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2-(Aralkylated/Arylated/Arenylatedthio) 5-Benzyl-1, 3, 4-Oxadiazoles: As Less Cytotoxic And Moderate Inhibitors Of Cholinesterases

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ABSTRACT:

1, 3, 4-Oxadiazoles are known to possess diverse biological activities and proposed synthetic methodology was aimed to synthesize biologically active 1, 3, 4-oxadiazole-2-thiols derivatives. It was instigated by converting 2-phenylacetic acid (**1**) to ethyl-2-phenylacetate (**2**) by Fischer esterification method. The ester underwent hydrazinolysis to afford 2-phenylacetohydrazide (**3**) which was transformed via ring closure with carbon disulfide in alcoholic base to achieve 5-benzyl-1,3,4-oxadiazole-2-thiol (**4**). Finally, the parent oxadiazole **4** was reacted with a variety of aralkylated/arylated/arenylated halides (**5a-i**) in polar aprotic solvent; *N*,*N*'-dimethylformamide (DMF) and lithium hydride (LiH) which acted as base under to afford 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles (**6a-i**). The synthesized derivatives were screened against acetyl/butyrylcholinesterases for enzyme inhibitory potential. The incorporation of 3-nitro/4-nitrophenyl moieties on *S*-position of parent oxadiaozle demonstrated decent inhibitory potential against cholinesterases while rest displayed weak inhibition relative to reference standard Eserine. The LD_{50} data revealed that most of the derivatives were found to be less cytotoxic relative to standard doxorubicin.

Keywords: 2-(Aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles, enzyme inhibition analysis, cytotoxicity, ^{1}H-NMR and EI-MS.

1. 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[1]. The derivatives of 1,3,4-oxadiazole have been found to demonstrate varied biological activities e.g. anti-microbial, anti-HIV[2], anti-tubercular, anti-malarial[3], analgesic[4], anti-inflammatory[5], anti-convulsant[6], hypoglycemic[7]. Moreover, numerous derivatives of 1,3,4-oxadiazole are recognized as potentially active anti-mycobacterial[8,9], anti-cancer[10] agents and are also inhibitors of tyrosinase enzyme[11]. 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[12-20].

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 different specificities for the substrates and inhibitors for these enzymes are due to the variances in amino acid residues of the active sites of AChE and BChE. These enzymes are responsible for the termination of acetylcholine at cholinergic synapses[21, 22]. The key role of these enzymes is to catalyze the hydrolysis of acetylcholine; a neurotransmitter as a result of which of the nerve impulse is terminated in cholinergic synapses[23]. Hence, it is considered imperative to search for new cholinesterase inhibitors to bring in new drug candidates for the treatment of Alzheimer's and other correlated diseases. 2,5-Disubstituted-1,3,4-oxadiazoles are associated with diverse biological activities[24] in which N=C-S linkage is responsible for a large number of pharmacological activities[25,26]. It is the major core of various therapeutics[27] e.g. Nesapidil; an anti-hypertensive agent and Raltegravir; A Merck HIV retroviral drug[28].

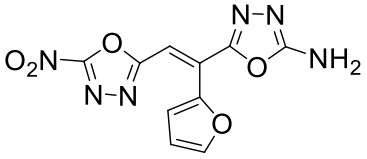

Fig-1: Structure of Nesapidil

They are a part of number of drugs which are used in last clinical trials e.g. Zibotentan[29]; anti-cancer agents, Ataluren[30]; treatment of cystic fibrosis. They are also known to possess anti-trypansomal, anti-helmintic, anti-proliferative (2-amino-5-aryl 1,3,4-oxadiazole) and splasmolytic activities[31-34].

The present research work is based on the recent advances on the discovery of allied bioactive compounds and a continuation of our preceding research efforts[35,36] to synthesize 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-

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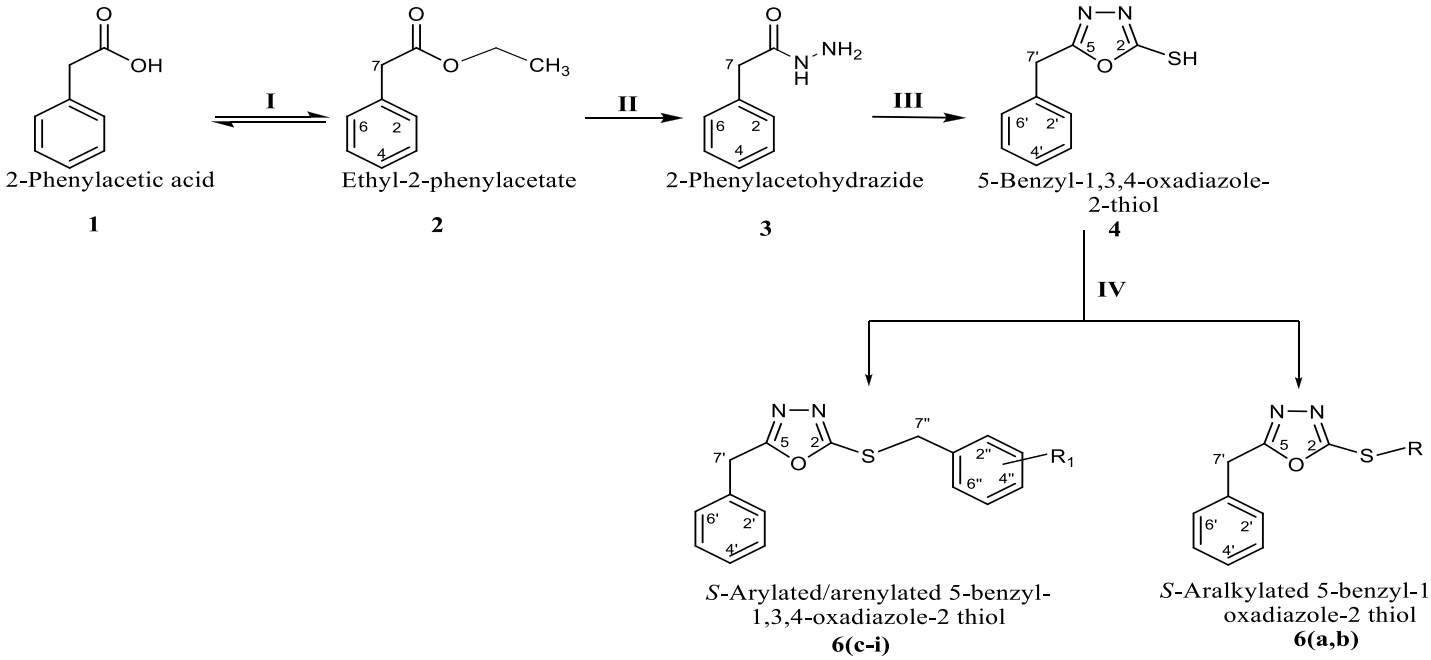
oxadiazoles. IR and ^{1}H-NMR analysis data endorsed the projected structures of synthesized compounds which depicted weak to moderate inhibition against cholinestrases. On basis of literature evidence[37] for determination of cytotoxicity brine shrimp assay was performed which revealed that most of the compounds are less cytotoxic in comparison to the standard used.

2. RESULTS AND DISCUSSION

2.1. Chemistry

2-Phenylacetic acid (**1**) was esterified using absolute ethanol and catalytic amount of sulfuric acid to ethyl-2-phenylacetate (**2**). The ester was reacted with hydrazine hydrate in methanol at 0 ºC to RT under stirring for an hour to form 2-phenylacetohydrazide (**3**) which was cyclized with carbon disulfide in alcoholic potassium hydroxide to yield 5-benzyl-1,3,4-oxadiazole-2-thiol (**4**). The oxadiazole was reacted with aralkylated/arylated/arenylated halides (**5a-i**) in DMF and LiH under stirring at RT for 3 h to afford 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles (**6a-i**) . The synthetic pathway is sketched in (**Scheme 1**; **Table 1**).



Scheme-1: Outline for the synthesis of 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles **(6a-i)**. Reagents & Conditions: (I) 2-Phenylacetic acid **(1)**/H_2SO_4/ethanol/reflux/6 h (II) Ethyl-2-phenylacetate **(2)**/$N_2H_4.H_2O$/methanol/stirring/0.5 h/0 °C to RT (III) 2-Phenylacetohydrazide **(3)**/CS_2/KOH/ethanol/reflux/6 h (IV) 5-benzyl-1,3,4-oxadiazole-2-thiol **(4)**/arylated/arenylated/aralkylated halides (**5a-i)**/DMF/LiH/stirring/3 h/RT.

Table-1: Different R groups in aralkylated/arylated/arenylated halides **(5a-i)**

Codes	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**
R	2-Br	2-Cl	3-Cl	4-Cl	4-F	3-NO_2	4-NO_2

Codes	**R**
5a	Benzyl
5b	Ethyl phenyl

2.2. Spectral Analysis

S-substituted derivatives (**6a-i**) were confirmed by observing characteristic IR absorption bands, molecular ion peaks and by counting the number of protons in NMR spectra The physical parameters of the synthesized *S*-substituted derivatives are tabulated in (**Table-2**) e.g. spectral data of **6a** revealed 2 multiplets at 7.32-30 and 7.29-25 for 10 protons of two phenyl rings and two set of singlets for two –CH_2 protons at 4.39 and 4.13 at 7″ and 7′ respectively. Similar pattern was observed for **6b** with exception of appearance of two triplets at 3.42 and 3.08 at 8″ and 7″ for 2 –CH_2 groups in neighborhood of one another. **6c** data confirmed the presence of ortho moiety attached to *S*-atom as evident from the appearance of two doublets at 7.53 for H-6″, 7.48 for H-3″, 2 broad triplets at 7.19 and 7.12 for H-5″ and H-4″ respectively. Similar pattern was observed for **6d** with little change in chemical shift values. **6e** NMR pattern showed the appearance of a br.s for H-2″ and a multiplet for H-4″-H-6″. **6f** and **6g** revealed A_2B_2 spin system confirming the para substituted moieties. **6h** showed pattern similar to 6e confirming the meta substituted moiety. **6i** also showed pattern similar to 6f/g by displaying a diorthocoupled pattern.

Table-2: Physical parameters of 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles (6a-i)

Code	Appearance	M.P. (°C)	Yield (%)	Mol. For./Wt. ($gmol^{-1}$)	IR KBr, v_{max} (cm^{-1})
6a	Off-white solid	70	93	$C_{16}H_{14}N_2OS$ 282	2870 (C-H str. of aromatic ring), 1456 (C=N str. of oxadiazole ring), 1430 (C=C aromatic ring str.)
6b	Off-white solid	89	95	$C_{17}H_{16}N_2OS$ 296	2860 (C-H str. of aromatic ring), 1496 (C=C str. of aromatic ring), 1445 (C=N str. of oxadiazole ring)
6c	Light yellow solid	65	95	$C_{16}H_{13}BrN_2OS$ 361	2889 (C-H str. of aromatic ring), 1478 (C=C aromatic ring str.), 1476 (C=N str. of oxadiazole ring)
6d	White solid	77	92	$C_{16}H_{13}ClN_2OS$ 316	2989 (C-H str. of aromatic ring), 1526 (C=C str. of aromatic ring), 1476 (C=N str. of oxadiazole ring)
6e	White solid	79	90	$C_{16}H_{13}ClN_2OS$ 316	2898 (C-H str. of aromatic ring), 1520 (C=C str. of aromatic ring), 1468 (C=N str. of oxadiazole ring)
6f	White solid	93	94	$C_{16}H_{13}ClN_2OS$ 316	2895 (C-H str. of aromatic ring), 1376 (C=C aromatic ring str.), 1432 (C=N str. of oxadiazole ring)
6g	White pallets	105	98	$C_{16}H_{13}FN_2OS$ 300	2900 (C-H str. of aromatic ring), 1537 (C=C str. of aromatic ring), 1457 (C=N str. of oxadiazole ring)
6h	Brownish pallets	67	91	$C_{16}H_{13}N_3O_3S$ 327	2877 (C-H str. of aromatic ring), 1537 (C=C str. of aromatic ring), 1457 (C=N str. of oxadiazole ring)
6i	Brown powder	105	94	$C_{16}H_{13}N_3O_3S$ 327	2900 (C-H str. of aromatic ring), 1537 (C=C str. of aromatic ring), 1457 (C=N str. of oxadiazole ring)

2.3. Biological Evaluation

2.3.1. Enzyme Inhibition Activity

The synthesized 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles (**6a-i**) were evaluated for enzyme inhibitory potential against acetyl/butyrylcholinesterase (AChE/BChE). The anti-enzymatic data revealed that *S*-substituted derivatives were found to be weak to moderate inhibitors of cholinesterases having IC_{50} values ranging from 460.31 ± 0.13 µM to 81.31 ± 0.59 µM for AChE and from > 500 µM to 50.31 ± 0.27 µM for BChE. The enzyme inhibition data is tabulated in (**Table-3**). The prominent results were demonstrated by the *S*-substituted derivatives where incorporation of 3-nitrophenyl and 4-nitrophenyl on 5-benzyl-1,3,4-oxadiazole-2-thiol were found to be fruitful i.e. **6i** having IC_{50} value of 81.31 ± 0.59 µM for AChE and 50.31 ± 0.27 µM for BChE and **6h** having IC_{50} value of 84.63 ± 0.41 µM for AChE and 65.93 ± 0.58 µM for BChE, when compared to standard, Eserine having IC_{50} value of 0.04 ± 0.0001 µM and 0.85 ± 0.0001 µM for AChE and BChE respectively.

Table-3: Enzyme Inhibition Activity of 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles **(6a-i)**

Codes	AChE			BChE	
	Conc. (mM)	Inhibition (%)	IC_{50} (µM)	Inhibition %	IC_{50} (µM)
6a	0.5	82.80±0.92	366.91±0.57	65.77±0.56	416.73±0.32
6b	0.5	63.20±0.32	444.92±0.25	50.90±0.36	>500
6c	0.5	57.71±0.29	460.31±0.13	75.86±0.73	275.93±0.39
6d	0.5	57.47±0.45	439.72±0.26	58.65±0.41	359.45±0.23
6e	0.5	84.23±0.96	333.34±0.56	64.68±0.59	226.01±0.21
6f	0.5	80.65±0.91	227.31±0.75	52.79±0.48	481.23±0.25
6g	0.5	74.19±0.73	357.11±0.37	21.08±0.22	>500
6h	0.5	87.60±0.79	84.63±0.41	89.93±0.87	65.93±0.58
6i	0.5	89.66±0.81	81.31±0.59	87.66±0.99	50.31±0.27
Eserine	**0.5**	**91.27±1.17**	**0.04±0.0001**	**82.82±1.09**	**0.85±0.0001**

2.3.2. Cytotoxic Analysis (Brine Shrimp Assay)

2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles (**6a-i**) also showed less cytotoxicity as compared to standard except **6a**, **6b**, **6d** and **6f** when compared to standard Doxorubicin having LD_{50} level of 5.21 μgml^{-1}**.** The data is tabulated in (**Table 4**).

3. EXPERIMENT

3.1. Measurements

The chemicals employed in the current research work were attained from Sigma/Fluka and all were of analytical grade. All solvents consumed herein were distilled prior to use. Melting points of the synthesized compounds were determined in open capillary tubes on electro-thermal Griffin and George melting point apparatus. The homogeneity of the synthesized compounds and the advancement in the reactions were monitored by ascending thin layer chromatographic technique which was executed on pre coated silica gel 60 F_{254} plates as adsorbent and UV light was

used as visualizing agent at 254 nm. Various percentages of *n*-hexane and ethyl acetate were used as mobile phase. IR spectra were recorded on a Jasco-320-A spectrophotometer by KBr disc method and absorption bands are expressed in wave number (cm^{-1}). ^{1}H-NMR was acquired in deuterated chloroform on a Bruker spectrometer operating at 300-500 MHz frequency and chemical shifts are expressed in parts per million (δ ppm) while coupling constants (*J*) are mentioned in Hertz (Hz). Mass spectra (EI-MS) were recorded on a JMS-HX-110 spectrometer, with a data system.

Table-4: Cytotoxic activity of 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles (**6a-i**)

Compounds	Cytotoxic Activity LD_{50} µgml^{-1}
6a	2.2
6b	2.7
6c	17.3
6d	2.5
6e	36.6
6f	2.3
6g	150.9
6h	160.4
6i	157.1
Doxorubicin	**5.21**

3.2. 2-(Arylated/arenylated/aralkylatedthio) 5-benzyl-1,3,4-oxadiazole-2-thiols (6a-i)

The parent scaffold i.e. 5-benzyl-1,3,4-oxadiazole-2-thiol (**4**; **Figure 2**) was synthesized from a valuable synthon; 2-phenylacetic acid by already published method[36].

5-benzyl-1,3,4-oxadiazole-2-thiol (**4**; 0.001 mol; 1 eq.) solubilized in 10mL DMF was taken in a 25 mL round-bottomed flask. Lithium hydride (0.002 moles) was added to the reaction mixture which was stirred at room temperature for 20 min. After 20 min arylated/arenylated/aralkylated halides (**5a-i**; 0.001 moles; 1 eq.) were further added to the reaction mixture and stirred for three hours at room temperature. Progress of the reaction was monitored by TLC till single spot. 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles (**6a-i**) by quenching the reaction mixture with crushed ice. Precipitates obtained were filtered, washed with distilled water and air-dried to afford pure product.

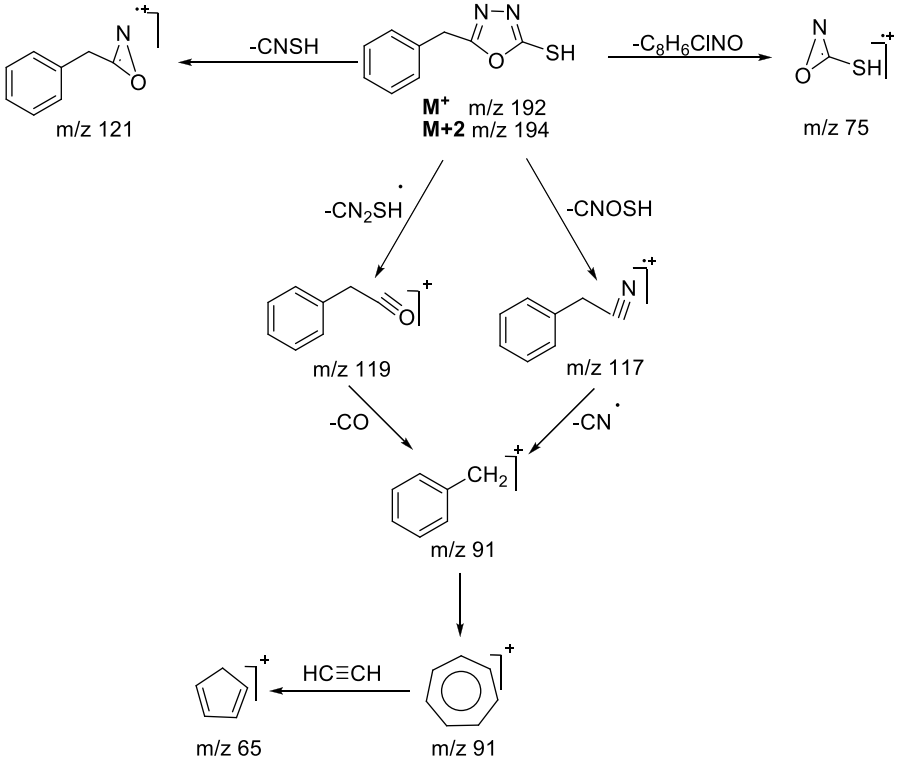


Fig-2: Proposed mass fragmentation pattern of 5-Benzyl-1,3,4-oxadiazole-2-thiol (**4**).

3.3. Spectral Characterization

3.3.1. 2-(Benzylthio) 5-benzyl-1,3,4-oxadiazole (6a)

HR-MS: $[M]^+$ 282.3601 (Calcd. for $C_{16}H_{14}N_2O_2S$; 282.4602); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.32-7.30 (m, 5H, H-2′ to H-6′), 7.29-7.25 (m, 5H, H-2″ to 6″), 4.39 (s, 2H, CH_2-7″), 4.13 (s, 2H, CH_2-7′); EIMS: *m/z* 284 (M+2; $C_{16}H_{14}N_2O_2S$), 282 (M^+; $C_{16}H_{14}N_2O_2S$), 159 $(C_9H_7N_2O)^+$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$.

3.3.2. 2-(Ethylphenylthio) 5-benzyl-1,3,4-oxadiazole (6b)

HR-MS: $[M]^+$ 296.3881 (Calcd. for $C_{17}H_{16}N_2OS$; 296.3885); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.46-7.30 (m, 5H, H-2′ to H-6′), 7.23-7.17 (m, 5H, H-2″ to 6″), 4.14 (s, 2H, CH_2-7′), 3.42 (t, J = 7.5 Hz, 2H, CH_2-8″), 3.08 (t, J = 7.5 Hz, 2H, CH_2-7″); EIMS: *m/z* 298 (M+2; $C_{17}H_{16}N_2OS$), 296 (M^+; $C_{17}H_{16}N_2OS$), 159 $(C_9H_7N_2O)^+$, 117 $(C_8H_7N)^+$, 105 $(C_8H_9)^+$, 91 $(C_7H_7)^+$, 77 $(C_6H_5)^+$.

3.3.3. 2-(2-Bromobenzylthio) 5-benzyl-1,3,4-oxadiazole (6c)

HR-MS: $[M]^+$ 360.2562 (Calcd. for $C_{16}H_{13}BrN_2OS$; 360.3563); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.53 (d, J = 8.0, 1H, H-6″), 7.48 (d, J = 8.0, 1H, H-3″), 7.31-7.25 (m, 5H, H-2′ & H-6′), 7.19 (br.t, J = 7.6 Hz, 1H, H-5″), 7.12 (br.t, J = 7.6 Hz, 1H, H-4″), 4.50 (s, 2H, CH_2-7″), 4.13 (s, 2H, CH_2-7′); EIMS: *m/z* 362 (M+2; $C_{16}H_{13}BrN_2OS$), 360 (M^+; $C_{16}H_{13}BrN_2OS$), 170 $(C_7H_6Br)^+$, 159 $(C_9H_7N_2O)^+$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$.

3.3.4. 2-(2-Chlorobenzylthio) 5-benzyl-1,3,4-oxadiazole (6d)

HR-MS: $[M]^+$ 316.8052 (Calcd. for $C_{16}H_{13}ClN_2OS$; 361.9053); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.53 (d, J = 4.0, 1H, H-6″), 7.48 (d, J = 8.0, 1H, H-3″), 7.31-7.25 (m, 5H, H-2′ & H-6′), 7.19 (br.t, J = 7.6 Hz, 1H, H-5″), 7.12 (br.t, J = 7.6 Hz, 1H, H-4″), 4.50 (s, 2H, CH_2-7″), 4.13 (s, 2H, CH_2-7′); EIMS: *m/z* 318 (M+2; $C_{16}H_{13}ClN_2OS$), 316 (M^+; $C_{16}H_{13}ClN_2OS$), 159 $(C_9H_7N_2O)^+$, 125 $(C_7H_6Cl)^+$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$.

3.3.5. 2-(3-Chlorobenzylthio) 5-benzyl-1,3,4-oxadiazole (6e)

HR-MS: $[M]^+$ 316.8052 (Calcd. for $C_{16}H_{13}ClN_2OS$; 361.9053); ^{1}H-NMR: (400 MHz, $CDCl_3$, δ / ppm): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.36 (br.s, 1H, H-2″), 7.34-7.27 (m, 3H, H-4″ to H-6″), 7.24-7.20 (m, 5H, H-2′ to H-6′), 4.35 (s, 2H, CH_2-7″), 4.13 (s, 2H, CH_2-7′); EIMS: *m/z* 318 (M+2; $C_{16}H_{13}ClN_2OS$), 316 (M^+; $C_{16}H_{13}ClN_2OS$), 159 $(C_9H_7N_2O)^+$, 125 $(C_7H_6Cl)^+$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$.

3.3.6. 2-(4-Chlorobenzylthio) 5-benzyl-1,3,4-oxadiazole (6f)

HR-MS: $[M]^+$ 316.8052 (Calcd. for $C_{16}H_{13}ClN_2OS$; 361.9053); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.42 (br.d, J = 8.0 Hz, 2H, H-3″ & H-5″), 7.27 (br.d, J = 8.0 Hz, 2H, H-2″ & H-6″), 7.30-7.26 (m, 5H, H-2′ to H-6′), 4.33 (s, 2H, CH_2-7″), 4.13 (s, 2H, CH_2-7′); EIMS: *m/z* 318 (M+2; $C_{16}H_{13}ClN_2OS$), 316 (M^+; $C_{16}H_{13}ClN_2OS$), 159 $(C_9H_7N_2O)^+$, 125 $(C_7H_6Cl)^+$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$.

3.3.7. 2-(4-Fluorobenzylthio) 5-benzyl-1,3,4-oxadiazole (6g)

HR-MS: $[M]^+$ 300.3506 (Calcd. for $C_{16}H_{13}FN_2OS$; 300.4507); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.38 (br.d, J = 8.0 Hz, 2H, H-3″ & H-5″), 7.32-7.25 (m, 5H, H-2′ to H-6′), 7.21 (br.d, J = 8.0 Hz, 2H, H-2″ & H-6″), 4.32 (s, 2H, CH_2-7″), 4.13 (s, 2H, CH_2-7′); EIMS: *m/z* 302 (M+2; $C_{16}H_{13}FN_2OS$), 300 (M^+; $C_{16}H_{13}FN_2OS$), 159 $(C_9H_7N_2O)^+$, 117 $(C_8H_7N)^+$, 109 $(C_7H_6F)^+$, 91 $(C_7H_7)^+$.

3.3.8. 2-(3-Nitrobenzylthio) 5-benzyl-1,3,4-oxadiazole (6h)

HR-MS: $[M]^+$ 327.3582 (Calcd. for $C_{16}H_{13}N_3O_3S$; 327.4507); ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.25 (br.s, 1H, H-2″), 8.11 (d, J = 8.0 Hz, 1H, H-6″), 7.73 (d, J = 7.6 Hz, 1H, H-4″), 7.44 (t, J = 8.0, 1H, H-5″), 7.31 (d, J = 7.2, 2H, H-2′ & H-6′), 7.27-7.25 (m, 3H, H-3′ to H-5′), 4.45 (s, 2H, CH_2-7″), 4.12 (s, 2H, CH_2-7′); EIMS: *m/z* 329 (M^+; $C_{16}H_{13}N_3O_3S$), 327 (M^+; $C_{16}H_{13}N_3O_3S$), 159 $(C_9H_7N_2O)^+$, 136 $(C_7H_6NO_2)^+$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$.

3.3.9. ***2-(4-Nitrobenzylthio) 5-benzyl-1,3,4-oxadiazole*** **(6i)**

HR-MS: $[M]^+$ 327.3582 (Calcd. for $C_{16}H_{13}N_3O_3S$; 327.4507); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.11 (br.d, J = 8.0 Hz, 2H, H-3″ & H-5″), 7.53 (br.d, J = 8.0 Hz, 2H, H-2″ & H-6″), 7.32-7.28 (m, 5H, H-2′ & H-6′), 4.42 (s, 2H, CH_2-7″), 4.12 (s, 2H, CH_2-7′); EIMS: *m/z* 329 (M^+; $C_{16}H_{13}N_3O_3S$), 327 (M^+; $C_{16}H_{13}N_3O_3S$), 159 $(C_9H_7N_2O)^+$, 136 $(C_7H_6NO_2)^+$, 117 $(C_8H_7N)^+$, 91 $(C_7H_7)^+$.

3.4. BIOLOGICAL ASSAYS

3.4.1. Cholinesterase assay

The AChE and BChE inhibition assays were done according to the method described by Ellman *et al*.[38] 100 µL reaction mixture contained 60 µL Na_2HPO_4 buffer having conc. of 50 mM with pH 7.7 was prepared. Test compound of 10 µL & conc. of 0.5 mM well^{-1} was poured, accompanied by the accession of 10 µL enzyme of concentration 0.005 unit/well. All contents were immixed and pre-read at a wavelength of 405 nm. After that contents were pre-incubated for 10 min at 37 ºC to initiate the reaction through 10 µL of conc. 0.5 mM well^{-1} substrate i.e. acetylthiocholine iodide (for AChE) or butyrylthiocholine chloride (for BChE). Then the 10 µL of DTNB with conc. 0.5 mM well^{-1} were added. After incubation of 15 min at 37 ºC, absorbance at 405 nm was measured by 96-well plate reader (Synergy HT, Biotek, USA). All the observations were carried out in triplicate with their respective controls. Eserine of conc. 0.5 mM well^{-1} was applied as a positive control. The percent inhibition was calculated by the help of following equation.

$$\%\ \boldsymbol{age\ Inhibition} = \frac{\boldsymbol{Control - Test}}{\boldsymbol{Control}} \times \mathbf{100}$$

IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA). IC_{50} values were determined by serial dilution of the compounds from 0.5 mM to 0.25, 0.125, 0.0625, 0.03125, 0.015625 mM. IC_{50} value was calculated from the graph, the concentration at which the enzyme inhibition was 50 %. Values are mean of 3 independent experiments.

3.4.2. Cytotoxic Assay

The assay was employed by the reported method[39]. Sea salt $34gL^{-1}$ was used to prepare artificial sea water. A shallow rectangular dish (22×32 cm) containing brine shrimps was maintained under constant aeration for 48 h at RT for hatching brine shrimp (*Artemia salina*) eggs (Sera, Heidelberg, Germany). After hatching, active shrimps were collected without eggs from brighter portion of the dish for analysis. 10 shrimps were transferred to each vial via pasture pipette vial containing 5 mL of artificial sea water with 200, 20, 2 and 0.2 μgmL^{-1} of test compound from their stock solution. The vials were maintained at 25-28 °C under illumination for 24 h. After 24 h, the number of survived shrimps was counted. Finney computer program was used to analyze data and for determining LD_{50} (lethal dose that killed 50 % shrimps) values.

3.4.3. Statistical analysis

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

4. CONCLUSION

A series of 2-(aralkylated/arylated/arenylatedthio) 5-benzyl-1,3,4-oxadiazoles (**6a-i**), was synthesized by reaction of different aralkylated/arylated/arenylated halides (**5a-i**) with 5-benzyl-1,3,4-oxadiazole-2-thiol (4) in polar aprotic solvent in the presence of catalytic amount LiH. The *2*-substituted oxadiazoles were structurally confirmed by modern spectral techniques. The studied molecules displayed moderate to weak enzyme inhibition activity against cholinesterases and most of them possess less cytotoxicity. Structural modifications amongst the substituent s used as electrophiles for substitution on *S*-atom of oxadiazole moiety may lead to enhanced bioactivity and the synthesized molecules may be utilized as suitable therapeutic agents for the treatment of different ailments.

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