‴ & H-6‴), 7.33-7.27

Table 4: MIC of 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated)acetamides **(8a-e)**.

Compounds	Bacterial Strains				
	S. typhi (-)	*E. coli (-)*	*P. aeruginosa (-)*	*S. auerus (+)*	*B. subtilis (+)*
8a	-	-	-	-	-
8b	100±1.61	100±1.14	100±2.36	150±1.87	150±1.59
8c	150±1.51	150±2.88	200±2.22	-	200±1.68
8d	150±1.32	100±1.85	200±1.49	200±2.56	150±2.63
8e	150±1.26	150±2.23	200±2.09	-	200±2.58
Roxithromycin	27.4±0.87	16.0±1.15	22.7±2.29	27.2±1.01	25.7±2.09

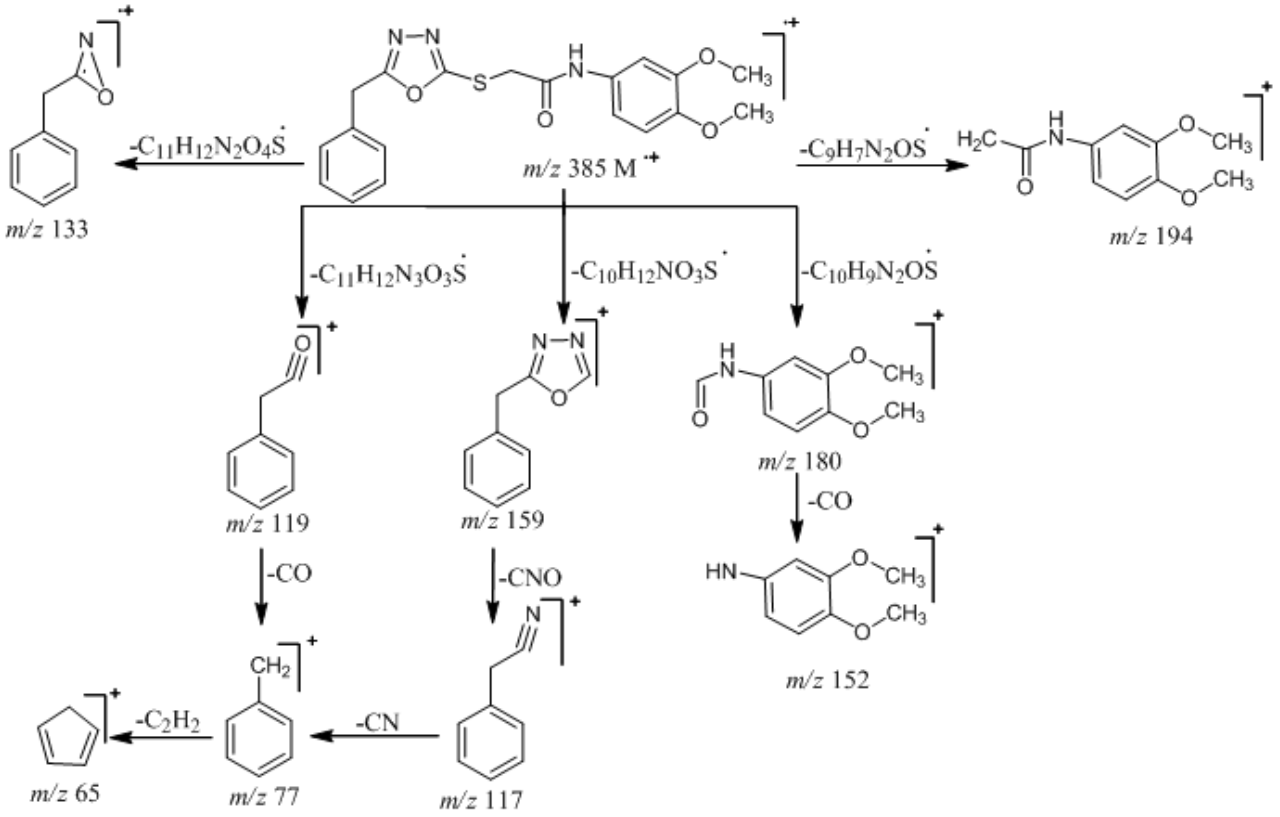

Fig. 3: Proposed mass fragmentation pattern of 2-[(5-Benzyl-1,3,4-oxadiazole-2-yl)sulfanyl]-*N*-(3,4-dimethoxyphenyl) acetamide (8e)

(m, 5H, H-2′ to H-6′), 6.81 (br.d, *J*=8.8 Hz, 2H, H-3‴ & H-5‴), 4.16 (s, 2H, CH_2-2″), 3.96 (q, *J*=7.0 Hz, 2H, CH_2-1‴′), 3.89 (s, 3H, CH_2-7′), 1.37 (t, *J*=7.0 Hz, 3H, CH_3-2‴′); EIMS: *m/z* 369 ($C_{19}H_{19}N_3O_3S$) $[M]^+$, 178 $(C_{10}H_{12}NO_2)^+$, 164 $(C_9H_{10}NO_2)^+$, 159 $(C_9H_7N_2O)^{\cdot+}$, 136 $(C_8H_{10}NO)^+$, 133 $(C_8H_7NO)^+$, 119 $(C_8H_7O)^+$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$, 65 $(C_5H_5)^+$.

Methyl 2-[(2-(5-benzyl-1,3,4-oxadiazol-2-yl)sulfanylacetamido]benzoate (8c)

HR-MS: $[M]^+$ 383.4209 (Calcd. for $C_{19}H_{19}N_3O_2S$; 383.5210); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 11.5 (s, 1H, NH), 8.62 (d, *J*=8.0 Hz, 1H, H-3‴), 8.00 (dd, *J*=7.6, 0.8 Hz, 1H, H-6‴), 7.51 (dt, *J*=8.0 Hz, 1H, H-4‴), 7.26-7.16 (m, 5H, H-2′ to H-6′), 7.10 (t, *J*=7.6 Hz, 1H, H-5‴), 4.14 (s, 2H, CH_2-2″), 4.12 (s, 2H, CH_2-7′), 3.84 (s, 3H, CH_3-1‴′); EIMS: *m/z* 383 ($C_{19}H_{17}N_3O_4S$) $[M]^+$, 178 $(C_9H_8NO_3)^+$, 159 $(C_9H_7N_2O)^+$, 150 $(C_8H_8NO_2)^+$, 133 $(C_8H_7NO)^{\cdot+}$, 119 $(C_8H_7O)^{\cdot+}$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$, 65 $(C_5H_5)^+$, 59 $(C_2H_3O_2)^+$.

2-[(5-Benzyl-1,3,4-oxadiazole-2-yl)sulfanyl]-N-(2-ethyl-6-methylphenyl)acetamide (8d)

HR-MS: $[M]^+$ 367.4646 (Calcd. for $C_{19}H_{19}N_3O_2S$; 367.5645); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.42 (s, 1H, NH), 7.14-7.03 (m, 3H, H-2′ to H-6′), 7.12 (t, *J* = 7.6 Hz,

1H, H-4‴), 7.04 (d, *J*=7.6 Hz, 2H, H-3‴ & H-5‴), 4.17 (s, 2H CH_2-2″), 3.99 (s, 3H, CH_2-7′), 2.45 (q, *J*=7.4 Hz, 2H, CH_2-1‴′), 2.12 (s, 3H, CH_3-3‴′), 1.02 (t, *J*=7.6 Hz, 3H, CH_3-2‴′); EIMS: *m/z* 367 ($C_{20}H_{21}N_3O_2S$) $[M]^+$, 176 $(C_{11}H_{14}NO)^+$, 162 $(C_{10}H_{12}NO)^+$, 159 $(C_9H_7N_2O)^+$, 134 $(C_9H_{12}N)^+$, 119 $(C_8H_7O)^{\cdot+}$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$ 65 $(C_5H_5)^+$.

Table 5: Cytotoxic Activity of 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated) acetamides (8a-e).

Compounds	Cytotoxic activity LD_{50} µgml^{-1}
8a	-
8b	22.39
8c	-
8d	18.69
8e	11.62
Doxorubicin	5.21

Note: (-) sign indicates that no activity was observed.

2-[(5-Benzyl-1,3,4-oxadiazole-2-yl)sulfanyl]-N-(3,4-dimethoxyphenyl)acetamide (8e)

HR-MS: $[M]^+$ 385.4386 (Calcd. for $C_{19}H_{19}N_3O_4S$; 385.4387); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 9.07 (s, 1H, NH), 7.36-7.29 (m, 5H, H-2′ to H-6′), 6.90 (dd, *J*=8.5, 2.5 Hz, 2H, H-5‴ and H-6‴), 6.78 (d, *J*=2.5 Hz, 1H, H-2‴), 4.18 (s, 3H, CH_2-7′), 3.92 (s, 2H CH_2-2″), 3.84 (s, 3H, CH_3-1‴′), 3.86 (s, 3H, CH_3-2‴′) (fig. 2); EIMS: *m/z* 385 $(C_{19}H_{19}N_3O_4S)$ $[M]^+$, 194 $(C_{10}H_{12}NO_3)^+$, 180 $(C_9H_{10}NO_3)^+$, 159 $(C_9H_7N_2O)^{\cdot+}$, 152 $(C_8H_{10}NO_2)^+$, 119 $(C_8H_7O)^+$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$ 65 $(C_5H_5)^+$ (fig. 3).

Biological evaluation

Anti-enzymatic potential

2-[(5-Benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/ arenylated)acetamides (8a-e) portrayed weaker anti-enzymatic potential against acetyl and butyrylcholinesterase and lipoxygenase enzymes. Compound 8a and 8d showed moderate inhibitory potential having MIC value of (74.71±0.05µM) and (70.84±0.05µM) against acetyl cholinesterase. Most prominent anti-enzymatic potential was demonstrated by 8e (17.5±0.11µM) when compared to standard Eserine (0.85±0.01µM). The results have been tabulated (table 3). 8e was the only but weak inhibitor of lipoxygenase having value of (157.2±0.07µM).

Antibacterial activity

2-[5-(Benzyl)-1,3,4-oxadiazole-2-ylthio)-*N*-(arylated/arenylated)]acetamides (8a-e) were screened for antibacterial potential against different Gram negative and Gram positive bacterial strains. All of them were found to possess weaker antibacterial potential when compared to standard Roxithromycin. The results are tabulated in table 4.

Cytotoxic analysis

2-[(5-Benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/arenylated)acetamides (8a-e) displayed less cytotoxicity when compared to standard; doxorubicin having LD_{50} of 5.21as demonstrated in table 5.

CONCLUSIONS

In conclusion, it was established from the aforesaid anti-enzymatic analysis and antibacterial action results that the 2-[(5-benzyl-1,3,4-oxadiazole-2yl)sulfanyl]-*N*-(arylated/ arenylated)acetamides (8a-e) are moderate to weak inhibitors of acetyl/ butyrylcholinesterase and lipoxygenase enzymes. Moreover, they also possessed weak antibacterial action except 8e and which may be due to the amalgamation of 3,4-dimethoxyphenylacetamide moiety with the parent oxadiazole core.

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