Synthesis and antibacterial screening of *S*-substituted derivatives of 5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazol-2-thiol

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Abstract: The most emerging class among the heterocyclic compounds is 1,3,4-oxadiazoles for their diverse biological activities. In the present research work, piperonylic acid (**1**) was converted consecutively into corresponding ester (**2**), hydrazide (**3**) and 1,3,4-oxadiazole (**4**) through intermolecular cyclization. The synthesized compound **4** was subjected further to *S*-alkylation/aralkylation, using alkyl/aralkyl halides (**5a-m**) and *S*-substituted-1,3,4-oxadiazole derivatives were synthesized (**6a-m**). The structure elucidation of the synthesized molecules was processed through ^{1}H-NMR, IR and mass spectral data. The antibacterial activity showed these molecules moderately good inhibitors of gram-negative and gram-positive bacteria.

Keywords: 1,3,4-oxadiazole, antibacterial activity, piperonylic acid.

INTRODUCTION

Pathogenic bacteria are showing antibacterial resistance against many prevailing antibiotics with the passage of time. This drastic situation has forced the scientists and chemists to explore something newer, better and safer which can combat with these resistant microbes effectively (Roy *et al.,* 2009). After an overview of literature survey, heterocyclic compounds are seen as gaining a crucial significance and making their meaningful place in drug development program. No doubt other classes of drugs are working very well but the compounds which have turned more than 90% of attention of the researchers towards themselves are the heterocyclic compounds (Somani and Shirodkar, 2009). Oxadiazole nucleus has a special capacity to undergo various types of reactions i.e. electrophilic substitution, nucleophilic substitution, thermal and photochemical reactions. This distinct property paved the way to construct potentially diverse compounds having broad spectrum activities (Jaiswal *et al.,* 2012).

In addition to the active oxadiazole ring, benzodioxol moiety also possesses crucial importance in the race of active potent drugs. Many of the natural products have competent role in the treatment of cancer. Among the various natural products used as anticancer and antitumor are narciclasine, lycoricidine and pancratistatin (Ingrassia *et al.,* 2008). The structural analysis reveals that they contain benzodioxole moiety. Moreover many of the antidepressant drugs, for example, paroxetine, escitalopram etc also contain benzodioxol moiety (Bar-Oz *et al.,* 2007).

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Owing to increased resistance of bacteria against the formal drugs, it is the demand of era to design new compounds with low side effects, high therapeutic activities, cost effectiveness and better patient compliance. Our previous work (Aziz-ur-Rehman *et al.,* 2012a; Aziz-ur-Rehman *et al.,* 2012b; Aziz-ur-Rehman *et al.,* 2013a; Aziz-ur-Rehman *et al.,* 2013b; Aziz-ur-Rehman *et al.,* 2013c), encompasses many acetamides and butanamides having 1,3,4-oxadiazole ring substituted with different aryl/aralkyl groups at its 5-position and many *S*-(alkyl/aralkyl) substituted 1,3,4-oxadiazoles having 3-nitrophenyl/4-nitrophenyl group at 5-position of oxadiazole ring. So in continuation of previous work and also in search of new drug candidates, an effort was made to synthesize different *S*-(alkyl/aralkyl) substituted derivatives of 5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazol-2-thiol. All the synthesized compounds bearing benzodioxole moiety at 5-position of oxadiazole ring were evaluated for their antibacterial potential against the certain strains of gram-negative and gram-positive bacteria taken into account.

MATERIALS AND METHODS

General

All the chemical reagents used for synthesis were purchased from Alfa Aesar and Sigma-Aldrich through commercial suppliers and further processed without purification. Melting points of the synthesized molecules were recorded by using Griffin and George apparatus using open capillary tube and all were uncorrected. The synthesized compounds were obtained as solid precipitates except of some of the compounds, which were obtained as gummy liquid states. The solid compounds were purified further by recrystallization with

methanol. Purity of the samples was checked by thin layer chromatography (TLC) using pre-coated silica gel G-25-UV_{254} plates with ethyl acetate and *n*-hexane solvent system giving single spot. Detection of spots was performed under UV at $_{254}$ (nm) and by Ce_2SO_4 after heating. The I.R. spectra were recorded by KBr (potassium bromide) pellet procedure on a Jasco-320-A spectrophotometer with wave number in cm^{-1}. ^{1}H-NMR spectra were computed in $CDCl_3$ by Bruker spectrometer working at 300/400 MHz. *δ*-values are given in ppm using TMS as internal reference standard. Mass spectra (EI-MS) were established on a JMS-HX-110 instrument, with a data system.

Procedure for the synthesis of ethyl 3,4-(methylenedioxy)benzoate (2)

Piperonylic acid (**1**, 1.0 g) was dissolved in absolute ethanol (4.0 mL) in a 100 mL round bottom flask. After the addition of conc. H_2SO_4 (0.5 mL), the reaction contents were refluxed for 2.0 hrs. The reaction progress was supervised by TLC. After completion, the contents were transferred to a separating funnel having distilled water (40 mL). Chloroform (15 mL) was poured into the separating funnel and mixture was neutralized by conc. aq. Na_2CO_3 solution till the pH of 8-9. The base is necessary to convert remaining organic/sulphuric acids into salts, which are taken away by aqueous layer. The solution was shaken and kept still for 5-10 min. Upper aqueous layer was discarded and lower chloroform layer containing required ester was taken in distillation flask. Chloroform was distilled off and the yellowish transparent ester **2** was collected. Yellowish transparent liquid; Yield: 90%; Molecular formula: $C_{10}H_{10}O_4$; Molecular mass: 194; IR (KBr): ν_{max} (cm^{-1}): 3006 (aromatic C-H), 1738 (C=O ester), 1605 (Ar C=C), 1110 (C-O ester); ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*/ppm): 7.64 (dd, *J* = 1.6, 8.4 Hz, 1H, H-6'), 7.44 (d, *J* =1.2 Hz, 1H, H-2'), 6.82 (d, *J* = 8.0 Hz, 1H, H-5'), 6.01 (s, 2H, H-7'), 4.32 (q, *J* = 7.2 Hz, 2H, -O$\underline{CH_2}$$CH_3$), 1.37 (t, *J* = 7.2 Hz, 3H, -OCH_2CH_3); EIMS (*m/z*): 194 $[M]^{\cdot+}$, 165 $[C_8H_5O_4]^+$, 149 $[C_8H_5O_3]^+$, 121 $[C_7H_5O_2]^+$, 29 $[C_2H_5]^+$.

Procedure for the synthesis of 3,4-(methylenedioxy) benzohydrazide (3)

The ester **2** (0.01 mol) was taken in 100 mL round bottom flask followed by addition of ethanol (10.0 mL). 80% Hydrazine hydrate (2.0 mL) was added gradually to the mixture. The whole mixture was refluxed for 5.0 hrs. Reaction completion was monitored by TLC. The cold distilled water was added after completion. The precipitated product was filtered off and washed out by distilled water to get the desired hydrazide (**3**). White amorphous solid; Yield: 97%; M.P: 170-173 ^oC; Molecular formula: $C_8H_8N_2O_3$; Molecular mass: 180; IR (KBr): ν_{max} (cm^{-1}): 3250 (N-H), 3010 (aromatic C-H), 1645 (C=O amide), 1610 (Ar C=C); ^{1}H-NMR ($CDCl_3$,400 MHz, *δ*/ppm): 7.66 (dd, *J* = 1.6, 8.4 Hz, 1H, H-6'), 7.45 (d, *J* = 1.2 Hz, 1H, H-2'), 6.87 (d, *J* = 8.0 Hz, 1H, H-5'), 6.06 (s, 2H, H-7'); EIMS (*m/z*): 180 $[M]^{\cdot+}$, 164 $[C_8H_6NO_3]^+$, 149 $[C_8H_5O_3]^+$, 121 $[C_7H_5O_2]^+$.

Procedure for the synthesis of 5-(3,4-Methylenedioxyphenyl)-1,3,4-oxadiazol-2-thiol (4)

Acid hydrazide **3** (0.01 mol) was taken in a 100 mL round bottom flask. 10.0 mL absolute ethanol was added followed by the addition of carbon disulfide (0.03 mol) and potassium hydroxide (0.02 mol). The reaction mixture was kept on refluxing for 6.0 hours and monitored *via* TLC. After single spot on TLC, distilled water (30 mL) and dilute HCl till the pH of 2-3 were poured to reaction mixture. The acid is essential to convert the salt form of 1,3,4-oxadiazole into acidic form but excess of it should be obviated because it decrements the yield by salt formation. The precipitated product **4** was acquired by filtration, washed off with distilled water and re-crystallized by using methanol. White amorphous solid; Yield: 83%; M.P: 237-238 ^oC; Molecular formula: $C_9H_6N_2O_3S$; Molecular mass: 222; IR (KBr): ν_{max} (cm^{-1}): 3011 (aromatic C-H), 1613 (Ar C=C), 1657 (C=N), 1109 (C-O-C);^{1}H-NMR ($CDCl_3$,400 MHz, *δ*/ppm): 7.52 (dd, *J* = 1.2, 8.4 Hz, 1H, H-6'), 7.44 (s, 1H, H-2'), 6.89 (d, *J* = 8.4 Hz, 1H, H-5'), 6.06 (s, 2H, H-7'); ^{13}C-NMR ($CDCl_3$, 100 MHz, *δ*/ppm): 165.1 (C-2), 156.7 (C-5), 153.0 (C-3□), 148.9 (C-4□), 121.4 (C-1□), 115.6 (C-6□), 110.7 (C-5□), 107.5 (C-2□), 101.0 (C-7□); EIMS (*m/z*): 236 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 147 $[C_8H_5NO_2]^{\cdot+}$, 121 $[C_7H_5O_2]^+$.

General procedure for the synthesis of S-substituted derivatives (6a-m)

The compound **4** (0.1 g, 4.5 mmol.) was homogeneously dissolved in 15 mL dimethyl formamide using 50 mL round bottom flask at room temperature. Solid NaH (0.002 g) was added as an activator followed by stirring for 0.5 hour. The different electrophiles, alkyl/aralkyl halides (**5a-m**, 4.5 mmol) were introduced and stirred for 3-5 hours to yield the *S*-substituted derivatives (**6a-m**). Reaction completion was managed through TLC. At the end of reaction, cold distilled water was added followed by aq. Na_2CO_3 till pH of 8-9, to get the precipitates. The base is used to remove the unreacted parent molecule **4**. Precipitates were filtered, washed with distilled water and recrystallized from methanol. Finally the products were dried.

2-Methylthio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6a)

White amorphous solid; Yield: 81%; M.P: 90-92 ^oC; Molecular formula: $C_{10}H_8N_2O_3S$; Molecular mass: 236; IR (KBr): ν_{max} (cm^{-1}): 3016 (aromatic C-H), 1615 (Ar C=C), 1679 (C=N), 1108 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*/ppm): 7.53 (dd, *J* = 1.2, 8.4 Hz, 1H, H-6'), 7.44 (s, 1H, H-2'), 6.89 (d, *J* = 8.4 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 2.74 (s, 3H, H-1"); EIMS (*m/z*): 236 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 147 $[C_8H_5NO_2]^{\cdot+}$, 121 $[C_7H_5O_2]^+$, 15 $[CH_3]^+$.

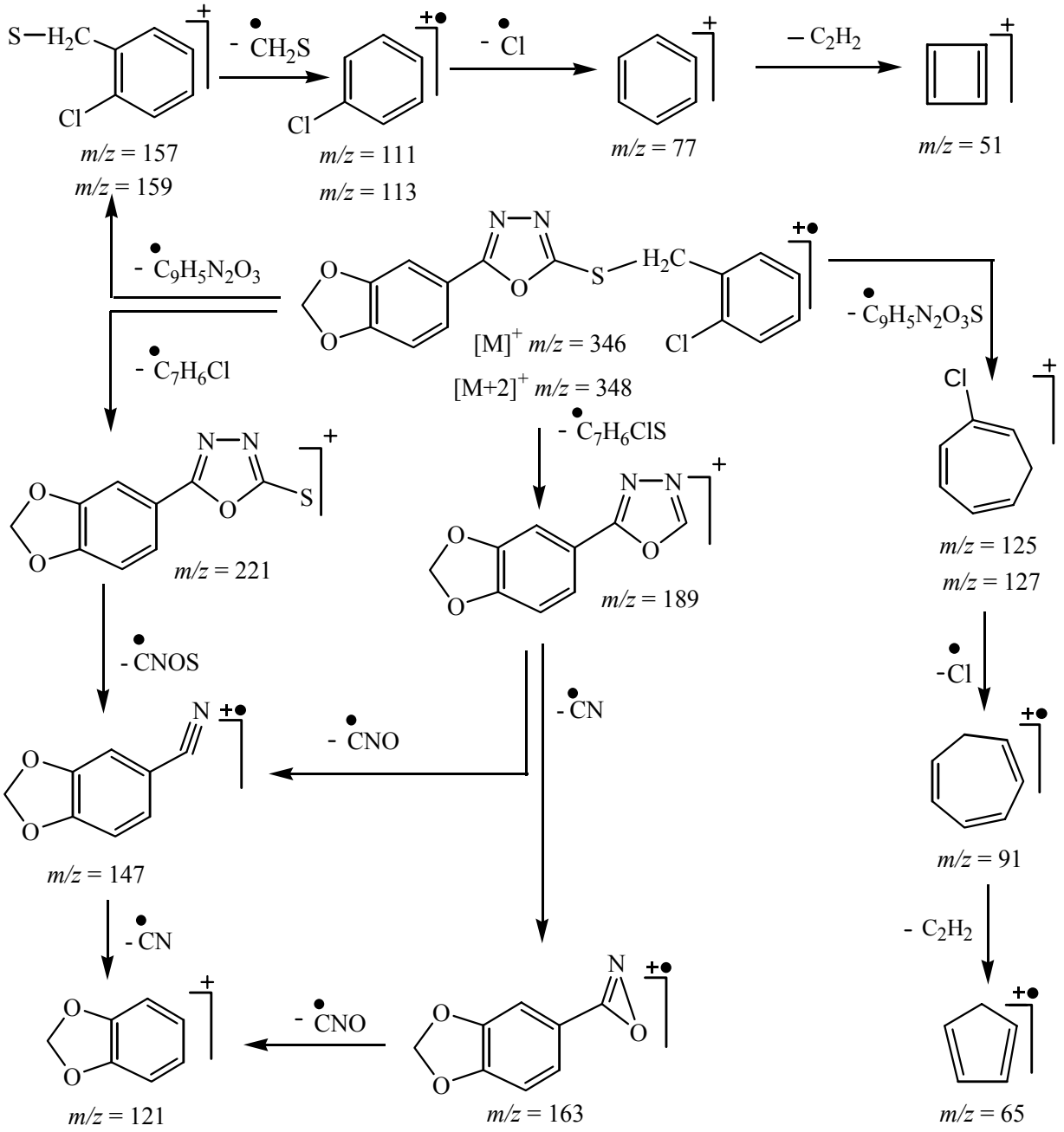


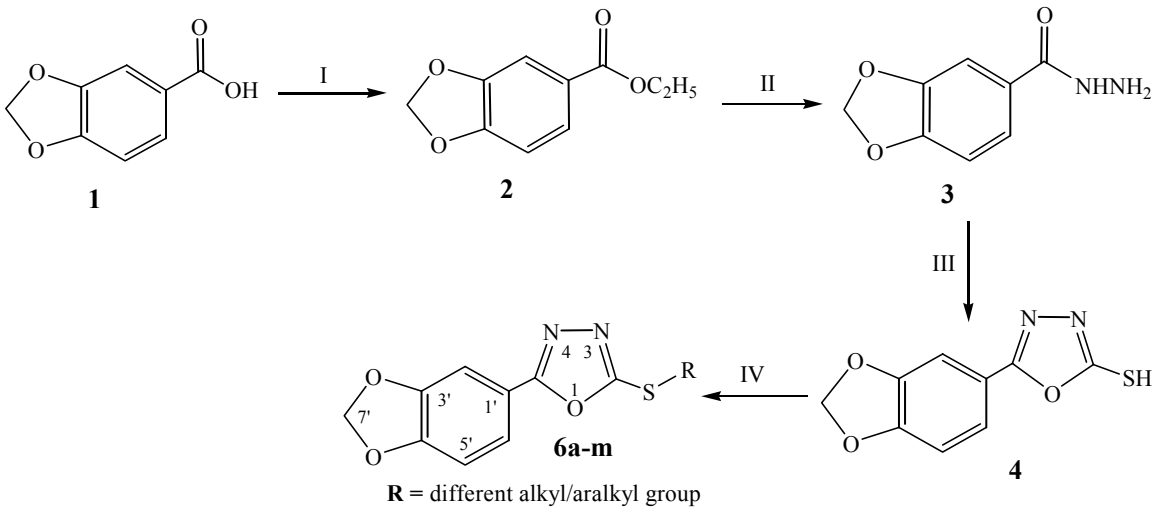
Fig. 1: Mass fragmentation pattern of the synthesized molecule 6f

2-Ethylthio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6b)

Grey solid; Yield: 87%; M.P: 68-70 °C; Molecular formula: $C_{11}H_{10}N_2O_3S$; Molecular mass: 250; IR (KBr): ν_{max} (cm^{-1}): 3018 (aromatic C-H), 1616 (Ar C=C), 1670 (C=N), 1110 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.53 (d, J = 8.0 Hz, 1H, H-6'), 7.44 (s, 1H, H-2'), 6.89 (d, J = 8.0 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 3.30 (q, J = 7.2 Hz, 2H, H-1"), 1.50 (t, J = 7.6 Hz, 3H, H-2"); EIMS (*m/z*): 250 $[M]^{+\cdot}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{+\cdot}$, 147 $[C_8H_5NO_2]^{+\cdot}$, 121 $[C_7H_5O_2]^+$, 29 $[C_2H_5]^+$.

2-(Propan-1-yl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6c)

Yellowish brown amorphous solid; Yield: 79%; M.P: 66-67 °C; Molecular formula: $C_{12}H_{12}N_2O_3S$; Molecular mass: 264; IR (KBr): ν_{max} (cm^{-1}): 3016 (aromatic C-H), 1617 (Ar C=C), 1676 (C=N), 1106 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.53 (dd, J = 1.6, 8.4 Hz, 1H, H-6'), 7.44 (d, J = 1.2 Hz, 1H, H-2'), 6.89 (d, J = 8.0 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 3.26 (t, J = 7.2 Hz, 2H, H-1"), 1.89 (sex, J = 7.2 Hz, 2H, H-2"), 1.07 (t, J = 7.2 Hz, 3H, H-3"); EIMS (*m/z*): 264 $[M]^{+\cdot}$, 221 $[C_9H_5N_2O_3S]^+$, 189



Scheme 1: Outline for the synthesis of *S*-substituted derivatives of 5-(3,4-methylenedioxyphenyl)-1,3,4-Oxadiazol-2-thiol. Reagents and conditions: (I). C_2H_5OH, conc. H_2SO_4, reflux for 2 hrs (II). $NH_2NH_2.H_2O$, C_2H_5OH, reflux for 5 hrs (III). CS_2, KOH, C_2H_5OH, reflux for 6 hr (IV). R-X (**5a-m**), NaH, DMF, stirring for 3-5 hrs.

$[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 147 $[C_8H_5NO_2]^{\cdot+}$, 121 $[C_7H_5O_2]^+$, 43 $[C_3H_7]^+$, 41 $[C_3H_5]^+$.

2-(Butan-1-yl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6d)

Creamy white liquid; Yield: 90%; Molecular formula: $C_{13}H_{14}N_2O_3S$; Molecular mass: 278; IR (KBr): ν_{max} (cm^{-1}): 3019 (aromatic C-H), 1620 (Ar C=C), 1676 (C=N), 1112 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.53 (dd, J = 1.6, 8.4 Hz, 1H, H-6'), 7.44 (d, J = 1.6 Hz, 1H, H-2'), 6.88 (d, J = 8.0 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 3.28 (t, J = 7.2 Hz, 2H, H-1"), 1.81 (qui, J = 7.2 Hz, 2H, H-2"), 1.50 (sex, J = 7.2 Hz, 2H, H-3"), 0.96 (t, J = 7.2 Hz, 3H, H-4"); EIMS (*m/z*): 278 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 147 $[C_8H_5NO_2]^{\cdot+}$, 121 $[C_7H_5O_2]^+$, 57 $[C_4H_9]^+$, 55 $[C_4H_7]^+$, 29 $[C_2H_5]^+$.

2-(Pentan-1-yl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6e)

Creamy white amorphous solid; Yield: 88%; M.P: 105-107 ^oC; Molecular formula: $C_{14}H_{16}N_2O_3S$; Molecular mass: 292; IR (KBr): ν_{max} (cm^{-1}): 3014 (aromatic C-H), 1616 (Ar C=C), 1678 (C=N), 1108 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.53 (dd, J = 1.6, 8.0 Hz, 1H, H-6'), 7.44 (d, J = 1.2 Hz, 1H, H-2'), 6.89 (d, J = 8.0 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 3.27 (t, J = 7.2 Hz, 2H, H-1"), 1.81 (qui, J = 7.2 Hz, 2H, H-2") 1.45-1.35 (m, 4H, H-3", H-4"), 0.89 (t, J = 7.2, 3H, H-5"); EIMS (*m/z*): 292 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 147 $[C_8H_5NO_2]^{\cdot+}$, 121 $[C_7H_5O_2]^+$, 71 $[C_5H_{11}]^+$, 69 $[C_5H_9]^+$, 43 $[C_3H_7]^+$.

2-(2-Chlorobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6f)

White amorphous solid; Yield: 83%; M.P: 96-98 ^oC; Molecular formula: $C_{16}H_{11}N_2O_3SCl$; Molecular mass: 346.5; IR (KBr): ν_{max} (cm^{-1}): 3437 (aromatic C-H), 1633 (Ar C=C), 1690 (C=N), 1109 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.60 (dd, J = 7.2, 2.4 Hz, 1H, H-3"), 7.50 (dd, J = 1.6, 8.4 Hz, 1H, H-6'), 7.41 (dd, J = 2.0, 7.2 Hz, 1H, H-6"), 7.37 (brd.s, 1H, H-2'), 7.23-7.18 (m, 2H, H-4", H-5"), 6.88 (d, J = 8.4 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.59 (s, 2H, H-7"); EIMS (*m/z*): 348 $[M+2]^{\cdot+}$, 346 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 157 $[C_7H_6ClS]^+$, 147 $[C_8H_5NO_2]^{\cdot+}$, 126 $[C_7H_7Cl]^+$, 121 $[C_7H_5O_2]^+$, 112 $[C_6H_5Cl]^{\cdot+}$, 90 $[C_7H_6]^{\cdot+}$, 76 $[C_6H_4]^+$, 65 $[C_5H_5]^{\cdot+}$, 51 $[C_4H_3]^+$.

2-(3-Chlorobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6g)

White amorphous solid; Yield: 85%; M.P: 109-112 ^oC; Molecular formula: $C_{16}H_{11}N_2O_3SCl$; Molecular mass: 346; IR (KBr): ν_{max} (cm^{-1}): 3339 (aromatic C-H), 1632 (Ar C=C), 1690 (C=N), 1109 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.50 (dd, J = 1.6, 8.0 Hz, 1H, H-6'), 7.43 (brd.s, 1H, H-2"), 7.41 (d, J = 1.2 Hz, 1H, H-2'), 7.33-7.26 (m, 3H, H-4" to H-6"), 6.88 (d, J = 8.4 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.43 (s, 2H, H-7"); EIMS (*m/z*): 348 $[M+2]^{\cdot+}$, 346 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 157 $[C_7H_6ClS]^+$, 147 $[C_8H_5NO_2]^{\cdot+}$, 126 $[C_7H_7Cl]^+$, 121 $[C_7H_5O_2]^+$, 112 $[C_6H_5Cl]^{\cdot+}$, 90 $[C_7H_6]^{\cdot+}$, 76 $[C_6H_4]^+$, 65 $[C_5H_5]^{\cdot+}$, 51 $[C_4H_3]^+$.

2-(4-Chlorobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6h)

White amorphous solid; Yield: 95%; M.P: 99-100 ^oC; Molecular formula: $C_{16}H_{11}N_2O_3SCl$; Molecular mass: 346; IR (KBr): ν_{max} (cm^{-1}): 3340 (aromatic C-H), 1630 (Ar C=C), 1692 (C=N), 1108 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.50 (dd, J = 1.2, 8.0 Hz, 1H, H-6'), 7.41 (brd.s, 1H, H-2'), 7.39 (d, J = 8.4 Hz, 2H, H-3", H-

5"), 7.28 (d, *J* = 8.4 Hz, 2H, H-2", H-6"), 6.88 (d, *J* = 8.4 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.43 (s, 2H, H-7"); EIMS (*m/z*): 348 $[M+2]^{\cdot+}$, 346 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 157 $[C_7H_6ClS]^+$, 147 $[C_8H_5NO_2]^{\cdot+}$, 126 $[C_7H_7Cl]^+$, 121 $[C_7H_5O_2]^+$, 112 $[C_6H_5Cl]^{\cdot+}$, 90 $[C_7H_6]^{\cdot+}$, 76 $[C_6H_4]^+$, 65 $[C_5H_5]^{\cdot+}$, 51 $[C_4H_3]^+$.

2-(4-Flourobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6i)

White amorphous solid; Yield: 87%; M.P: 120-123 ^oC; Molecular formula: $C_{16}H_{11}N_2O_3SF$; Molecular mass: 330; IR (KBr): ν_{max} (cm^{-1}): 3244 (aromatic C-H), 1630 (Ar C=C), 1690 (C=N), 1109 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*/ppm): 7.51 (dd, *J* = 1.2, 7.2 Hz, 1H, H-6'), 7.41 (d, *J* = 2.0 Hz, 1H, H-2'), 7.39 (t, *J* = 8.0 Hz, 2H, H-3", H-5"), 7.03 (t, *J* = 8.4 Hz, 2H, H-2", H-6"), 6.87 (d, *J* = 8.0 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.45 (s, 2H, H-7"); EIMS (*m/z*): 330 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 147 $[C_8H_5NO_2]^{\cdot+}$, 141 $[C_7H_6SF]^+$, 121 $[C_7H_5O_2]^+$, 110 $[C_7H_6F]^+$, 96 $[C_6H_5F]^{\cdot+}$, 91 $[C_7H_7]^{\cdot+}$, 77$[C_6H_5]^+$, 65 $[C_5H_5]^{\cdot+}$, 51 $[C_4H_3]^+$.

2-(3-Nitrobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6j)

White amorphous solid; Yield: 90%; M.P: 112-113 ^oC; Molecular formula: $C_{16}H_{11}N_3O_5S$; Molecular mass: 357; IR (KBr): ν_{max} (cm^{-1}): 3339 (aromatic C-H), 1635 (Ar C=C), 1695 (C=N), 1108 (C-O-C); ^{1}H-NMR ($CDCl_3$, 300 MHz, *δ*/ppm): 8.32 (s, 1H, H-2"), 8.15 (d, *J* = 8.1 Hz, 1H, H-4"), 7.85 (d, *J* = 7.5 Hz, 1H, H-6'), 7.53-7.47 (m, 2H, H-5", H-6"), 7.40 (d, *J* = 1.5 Hz, 1H, H-2'), 6.88 (d, *J* = 8.1 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.54 (s, 2H, H-7"); EIMS (*m/z*): 357 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 147 $[C_8H_5NO_2]^{\cdot+}$, 136 $[C_7H_6NO_2]^+$, 121 $[C_7H_5O_2]^+$, 90 $[C_7H_6]^+$, 65 $[C_5H_5]^{\cdot+}$, 51 $[C_4H_3]^+$.

2-(4-Nitrobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6k)

Creamy white amorphous solid; Yield: 80%; M.P: 190-195 ^oC; Molecular formula: $C_{16}H_{11}N_3O_5S$; Molecular mass: 357; IR (KBr): ν_{max} (cm^{-1}): 3338 (aromatic C-H), 1634 (Ar C=C), 1694 (C=N), 1108 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*/ppm): 8.18 (d, *J* = 8.8 Hz, 2H, H-3", H-5"), 7.65 (d, *J* = 8.8 Hz, 2H, H-2", H-6"), 7.49 (dd, *J* = 2.0, 8.4 Hz, 1H, H-6'), 7.39 (d, *J* = 1.6 Hz, 1H, H-2'), 6.88 (d, *J* = 8.0 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 4.53 (s, 2H, H-7"); EIMS (*m/z*): 357 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 147 $[C_8H_5NO_2]^{\cdot+}$, 136 $[C_7H_6NO_2]^+$, 121 $[C_7H_5O_2]^+$, 90 $[C_7H_6]^+$, 65 $[C_5H_5]^{\cdot+}$, 51 $[C_4H_3]^+$.

2-(2-Phenylethyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6l)

Golden brown gummy liquid; Yield: 92%; Molecular formula: $C_{17}H_{14}N_2O_3S$; Molecular mass: 326; IR (KBr): ν_{max} (cm^{-1}): 3436 (aromatic C-H), 1633 (Ar C=C), 1692 (C=N), 1109 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*/ppm): 7.52 (dd, *J* = 1.2, 8.0, Hz, 1H, H-6'), 7.44 (d, *J* = 1.2 Hz, 1H, H-2'), 7.30-7.25 (m, 5H, H-2" to H-6"), 6.89 (d, *J* = 8.0 Hz, 1H, H-5'), 6.04 (s, 2H, H-7'), 3.52 (t, *J* = 7.2 Hz, 2H, H-8"), 3.15 (t, *J* = 7.2 Hz, 2H, H-7"); EIMS (*m/z*): 326 $[M]^{\cdot+}$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 147 $[C_8H_5NO_2]^{\cdot+}$, 136 $[C_8H_9S]^+$, 121 $[C_7H_5O_2]^+$, 91 $[C_7H_7]^+$, 77 $[C_6H_5]^{\cdot+}$, 65 $[C_5H_5]^{\cdot+}$, 51 $[C_4H_3]^{\cdot+}$.

2-(3-Phenylpropyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (6m)

White amorphous solid; Yield: 90%; M.P: 73-76^oC; Molecular formula: $C_{18}H_{16}N_2O_3S$; Molecular mass: 340; IR (KBr): ν_{max} (cm^{-1}): 3290 (aromatic C-H), 1630 (Ar C=C), 1687 (C=N), 1110 (C-O-C); ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*/ppm): 7.51 (dd, *J* = 1.6, 8.0 Hz, 1H, H-6'), 7.42 (d, *J* = 1.2 Hz, 1H, H-2'), 7.27 (d, *J* = 7.6 Hz, 2H, H-2", H-6"), 7.20-7.17 (m, 3H, H-3" to H-5"), 6.89 (d, *J* = 8.4 Hz, 1H, H-5'), 6.03 (s, 2H, H-7'), 3.27 (t, *J* = 7.6 Hz, 2H, H-9"), 2.80 (t, *J* = 7.6 Hz, 2H, H-7"), 2.18 (qui, *J* = 7.2 Hz, 2H, H-8"); EIMS (*m/z*): 340 $[M]^+$, 221 $[C_9H_5N_2O_3S]^+$, 189 $[C_9H_5N_2O_3]^+$, 163 $[C_8H_5NO_3]^{\cdot+}$, 151 $[C_9H_{11}S]^+$, 147 $[C_8H_5NO_2]^{\cdot+}$, 121 $[C_7H_5O_2]^+$, 119 $[C_9H_{11}]^+$, 105 $[C_8H_9]^+$, 91 $[C_7H_7]^{\cdot+}$, 77 $[C_6H_5]^{\cdot+}$, 65 $[C_5H_5]^{\cdot+}$, 51 $[C_4H_3]^{\cdot+}$.

Antibacterial activity

Microbial cell number is proportional to the logarithm of growth rate, which increases with the increase in absorbance of broth medium. This is the principle for antibacterial activity method (Kaspady *et al.,* 2009; Khalid *et al.,* 2013; Yang *et al.,* 2006). The clinically isolated two gram-positive and four gram-negative bacteria were kept in arsenal on stock culture agar medium and 180 µL of it after dilutions with fresh nutrient broth were mixed with 20 µg diluted test samples by suitable solvents. The change in absorbance (from 0.12-0.19, maintained at start) measured at 540 nm, before and after incubation at 37^oC for 16-24 hrs with lid on the micro plate, was index for bacterial growth. The percent inhibition was calculated by the formula:

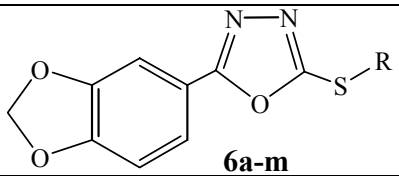
$$\text{Inhibition (\%)} = \frac{X - Y}{Y} \times 100$$

where X = Absorbance in control with bacterial culture and Y = Absorbance in test sample. Results are mean of triplicate (n = 3, ± SEM). Ciprofloxacin was taken as reference standard. Minimum inhibitory concentration (MIC) was computed with suitable dilutions (5-30 µg/well) for each sample and results were calculated using EZ-Fit Perrella Scientific Inc. Amherst USA software.

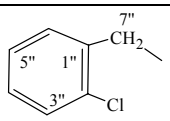
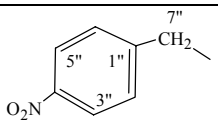
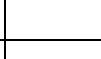
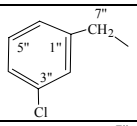
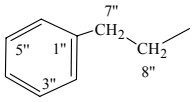
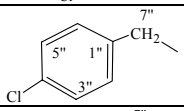
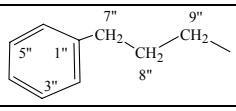
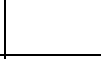
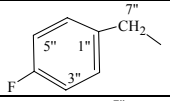
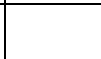
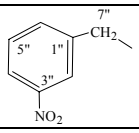
STATISTICAL ANALYSIS

The threefold calculation was statistically analyzed by Microsoft Excel 2010. Results are given as mean ± SEM.

Table 1: Different alkyl/aralkyl groups



6a-m

Comp.	R	Comp.	R	Comp.	R
6a		**6f**		**6k**	
6b		**6g**		**6l**	
6c		**6h**		**6m**	
6d		**6i**			
6e		**6j**			

RESULTS

The *S*-substituted 1,3,4-oxadiazole derivatives **6a-m** were synthesized according to the protocol sketched in scheme-1 and different *S*-substituted aryl/aralky groups are mentioned in table 1. The synthesized compounds were tested further for antibacterial activity against gram-positive and gram-negative bacterial strains. The general reaction conditions and the structure characterization are described in experimental section.

Prime objective of this research work was to introduce some *S*-substituted 1,3,4-oxadiazoles with less toxicity and high potential against different bacterial strains taken into account. The synthesized compounds were obtained in good yields with moderate antibacterial activities. The synthesis constituted different steps. In first step, ethyl 3,4-(methylenedioxy)benzoate (**2**) was synthesized from piperonylic acid (**1**) in the presence of conc. H_2SO_4 and ethanol by refluxing for 2 hours. In second step, ethyl 3,4-(methylenedioxy)benzoate (**2**), was converted into 3,4-(methylenedioxy)benzohydrazide (**3**) by refluxing for 5 hours with 80% hydrated hydrazine in ethanol. Third step involves intermolecular cyclization of **3** to yield 5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazol-2-thiol (**4**) by refluxing it for 6 hours in ethanol in the presence of CS_2/KOH. Finally *S*-substituted 1,3,4-oxadiazole (**6a-m**) were synthesized by gearing up various alkyl/aralkyl halides (**5a-m**) with **4** in DMF using NaH as weak base and activator (scheme 1). All the proposed structures of synthesized compounds were corroborated by ^{1}H-NMR, IR and EIMS spectroscopic analysis and the physical characteristic data is reported in experimental section.

DISCUSSION

The compounds were synthesized using the known methodology in search of new molecules with better antibacterial potential. All the compounds were obtained in a very good yield. The synthesized compounds were structurally corroborated through spectral data of IR, ^{1}H-NMR and EIMS. One of the compounds is discussed in detail for the reader. The compound, 2-(2-Chlorobenzyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (**6f**), was synthesized as white amorphous solid having 83% yield and melting point of 96-98 ^oC. The molecular formula $C_{16}H_{11}N_2O_3SCl$ was erected by EIMS showing $[M]^{+\bullet}$ peak at *m/z* 346 and [M+2] at 348 and by counting the number of protons in ^{1}H-NMR spectrum. In the IR spectrum, two stretching bands at 1695-1670 cm^{-1} for (C=N-N=C) and at 1112-1106 cm^{-1} for (C-O-C) in all the synthesized compounds affirmed the presence of 1,3,4-oxadiazole ring. The EIMS also gave two distinct peaks at *m/z* 221 because of 3,4-methylenedioxyphenyl-1,3,4-oxadiazole 2-thio cation and also supported by the other nominated distinct peaks. The base peak appeared at *m/z* 121 for 3,4 methylenedioxyphenyl cation.

A very prominent peak at *m/z* 91 corresponds to the tropylium radical cation. The ^{1}H-NMR spectrum showed three signals at δ 7.50 (dd, J = 1.6, 8.4 Hz, 1H, H-6'), 7.37 (brd.s, 1H, H-2') and 6.88 (d, J = 8.4 Hz, 1H, H-5') in aromatic region of the spectrum for three protons of methylenedioxy ring and one signal at δ 6.03 (s, 2H, H-7') for the two methylenic protons of the moiety. The signal appeared in highly downfield aliphatic region showing the presence of electronegative oxygen atoms surrounding the methylene group. There are three more signals in aromatic region for four protons of chlorobenzyl group attached at sulfur atom of oxadiazole ring i.e. at δ 7.60 (dd, J = 7.2, 2.4 Hz, 1H, H-3"), 7.23-7.18 (m, 2H, H-4", H-5") and 7.41 (dd, J = 2.0, 7.2 Hz, 1H, H-6"). The signal at δ 4.59 (s, 2H, H-7") corresponds to the two methylenic protons, which are attached at sulfur atom. On the ground of all these manifests, the structures of other synthesized products were confirmed *via* spectral evidences from IR, EIMS and ^{1}H-NMR as described in experimental section. The mass fragmentation pattern of 2-(2-chlorobenzyl) thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (**6f**) is given in fig. 1 for the convenience of reader.

Antibacterial activity (in vitro)

According to the objective of the work, we tried to explore the inhibition potential of the present compounds as was observed in our previous group work (Siddiqa *et al.,* 2014; Irshad *et al.,* 2014; Aziz-ur-Rehman *et al.,* 2014a; Aziz-ur-Rehman *et al.,* 2014b). The results obtained are quite satisfactory. All the *S*-substituted molecules, **6a-m**, were assessed for their inhibition activity against gram-bacteria (positive and negative). The results are presented both in the form of percentage inhibition and minimum inhibitory concentration (MIC) values collectively in table 2. Overall the results were found to be varying from moderate to excellent as shown by their minimum inhibitory concentration MIC values.

The synthesized compound, 2-(2-Phenylethyl)thio-5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole (**6l**), exhibited the highest percentage inhibition and the least MIC values against all the bacterial strains taken into account. It showed almost the same activity as that of the reference standard ciprofloxacin against all the bacterial strains. The excellent activity of this compound was conjectured to be due to the presence of aralkyl group i.e. phenyl ring linked to the main part of molecule through two methylenic ($-CH_2$) groups. The compounds **6h**, **6i** and **6k** remained inactive against all the bacterial strains. Might this inactivity be due to the para substituted phenyl ring with chloro ($-Cl^-$), flouro ($-F^-$) and nitro groups ($-NO_2$) in **6h**, **6i** and **6k** compounds, respectively.

It was observed that **6d** and **6l** exhibited valuable activity against all the four gram-negative bacterial strains but **6h**, **6i** and **6k** were rendered inactive. Most of the compounds showed positive results against *S. typhi* and *P. aeruginosa* but a few against *E. coli* and *K. pneumoniae*. The synthesized compound, **6c** showed good activity results for the gram-positive bacteria, *B. subtilis*, compared to the reference standard.

CONCLUSION

All the synthesized molecules were acquired in good yields and well supported by the spectroscopic data. The series of new molecules were synthesized to inaugurate the potent inhibitors of various bacterial strains. The reaction methodology applied was environment friendly, easy and facile. The antibacterial activity results rendered the synthesized molecules as valuable for pharmacological research. It was concluded that simple phenyl rings might prove to be the better antibacterial agents rather than the substituted ones as observed through the activity results. The compound **6l** showed the highest excellent activity might be due to the presence of unsubstituted phenyl ring. It was observed that compounds **6h**, **6i** and **6k** did not show any activity that might be due to the presence of substituted phenyl rings with flouro, chloro and nitro groups. Moreover, surprisingly these compounds were para substituted. However overall the compounds showed good activities and they may be proved to be the effective drug candidates in advance research.

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