

SYNTHESIS, ANTIBACTERIAL SCREENING AND HEMOLYTIC ACTIVITY OF *S*-SUBSTITUTED DERIVATIVES OF 5-BENZYL-1,3,4-OXADIAZOLE-2-THIOL

AZIZ-UR-REHMAN^{1,*}, SIDDIQUI S.Z.¹, ABBASI M.A.¹, ABBAS N.¹, KHAN K.M.², SHAHID M.³, MAHMOOD Y.³
AKHTAR M. N.⁴ AND LAJIS N. H.⁴

¹Department of Chemistry, Government College University, Lahore-54000, ²HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, ³Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan, ⁴Laboratory of Natural Products, Institute of Bioscience, University Putra Malaysia, Malaysia.
Email: azizryk@yahoo.com

Received: 26 Dec 2011, Revised and Accepted: 2 Feb 2012

ABSTRACT

In the present study, a series of *S*-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2-thiol were synthesized by converting phenyl acetic acid successively into ester, hydrazide and 1,3,4-oxadiazole. Finally the target compounds were obtained by stirring 5-benzyl-1,3,4-oxadiazole-2-thiol with different electrophiles in the presence of sodium hydride (NaH) and dimethyl formamide (DMF). The structure of synthesized compounds has been established by spectral data. All the compounds have been screened for their antimicrobial and hemolytic activity.

Keywords: Phenyl acetic acid, 1,3,4-oxadiazole, antimicrobial activity, hemolytic activity, ¹H-NMR and EI-MS.

INTRODUCTION

1,3,4-oxadiazoles concerned to the group of heterocyclic compounds that have appealing attention for last two decades due to their extensive range of biological concentration.¹ 2,5-disubstituted-1,3,4-Oxadiazole derivatives have attractive concentration due to broad range of biological interactions such as anti-inflammatory² antifungal^{3,4} antiparasitic⁵ and antimicrobial^{6,7} effects. These derivatives have inhibitory effect against HIV replication.⁸ Some of 2,5-disubstituted-1,3,4-oxadiazole derivatives potent against 60 tumor cell lines. Biological results demonstrate a very remarkable anti-tumor activity against leukemia, colon and breast cancer.^{9,11}

Literature survey revealed that minor modification in the structure of 1,3,4-oxadiazole can lead to quantitative as well as qualitative changes in the biological activity. It prompted us to synthesize the various *S*-substituted derivatives of oxadiazole derived from phenyl acetic acid with the goal of having lesser toxicity and improved activity. For this, the parent compound 5-benzyl-1,3,4-oxadiazole-2-thiol (**4**), was prepared by converting successively phenyl acetic acid into ester, hydrazide and 1,3,4-oxadiazole. Finally the title compounds were obtained by reacting 5-benzyl-1,3,4-oxadiazole-2-thiol with different alkyl halides to acquire *S*-alkyl substituted derivatives of oxadiazole.

MATERIALS AND METHODS

Reagents

Melting points of the synthesized compounds were recorded on a Griffin and George melting point apparatus by open capillary tube and were uncorrected. Purity was checked on thin layer chromatography (TLC) on pre-coated silica gel G-25-UV₂₅₄ plates with different solvent systems using ethyl acetate and *n*-hexane giving single spot. Detection was carried out at 254 nm, and by ceric sulphate reagent. The I.R. spectra were recorded in KBr pellet method on a Jasco-320-A spectrophotometer (wave number in cm⁻¹). Nuclear magnetic resonance spectra were recorded in CDCl₃ on a Bruker spectrometers operating at 500 MHz. Chemical shifts are given in ppm. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer, with a data system.

Procedure for the preparation of ethyl phenyl acetate (**2**)

Phenylacetic acid **1** (1g) was taken in round bottom flask fitted with reflux condenser. Absolute ethyl alcohol (4 mL) and concentrated sulphuric acid (1/2 mL) was added and reaction mixture was refluxed for 1 hour. The reflux time was checked by thin layer chromatography (TLC). After completion of reaction, reaction mixture was poured into the separating funnel that contained 50 mL

distilled water. Diethyl ether (20 mL) was added to the separating funnel and mixture was shaken vigorously, neutralization of reaction mixture was done by using concentrated solution of sodium carbonate (Na₂CO₃) until all free acids was removed. The solution was allowed to stand for some time, two layers were formed i.e. lower layer was aqueous and upper layer was organic that contained ethyl phenylacetate. Two layers were carefully separated and lower aqueous layer was discarded. Upper layer was taken in distillation flask. The flask was fitted with thermometer, a condenser and a receiving flask. Diethyl ether was distilled off and the transparent liquid, ethyl phenylacetate was obtained from the flask.

Procedure for the preparation of phenyl acetic acid hydrazide (**3**)

Reaction mixture ethyl phenylacetate (0.01 mol, 1.64 mL) and 5 mL of methanol was added in the round bottom flask and mixture was cooled to 0-5°C. Hydrazine hydrate (80% 1.5 mL) was added drop wise in reaction mixture and then this solution was stirred for 40 mins at temperature 0-5°C. Reaction completion was monitored by TLC. After completion of reaction precipitate was formed, filtered out and washed with hexane.

Procedure for the preparation of 5-benzyl-1,3,4-oxadiazole-2-thiol (**4**)

Acid hydrazide **3** (1.5 g, 0.01 mol) was dissolved in absolute ethanol (10 mL) in a 250 mL round bottom flask. Carbon disulfide (1.89 mL, 0.03 mol) was then added to the solution followed by the addition of excess potassium hydroxide (1.12 g, 0.02 mol) in water (5 mL). This reaction mixture was properly stirred and reflux for 6 hours. Color of solution was changed; initially it was yellow which turned to brownish yellow with the progress of reaction. Hydrogen sulfide gas was evolved during this reaction. The mixture was diluted with distilled water (50 mL) and acidified with dilute hydrochloric acid to pH 2-3. It was then filtered, washed with water and recrystallized from ethanol.

General procedure for the synthesis of *S*-substituted of 5-benzyl-1,3,4-oxadiazole-2-thiol (**4a-j**)

The calculated amount of 5-benzyl-1,3,4-oxadiazole-2-thiol (0.1 mmol) was taken in a round bottom flask (50 mL), then *N,N*-dimethyl formamide (DMF) (10 mL) was added to dissolve it followed by the addition of sodium hydride (0.1 mmol) to the mixture. The mixture was stirred for 30 minutes at room temperature and then slowly added the alkyl halide to the mixture and the solution was further stirred for three hours. The progress of reaction was monitored *via* TLC till single spot. Distilled water was added in the flask and the product was received by solvent extraction.

5-benzyl-1,3,4-oxadiazole-2-thiol (4)

Yellowish brown amorphous powder, Yield 89%, m.p. 130°C. Molecular formula $C_9H_8N_2OS$; Mol. Wt. 192. IR (KBr): ν_{max} : 3058 (C-H stretching of aromatic ring), 1575 (C=C str. of aromatic ring), 1518 (C=N stretching); 1H -NMR (500 MHz, $CDCl_3$): δ 7.46 (m, 2H, H-2', H-6'), 7.34 (m, 2H, ArH-3', H-5'), 7.26 (m, 1H, H-4'), 4.02 (s, 2H, CH_2 -7); EIMS: m/z 192 (45%) [M]⁺, 117 (100%; loss of benzyl cyanide cation fragment), 91 (32%; loss of tropylium ion fragment).

5-benzyl-2-(methylsulfanyl)-1,3,4-oxadiazole (4a)

Off white amorphous powder, Yield 79%, m.p. 38°-40°C. Molecular formula $C_{10}H_{10}N_2OS$; Mol. Wt. 206. IR (KBr): ν_{max} : 3058 (C-H of aromatic ring), 1557 (C=C stretching of aromatic ring), 1519 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.42 (m, 2H, H-2', H-6'), 7.32 (m, 2H, ArH-3', H-5'), 7.25 (m, 1H, H-4'), 4.16 (s, 2H, CH_2 -7), 2.67 (s, 3H, CH_3 -1"); EIMS: m/z 206 (10%) [M]⁺, 191 (26%), 117 (100%; loss of benzyl cyanide cation fragment), 91 (47%; loss of tropylium ion fragment).

5-benzyl-2-(ethylsulfanyl)-1,3,4-oxadiazole (4b)

Light brown greasy liquid, Yield 72%, Molecular formula $C_{11}H_{12}N_2OS$; Mol. Wt. 220. IR (KBr): ν_{max} : 3057 (C-H of aromatic ring), 1566 (C=C stretching of aromatic ring), 1519 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.44 (m, 2H, H-2', H-6'), 7.35 (m, 2H, ArH-3', H-5'), 7.26 (m, 1H, H-4'), 4.11 (s, 2H, CH_2 -7), 3.22 (s, 2H, CH_2 -1") 1.44 (s, 3H, CH_3 -2"); EIMS: m/z 220 (12%) [M]⁺, 117 (100%; loss of benzyl cyanide cation fragment), 91 (29%; loss of tropylium ion fragment), 29 (15%).

5-benzyl-2-(iso-propan-2-ylsulfanyl)-1,3,4-oxadiazole (4c)

Light green greasy liquid, Yield 65%, Molecular formula $C_{12}H_{14}N_2OS$; Mol. Wt. 234. IR (KBr): ν_{max} : 3059 (C-H of aromatic ring), 1574 (C=C stretching of aromatic ring), 1525 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.43 (m, 2H, H-2', H-6'), 7.35 (m, 2H, ArH-3', H-5'), 7.25 (m, 1H, H-4'), 4.13 (s, 2H, CH_2 -7), 3.85 (m, 1H, H-1") 1.46 (d, J = 6.0 Hz, 6H, CH_3 -2", CH_3 -3"); EIMS: m/z 234 (12%) [M]⁺, 117 (100%; loss of benzyl cyanide cation fragment), 91 (44%; loss of tropylium ion fragment), 43 (26%).

5-benzyl-2-(butylsulfanyl)-1,3,4-oxadiazole (4d)

Brown greasy liquid, Yield 85%, Molecular formula $C_{13}H_{16}N_2OS$; Mol. Wt. 248. IR (KBr): ν_{max} : 3055 (C-H of aromatic ring), 1520 (C=C stretching of aromatic ring), 1582 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.46 (m, 2H, H-2', H-6'), 7.33 (m, 2H, ArH-3', H-5'), 7.24 (m, 1H, H-4'), 4.16 (s, 2H, CH_2 -7), 3.21 (br.s, 2H, CH_2 -1") 1.74 (m, 2H, CH_2 -2") 1.42 (m, 2H, CH_2 -3") 0.91 (t, J = 6.5 Hz, 3H, CH_3 -4"); EIMS: m/z 248 (19%) [M]⁺, 191 (23%), 117 (100%; loss of benzyl cyanide cation fragment), 91 (35%; loss of tropylium ion fragment), 57 (39%).

5-benzyl-2-[(2-methylpropyl)sulfanyl]-1,3,4-oxadiazole (4e)

Brown greasy liquid, Yield 82%, Molecular formula $C_{13}H_{16}N_2OS$; Mol. Wt. 248. IR (KBr): ν_{max} : 3059 (C-H of aromatic ring), 1509 (C=C stretching of aromatic ring), 1567 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.47 (m, 2H, H-2', H-6'), 7.32 (m, 2H, ArH-3', H-5'), 7.27 (m, 1H, H-4'), 4.14 (s, 2H, CH_2 -7), 3.19 (d, J = 6.0 Hz, 2H, CH_2 -1") 1.77 (m, 2H, H-2"), 0.92 (d, J = 6.5 Hz, 6H, CH_3 -3", CH_3 -4"); EIMS: m/z 248 (10%) [M]⁺, 117 (100%; loss of benzyl cyanide cation fragment), 91 (62%; loss of tropylium ion fragment), 57 (55%).

5-benzyl-2-(pentylsulfanyl)-1,3,4-oxadiazole (4f)

Brown greasy liquid, Yield 85%, Molecular formula $C_{14}H_{18}N_2OS$; Mol. Wt. 262. IR (KBr): ν_{max} : 3055 (C-H of aromatic ring), 1520 (C=C stretching of aromatic ring), 1582 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.46 (m, 2H, H-2', H-6'), 7.33 (m, 2H, ArH-3', H-5'), 7.24 (m, 1H, H-4'), 4.16 (s, 2H, CH_2 -7), 3.58 (br.s, 2H, CH_2 -1"), 1.36 (m, 2H, CH_2 -2"), 1.33 (m, 2H, CH_2 -3" & CH_2 -4") 0.86 (t, J = 7.5 Hz, 3H CH_3 -5"); EIMS: m/z 262 (12%) [M]⁺, 117 (100%; loss of benzyl cyanide cation fragment), 91 (49%; loss of tropylium ion fragment), 71 (24%).

[(5-benzyl-1,3,4-oxadiazol-2-yl)sulfanyl]acetic acid (4g)

Light brown amorphous powder, Yield 68%, m.p. 60°-62°C, Molecular formula $C_{11}H_{10}N_2O_3S$; Mol. Wt. 250. IR (KBr): ν_{max} : 3056 (C-H of aromatic ring), 1575 (C=C stretching of aromatic ring), 1518

(C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.40 (m, 2H, H-2', H-6'), 7.28 (m, 2H, H-3', H-5'), 7.20 (m, 1H, H-4'), 4.18 (s, 2H, CH_2 -7), 3.69 (s, 2H, CH_2 of acetic acid); EIMS: m/z 250 [M]⁺, 191 (34%), 117 (100%; loss of benzyl cyanide cation fragment), 91 (45%; loss of tropylium ion fragment).

5-benzyl-2-[(2-bromoethyl)sulfanyl]-1,3,4-oxadiazole (4h)

Creamy brown amorphous powder, Yield 79%, m.p. 54°-56°C, Molecular formula $C_{11}H_{11}BrN_2OS$; Mol. Wt. 299. IR (KBr): ν_{max} : 3059 (C-H of aromatic ring), 1570 (C=C stretching of aromatic ring), 1520 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.43 (m, 2H, H-2', H-6'), 7.29 (m, 2H, H-3', H-5'), 7.21 (m, 1H, H-4'), 4.14 (s, 2H, CH_2 -7), 3.90 (br.s, 2H, CH_2 -1"), 3.43 (t, J = 6.5, 2H CH_2 -2"); EIMS: m/z 299 (12%) [M]⁺, 117 (100%; loss of benzyl cyanide cation fragment), 107 (14%), 91 (53%; loss of tropylium ion fragment).

5-benzyl-2-[(2-phenylmethyl)sulfanyl]-1,3,4-oxadiazole (4i)

Transparent greasy liquid, Yield 70%, Molecular formula $C_{16}H_{14}N_2OS$; Mol. Wt. 282. IR (KBr): ν_{max} : 3052 (C-H of aromatic ring), 1576 (C=C stretching of aromatic ring), 1518 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.42 (m, 2H, H-2', H-6'), 7.32 (m, 2H, H-3', H-5'), 7.22 (m, 1H, H-4'), 7.14-7.20 (m, 5H, H-2" to H-6"), 4.12 (s, 2H, CH_2 -7), 3.51 (s, 2H, H-8").

EIMS: m/z 282 (16%) [M]⁺, 191 (16%), 117 (100%; loss of benzyl cyanide cation fragment), 91 (32%; loss of tropylium ion fragment).

5-benzyl-2-[(4-chlorobenzyl)sulfanyl]-1,3,4-oxadiazole (4j)

Transparent greasy liquid, Yield 54%, Molecular formula $C_{16}H_{13}ClN_2OS$; Mol. Wt. 316. IR (KBr): ν_{max} : 3048 (C-H of aromatic ring), 1570 (C=C stretching of aromatic ring), 1523 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.46 (m, 2H, H-2', H-6'), 7.44 (d, J = 8.1 Hz, 2H, H-3" & H-5"), 7.30 (m, 2H, H-3' & H-5'), 7.11 (d, J = 8.1 Hz, 2H, H-2" & H-6"), 7.17-4.10 (s, 2H, CH_2 -7), 3.56 (s, 2H, H-7"); EIMS: m/z 316 (36%) [M]⁺, 125 (23%), 117 (100%; loss of benzyl cyanide cation fragment), 91 (64%; loss of tropylium ion fragment).

5-benzyl-2-[(2-phenylethyl)sulfanyl]-1,3,4-oxadiazole (4k)

Transparent greasy liquid, Yield 78%, Molecular formula $C_{17}H_{16}N_2OS$; Mol. Wt. 296. IR (KBr): ν_{max} : 3056 (C-H of aromatic ring), 1575 (C=C stretching of aromatic ring), 1518 (C=N); 1H -NMR (500 MHz, $CDCl_3$): δ 7.46 (m, 2H, H-2', H-6'), 7.30 (m, 2H, H-3', H-5'), 7.24 (m, 1H, H-4'), 7.17-7.23 (m, 5H, H-2" to H-6"), 4.14 (s, 2H, CH_2 -7), 3.42 (t, 2H, H-8"), 3.06 (d, 2H, H-7"); EIMS: m/z 296 (46%) [M]⁺, 191 (44%), 117 (100%; loss of benzyl cyanide cation fragment), 105 (35%; loss of phenylethyl cation fragment), 91 (54%; loss of tropylium ion fragment).

Antimicrobial Assay**Microbial strains**

The extracted oils of both cultivars were individually tested against a set of microorganisms, including two Gram-positive bacteria: *Staphylococcus aureus* (*S. aureus*), API Staph TAC 6736152, *Bacillus subtilis* (*B. subtilis*) JS 2004, two Gram-negative bacteria: *Escherichia coli* (*E. coli*) ATCC 25922, and *Pasteurella multocida* (*P. multocida*) (local isolate) The pure bacterial strains were obtained from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Purity and identity were verified by the Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured overnight at 37°C in Nutrient agar (NA, Oxoid).

Disc diffusion method

The antibacterial activity of the compounds was determined by disc diffusion method. Briefly, 100 μ L of suspension of tested microorganisms, containing 10^7 colony-forming units (CFU)/mL of bacteria cells on NA medium. The filter discs (6 mm in diameter) were individually impregnated with compound solution, placed on the agar plates which had previously been inoculated with the tested microorganisms. Discs without samples were used as a negative control. Streptomycin sulphate (30 μ g/dish) (Oxoid, UK) were used as positive reference for bacteria to compare sensitivity of

strain/isolate in analyzed microbial species. Plates, after 2 h at 4°C, were incubated at 37°C for 18 h for bacteria strains. Antibacterial activity was evaluated by measuring the diameter of the growth inhibition zones (zone reader) in millimeters for the organisms and comparing to the controls.¹²

Hemolytic Activity

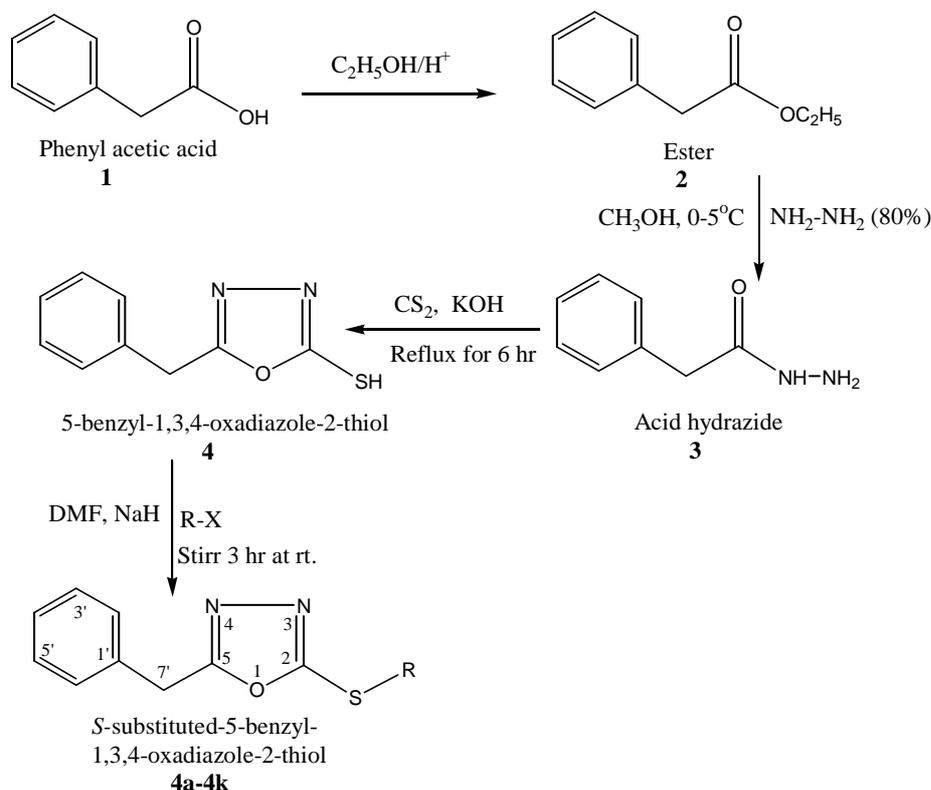
Hemolytic activity of the compound was studied by the method used by Sharma and Powell.^{13,14} Three mL freshly obtained heparinized human blood was collected from volunteers after consent and counseling and bovine from the Department of Clinical Medicine and Surgery, University of Agriculture. Blood was centrifuged for 5 min at 1000xg plasma was discarded and cells were washed with three times with 5 mL of chilled (4°C) sterile isotonic Phosphate-buffered saline (PBS) pH 7.4. Erythrocytes were maintained 10⁸ cells per mL for each assay. Hundred µL of each compound was mixed with human (10⁸cells/mL) separately. Samples were incubated for 35 min at 37°C and agitated after 10 min. Immediately after incubation the samples were placed on ice for 5 min then centrifuged for 5 min at 1000 xg. Supernatant 100 µL were taken from each tube and diluted 10 time with chilled (4°C) PBS. Triton X-100 (0.1% v/v) was taken as positive control and phosphate buffer saline (PBS) was taken as negative control and pass through the same process. The absorbance was noted at 576 nm using µQuant (Biotech, USA). The % RBCs lysis for each sample was calculated.

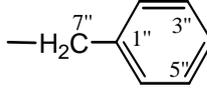
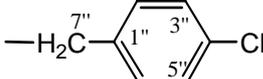
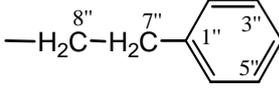
RESULTS AND DISCUSSION

In the present investigation *S*-substituted-1,3,4-oxadiazole-2-thiol were obtained in 70-85% yield by converting organic acid 1 to the corresponding esters 2 and acid hydrazide 3 by reaction with ethanol and hydrazine hydrate, respectively. The hydrazide was converted to 5-benzyl-1,3,4-oxadiazole-2-thiols 4 by using carbon disulfide and potassium hydroxide as outlined in Scheme 1. Compound 4 was synthesized as yellowish brown amorphous powder. The molecular formula C₉H₈N₂OS was established by HR-MS showing molecular ion peak at *m/z* 192.239 (calcd for C₉H₈N₂OS, 192.235). The IR spectrum showed absorption bands at 3058 cm⁻¹, 1575 cm⁻¹, and 1518 cm⁻¹ which were assigned to C-H (stretching of aromatic ring), C=C (aromatic stretching) and C=N (stretching),

respectively. The EI-MS gave a characteristic peak at *m/z* 91 which was attributed to the tropylium ion produced by the loss of 1,3,4-oxadiazole-2-thiol moiety. In the aromatic region of the ¹H-NMR spectrum signals appeared at δ 7.46 (m, 2H, H-2', H-6'), δ 7.34 (m, 2H, ArH-3', H-5'), 7.26 (m, 1H, H-4') which were assigned to the mono substituted aromatic ring. In the aliphatic region of the ¹H-NMR spectrum, a singlet signal appeared at δ 4.02 (s, 2H, CH₂-7') indicated the presence of one methylene group in the molecule. On the basis of above cumulative evidences, the structure of 4 was assigned as 5-benzyl-1,3,4-oxadiazole-2-thiol. Further, *S*-alkyl substituted oxadiazoles were obtained by stirring 5-benzyl-1,3,4-oxadiazole-2-thiol with different electrophile in the presence of sodium hydride and *N,N*-dimethyl formamide. All the synthesized *S*-substituted derivatives of oxadiazole were new except two compounds 4a and 4g.^{15,16} On the basis of evidences from IR, EIMS and ¹H-NMR, the structures of *S*-substituted oxadiazole derivatives were elucidated as described in experimental section.

In vitro antibacterial activities were performed against two Gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis* and two Gram-negative bacteria: *Escherichia coli* and *Pasteurella multocida* keeping Streptomycin Sulfate as a standard. Out of twelve only five compounds which are 4, 4g, 4h, 4j and 4k (Table 1) showed the antibacterial activity against the selected panel of bacterial species. The order of activity of these compounds were 4j>4k>4g>4h>4 from the higher activity to lowest activity against the selected strains but lower antibacterial activity than the standard compounds used in the assay. One more concrete information from this data reflected that these entire five compounds showed the higher antibacterial activity against the Gram positive bacteria than the Gram negative strains. Other remaining compounds acquire very low or no activity against the test microbes. Hasan et al. (2011) also reported the antimicrobial activity of such nature of compounds. The highest hemolytic activity was reported by the 4c (96.7%) and 4 (97.7%) but less than the positive control (Triton-X-100). Lowest activity by the 4g (4.0%) followed by 4j (9.0%) but higher than the negative control (Table 1). All other compounds also presented the hemolytic activity between this given range of minimum and maximum values. The compounds showed the highest activity may also be considered for the antitumor activity.



Compound	R	Compound	R
4a	---CH_3 1"	4g	$\text{---CH}_2\text{---COOH}$ 1" 2"
4b	$\text{---CH}_2\text{---CH}_3$ 1" 2"	4h	$\text{---CH}_2\text{---CH}_2\text{---Br}$ 1" 2"
4c	---HC 1" $\begin{matrix} \text{CH}_3 \\ \text{2"} \\ \text{CH}_3 \\ \text{3"} \end{matrix}$	4i	$\text{---H}_2\text{C}$ 
4d	$\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_3$ 1" 2" 3" 4"	4j	$\text{---H}_2\text{C}$ 
4e	$\text{---CH}_2\text{---CH}$ 1" 2" $\begin{matrix} \text{3"} \\ \text{CH}_3 \\ \text{4"} \\ \text{CH}_3 \end{matrix}$	4k	$\text{---H}_2\text{C}^8\text{---H}_2\text{C}^7$ 
4f	$\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_3$ 1" 2" 3" 4" 5"		

Scheme 1: Synthetic scheme for S-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2-thiol

Table 1: Antibacterial activity of S-substituted derivatives of 5-benzyl-1,3,4-oxadiazole-2-thiol

Compd. No.	Gram-positive bacteria		Gram-negative bacteria		Hemolytic activity (% ± S.D)
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Pasturella multocida</i>	<i>Escherichia coli</i>	
4	10	10	10	10	96.7±0.93
4a	-	-	-	-	83.5±0.80
4b	-	-	-	-	76.2±1.00
4c	-	-	-	-	97.7±0.93
4d	-	-	-	-	85.0±0.94
4e	-	-	-	-	73.4±0.95
4f	-	-	-	-	67.5±0.16
4g	16	14	10	10	04.0±0.21
4h	12	10	08	08	43.2±0.26
4i	-	-	-	-	85.6±0.52
4j	20	18	14	13	09.0±0.62
4k	18	18	16	16	28.5±0.96
Streptomycin	22	28	26	26	
PBS					0.00
Triton (toxicity)					100±0.0

Note: PBS = Phosphate-buffered saline

CONCLUSION

The proposed structure of the synthesized compound is well supported by spectroscopic data. From the antimicrobial activity data (Table 1), it may be concluded that only five synthesized compound showed promising activity but all others remains inactive.

REFERENCES

- F.A. Omar, N.M. Mahfouz, M.A. Rahman. Design, synthesis and anti-inflammatory activity of some 1,3,4-oxadiazole derivatives. *European Journal of Medicinal Chemistry*. 1996; 31: 819-825.
- B.N. Goswami, J.C.S. Katakya, J.N. Baruah. Synthesis of 3,4-disubstituted 1,3,4-oxadiazole-2-thiones as potential fungicidal agents. *Journal of Heterocycle Chemistry*. 1984; 21: 205-208.
- B.S. Holla, K.N. Poojary, B. Kalluraya, P.V. Gowda. 5-Substituted-1,3,4-oxadiazole-2-thiones. *Indian Journal of Heterocycle Chemistry*. 1996; 5: 273-276.
- Hasan, N. F. Thomas, S. Gopil. Synthesis, characterization and antifungal evaluation of 5-substituted-4-amino-1,2,4-triazole-3-thioesters. *Molecules*. 2011; 16: 1297-1309.
- M.T. Omar. Synthesis of new xanthenone derivatives of expected antibilharzial activity. *Archives of Pharmacal Research (Seoul)*. 1997; 20: 602-609.
- K.Y. Matsumoto, Y. Yasuda, T. Tanimoto, K. Matsumoto, T. Yoshida, J. Shoji. Isolation and characterization of thioxamycin. *Journal of Antibiotics (Tokyo)*. 1998; 42: 1465-1469.
- S.S. Shafi, T.R. Radhakrishnan. Studies on biological active heterocycles. Part I. Synthesis and antibacterial activity of some 2,5-di-substituted-1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,2,4-triazole and 4-thiazolidinone. *Indian Journal of Heterocycle Chemistry*. 1995; 5: 133-138
- T.M. Tan, Y. Chen, K.H. Kong, J. Bai, S.G. Li Lim, H. Ang, Y. Lam. Synthesis and the biological evaluation of 2-benzenesulfonylalkyl-5-substituted-sulfanyl-(1,3,4)-oxadiazoles as potential anti-hepatitis B virus agents. *Antiviral Research* 2006; 71: 7-14.
- S. Wagle, A.A. Vasudeva, N.K. Suchetha. Synthesis of some new 2-(3-Methyl-7-substituted-2-oxoquinoxaliny)-5-(aryl)-1,3,4-oxadiazoles as potential non-steroidal anti-inflammatory and analgesic agents. *Indian Journal of Chemistry*. 2008; 47B: 439-48.
- K.M. Lokanatha Rai, B.K. Vishu Kumar, S.N. Hemavathi. Synthesis of biological screening of some new 2,5-disubstituted 1,3,4-oxadiazoles. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011; 3: 110-114.
- Nagaraj, K.C. Chaluvvaraju, M. S. Niranjana, S. Kiran, 1,3,4-oxadiazole: a potent drug candidate with various

- pharmacological activities. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(3): 9-16.
12. CLSI (The clinical Laboratory Standard Institute). Agar dilution and disk diffusion susceptibility testing of campylobacter spp. Journal of Clinical Microbiology. 2010; 45(8): 2758-2759.
 13. P. Sharma, J.D. Sharma. In vitro hemolysis of human erythrocytes by plant extracts with antiplasmodial activity. Journal of Ethnopharmacol. 2001; 74: 239-243.
 14. W.A. Powell, C.M. Catranis, C.A. Maynard. Design of self-processing antimicrobial peptide for plant protection. Letters in Applied Microbiology. 2000; 31(2): 163-165.
 15. P.N. Confalone, R. B. Woodward, A novel synthesis of peptides based on the photochemistry of 5-azido-1,3,4-oxadiazoles. Journal of the American Chemical Society. 1983; 105(4): 902-906.
 16. M.M. Hamad, Synthesis and reactions of 2(5)-[benzyl or cyanomethyl]-1,3,4-oxadiazoles. Archiv der Pharmazie. 1990; 323(9): 595-599.