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Original Research Article

Preliminary structure-activity relationship studies on some novel s-substituted aliphatic analogues of 5-{1-[(4-chlorophenyl) sulfonyl]-3-piperidinyl}-1, 3, 4-oxadiazol-2-yl sulfide

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Abstract

Purpose: *To study the structure-activity relationships of synthetic multifunctional sulfides through evaluation of lipoxygenase and anti-bacterial activities.*
Methods: *S-substituted derivatives of the parent compound 5-(1-(4-chlorophenylsulfonyl) piperidin-3-yl)-1, 3, 4-oxadiazole-2-thiol were synthesized through reaction with different saturated and unsaturated alkyl halides in DMF medium, with NaH catalyst. Spectral characterization of each derivative was carried out with respect to IR, ^{1}H - NMR, ^{13}C - NMR and EI - MS. The lipoxygenase inhibitory and antibacterial activities of the derivatives were determined using standard procedures.*
Results: *Compound **5e** exhibited higher lipoxygenase inhibitory potential than the standard (Baicalein®), with % inhibition of 94.71 ± 0.45 and IC_{50} of 20.72 ± 0.34 µmoles/L. Compound **5b** showed significant antibacterial potential against all the bacterial strains with % inhibition ranging from 62.04 ± 2.78, 69.49 ± 0.41, 63.38 ± 1.97 and 59.70 ± 3.70 to 78.32 ± 0.41, while MIC ranged from 8.18 ± 2.00, 10.60 ± 1.83, 10.84 ± 3.00, 9.81 ± 1.86 and 11.73 ± 5.00 µmoles/L for S. typhi, E. coli, P. aeruginosa, B. subtilis and S. aureus, respectively. Compounds **5d**, **5e** and **5g** showed good antibacterial activity against S. typhi and B. subtilis bacterial strains.*
Conclusion: *The results suggest that compound **5e** bearing n-pentyl group is a potent lipoxygenase inhibitor, while compound **5b** with n-propyl substitution is a strong antibacterial agent. In addition, compounds **5d**, **5e** and **5g** bearing n-butyl, n-pentyl and n-octyl groups, respectively, are good antibacterial agents against S. typhi and B. subtilis.*

Keywords: *Sulfides, Antibacterial activity, Lipoxygenase activity, Spectral analysis*

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INTRODUCTION

Millions of people suffer from bacterial infectious diseases which are among the leading causes of morbidity and mortality worldwide [1,2]. Thus infectious diseases of bacterial aetiology are of serious public health significance. However, the treatment and management of bacterial infectious diseases are hampered by numerous factors, including the emergence of multi-drug resistant strains of bacteria [3]. The phenomenon of multi-drug resistance has led to increasing

attention on the search for new, synthetic compounds with potential anti-microbial properties. Indeed a large number of compounds have been synthesized and evaluated for their anti-microbial potential [4,5].

Oxadiazole compounds have potent pharmacological properties. They are 5-membered heteroaromatic rings which exist in different isomeric forms. Oxadiazoles have been characterized as frequently occurring motifs in drug-like compounds [6]. The 2,5-di-substituted - 1, 3 4- oxadiazoles associated with a wide range of hetero-atom rings are important parts of a variety of clinical drugs used for management of different diseases.

Oxadiazole-derived compounds have a wide range of medical applications such as anti-tussive, anaesthetic, anti-inflammatory, anti-allergic, vasodilator and anti-helmintic [7] 2, 5-di-substituted-1, 3, 4-oxadiazoles are known to have fungicidal [3], anti-inflammatory [4,5], antitumor [8], insecticidal, herbicidal, antiviral, analgesic [9], antibacterial [10], anti-tubercular [11], herbicidal, anti-malarial, anti-convulsant and cytotoxic [12-14] activities. The present study was aimed at synthesizing S-substituted derivatives of 5-(1-(4–chlorophenysulfonyl) piperidin-3-yl)-1,3,4-oxadiazole-2-thiol, and investigating the lipoxygenase inhibitory and antimicrobial properties of the derivatives.

EXPERIMENTAL

Materials

All chemicals were products of either Merck (Darmstadt) or Sigma Aldrich (St Louis). For column chromatography (CC), silica gel (70 - 230 mesh) was used. Silica plates (0.25 mm) coated on alumina were used for thin layer chromatography (TLC) to check the purity of synthesized compounds. Ethyl acetate:n-hexane (30:70) was used as mobile phase. To visualize the fluorescent spots UV lamp was utilized at 254 nm. Using KBr pellet method Jasco FTIR spectrometer recorded the IR spectra. Bruker spectrometers working at 300 and 400 MHz were employed in recording the ^{1}H-NMR and ^{13}C-NMR spectra. $CDCl_3$ was used as solvent and TMS was the reference standard. Chemical shifts (δ) were given in ppm, while coupling constants (J) were recorded in Hz. EIMS spectra were recorded on JMS-HX 110 spectrometer. Melting points were determined on Griffin and George melting point apparatus using open capillary tube method.

Synthesis of Ethyl-1-[(4-chlorophenyl) sulfonyl] piperidine-3-carboxylate (1)

Ethyl piperidine-3-carboxylate (a) (30 mmol) (Scheme 1) was mixed with 4-chlorobenzene sulfonyl chloride (30 mmol) in 30 mL distilled water contained in 100 mL round bottom flask. During the reaction, the pH of reaction medium was maintained at 10 - 11 by addition of a small amount of 15 % Na_2CO_3 solution and the reaction mixture was stirred at room temperature. TLC was utilized for monitoring reaction progress, with n-hexane and EtOAc as mobile phase. At the end of the reaction, the flask contents were neutralized and precipitates were filtered, washed with distilled water and re-crystallized in ethanol. The resultant crystalline product was designated as compound **1**.

Synthesis of 1-[(4-chlorophenyl) sulfonyl] piperidine-3-carbohydrazide (2)

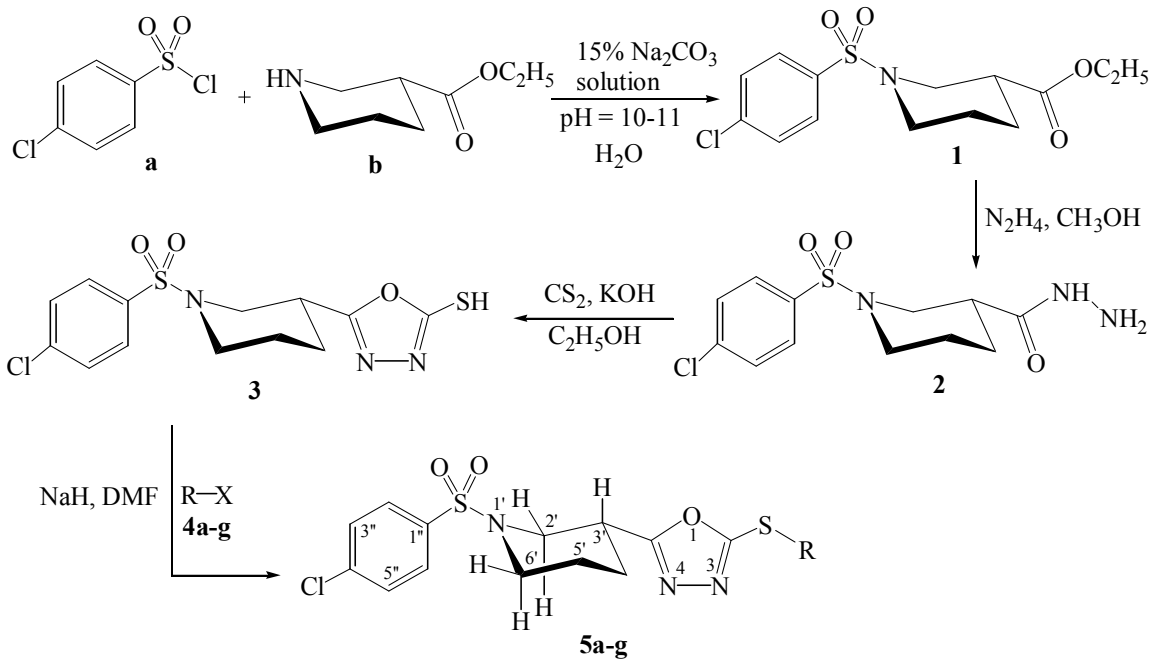
Compound **1** (25 mmol) was refluxed with 80 % hydrazine hydrate (40 mmol) in 50 mL methanol contained in 100 mL round bottom flask for 2 - 3 h. Reaction was monitored by TLC and on completion of the reaction, excess methanol was distilled off. Cold distilled water was introduced to the reaction mixture and the precipitates were filtered, washed with water and recrystallized from ethanol. The product was tagged compound **2**.

Synthesis of 5-(1-(4-chlorophenylsulfonyl)-3-piperidinyl)-1, 3, 4-oxadiazole-2-thiol (3)

Compound **2** (65 mmol) was dissolved in 60 mL ethanol along with CS_2 (65 mmol) and KOH (0.13 mol) in 100 mL round bottom flask. KOH was used to provide an alkaline environment to enhance the electrophilicity of CS_2. The reaction mixture was refluxed for 5 h and reaction progress was monitored with TLC. Excess ethanol was distilled off at the completion of reaction. The reaction contents were dissolved in distilled water and acidified to obtain the oxadiazole precipitates. The precipitates were filtered and washed with distilled water to yield compound **3**.

Synthesis of S-substituted derivatives of 5-{1-[(4-chlorophenyl) sulfonyl]-3-piperidinyl}-1, 3, 4-oxadiazol-2-yl sulfide

Compound **3** (1 mmol) was taken in 50 mL round bottom flask along with 7 - 10 mL of DMF. Equimolar NaH was introduced to the reaction flask and the contents were stirred for 30 min at room temperature.



Scheme-1: Outline for the synthesis of *S*-substituted derivatives of 5-(1-(4-chlorophenylsulfonyl) piperidin-3-yl)-1, 3, 4-oxadiazole-2-thiol

Table 1: Different *S*-substituted alkyl groups

Compd.	R
5a	$H_3C^{2'''}—CH_2^{1'''}—$
5b	$H_3C^{3'''}—H_2C^{2'''}—CH_2^{1'''}—$
5c	$H_2C^{3'''}=HC^{2'''}—CH_2^{1'''}—$
5d	$H_3C^{4'''}—H_2C^{3'''}—H_2C^{2'''}—CH_2^{1'''}—$
5e	$H_3C^{5'''}—H_2C^{4'''}—H_2C^{3'''}—H_2C^{2'''}—CH_2^{1'''}—$
5f	$H_3C^{7'''}—H_2C^{6'''}—H_2C^{5'''}—H_2C^{4'''}—H_2C^{3'''}—H_2C^{2'''}—CH_2^{1'''}—$
5g	$H_3C^{8'''}—H_2C^{7'''}—H_2C^{6'''}—H_2C^{5'''}—H_2C^{4'''}—H_2C^{3'''}—H_2C^{2'''}—CH_2^{1'''}—$

Different saturated/unsaturated alkyl halides (1 mmol) were added separately to the reaction mixture in the round bottom flask and the mixture was stirred for 4 h. At the end of reaction, cold distilled water was added and the precipitates were washed thoroughly with water. Seven different S-substituted derivatives resulted from the different alkyl halides (Table 1).

Assessment of biological activities

Antibacterial assay

The antibacterial activity of each S-substituted alkyl derivative was evaluated using the methods of Kaspady *et al* [15] and Yang *et al* [16]. Two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), and three gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) were clinically isolated and stored on appropriate agar media to facilitate bacterial growth. All the strains were obtained from a local hospital. Test samples (20 µg), after suitable dilution was added to 180 µL of diluted fresh bacterial cultures in nutrient broth in a microplate. The initial absorbance at 540 nm was taken and kept at values between 0.12 - 0.19. Ciprofloxacin^R was used as standard drug. The microplates with lids were incubated at 37 ^oC for 16 - 24 h. Absorbance was read at 540 nm in a microplate reader, before and after incubation and the difference was used as an index of bacterial growth. Percent inhibition was calculated as in Eq 1.

$$\text{Inhibition (\%)} = \{(C - T)/C\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where C (i.e., control) = total enzyme activity without inhibitor, and T (i.e., test sample) = activity in the presence of test compound. The results are expressed as mean ± SEM (n = 3). Minimum inhibitory concentration (MIC) was measured with suitable dilutions (5 - 30 µg/well) and the results were analyzed using EZ-Fit (Perrella Scientific Inc. Amherst USA) software.

Lipoxygenase assay

Lipoxygenase activity was assayed using the methods as described previously [17,18], with slight modifications. A total volume of 200 µL assay mixture contained 150 µL sodium phosphate buffer (100 mM, pH 8.0), 10 µL test compound and 15 µL purified lipoxygenase (Sigma, USA). The contents were mixed and pre-read at 234 nm and pre-incubated for 10 min at 25 ^{o}C. Reaction was initiated by the addition of 25 µL substrate solution and the change in absorbance was read after 6 min at 234 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All reactions were carried out in triplicates. Positive and negative controls were included in the assay. Baicalein^{R} (0.5 mM well^{-1}) was used as a positive control. Percentage inhibition and IC_{50} values were calculated as in Eq 2.

Inhibition (%) = {(C – T)/C}100 …… (2)

where C (i.e., control) = total enzyme activity without inhibitor, and T (i.e., test sample) = activity in the presence of test compound. IC_{50} values (concentration at which enzyme inhibition is 50 %) were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Statistical analysis

All the measurements were done in triplicate and statistical analysis was performed using Microsoft Excel 2010. Results are presented as mean ± SEM with 85 % confidence limit.

RESULTS

Target compounds were synthesized (Table 1) by following a series of reactions as described in experimental section and shown in Scheme-1. All the molecules were characterized by spectral data of IR, ^{1}H-NMR, ^{13}C-NMR and EIMS.

5-[1-[(4-Chlorophenyl) sulfonyl]-3-piperidinyl]-1, 3, 4-oxadiazole-2-thiol (Compound 3)

White amorphous solid; Yield: 85 %; M.P. 145 - 146 °C; Molecular formula: $C_{13}H_{14}ClN_3O_3S_2$; Molecular Mass: 359 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3033 (Ar-H), 2252 (S-H stretching), 1591 (C=N stretching), 1524 (Ar C=C stretching), 1327 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.70 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.52 (d, J = 8.7 Hz, 2H, H-3" & H-5"), 3.90 (dd, J = 11.7, 3.6 Hz, 1H, H_e-2'), 3.65 (br.d, J = 11.7 Hz, 1H, H_a-2'), 3.10-3.02 (m, 1H, H-3'), 2.65 (br.t, J = 9.9 Hz, 1H, H_e-6'), 2.49 (td, J = 11.4, 3.0 Hz, 1H, H_a-6'), 2.10-2.06 (m, 1H, H_a-5'), 1.90-1.82 (m, 1H, H_a-4'), 1.81-1.70 (m, 1H, H_e-5'), 1.69-1.58 (m, 1H, H_e-4'); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ/ppm): 178.4 (C-2), 163.6 (C-5), 139.7 (C-1"), 134.6 (C-4"), 129.5 (C-2" & C-6"), 128.9 (C-3" & C-5"), 47.5 (C-2'), 46.1 (C-6'), 33.7 (C-3'), 26.5 (C-4'), 23.5 (C-5'); EIMS (*m/z*): 359 $[M]^+$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl) sulfonyl]-3-piperidinyl} -1, 3, 4-oxadiazol-2-yl ethyl sulfide (Compound 5a)

White solid; Yield: 76 %; M.P. 97 - 99 °C; Molecular formula: $C_{15}H_{18}ClN_3O_3S_2$; Molecular Mass: 387 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3025 (Ar-H), 1585 (C=N stretching), 1534 (Ar C=C stretching), 1321 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.65 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.49 (d, J = 8.7 Hz, 2H, H-3" & H-5"), 3.91 (dd, J = 8.7, 3.6 Hz, 1H, H_e-2'), 3.63 (br.d, J = 11.7 Hz, 1H, H_a-2'), 3.21 (q, J = 7.2 Hz, 2H, H-1'"), 3.08-3.02 (m, 1H, H-3'), 2.68 (br.t, J = 9.9 Hz, 1H, H_e-6'), 2.47 (td, J = 11.4, 3.0 Hz, 1H, H_a-6'), 2.07-2.01 (m, 1H, H_a-5'), 1.87-1.81 (m, 1H, H_a-4'), 1.78-1.70 (m, 1H, H_e-5'), 1.68-1.58 (m, 1H, H_e-4'), 1.43 (t, J = 7.2 Hz, 3H, H-2'"); EIMS (*m/z*): 387 $[M]^+$, 359 $[C_{13}H_{14}ClN_3O_3S_2]^{\bullet+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 212 $[M\text{-}C_6H_4ClSO_4]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl) sulfonyl]-3-piperidinyl} -1, 3, 4-oxadiazol-2-yl propyl sulfide (Compound 5b)

Grey solid; Yield: 73 %; M.P. 102 - 104 °C; Molecular formula: $C_{16}H_{20}ClN_3O_3S_2$; Molecular Mass: 401 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3027 (Ar-H), 1589 (C=N stretching), 1537 (Ar C=C stretching), 1324 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.70 (d, J = 8.4 Hz, 2H, H-2" & H-6"), 7.50 (d, J = 8.4 Hz, 2H, H-3" & H-5"), 3.99 (dd, J = 11.6, 3.2 Hz, 1H, H_e-2'), 3.71 (br.d, J = 11.6 Hz, 1H, H_a-2'), 3.20 (t, J = 6.8 Hz, 2H, H-1'"), 3.18-3.16 (m, 3H, H-3'merged with propyl signals), 2.62 (br.t, J = 11.2 Hz, 1H, H_e-6'), 2.44 (td, J = 11.6, 2.8 Hz, 1H, H_a-6'), 2.15-2.10 (m, 1H, H_e-5'), 1.86-1.83 (m, 3H, H_a-5' & H-4'), 1.80

(sext, *J* = 7.2 Hz, 2H, H-2'''), 1.03 (t, *J* = 7.2 Hz, 3H, H-3'''); EIMS (*m/z*): 401 $[M]^+$, 359 $[C_{13}H_{14}ClN_3O_3S_2]^{\bullet+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 226 $[M-C_6H_4ClSO_4]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl) sulfonyl]-3-piperidinyl} -1, 3, 4-oxadiazol-2-yl allyl sulfide (Compound 5c)

Greyish white solid; Yield: 79 %; M.P. 92 - 94 °C; Molecular formula: $C_{16}H_{18}ClN_3O_3S_2$; Molecular Mass: 399 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3027 (Ar-H), 1582 (C=N stretching), 1531 (Ar C=C stretching), 1319 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.67 (d, *J* = 8.4 Hz, 2H, H-2" & H-6"), 7.50 (d, *J* = 8.4 Hz, 2H, H-3" & H-5"), 5.99-5.90 (m, 1H, H-2'''), 5.29 (dd, *J* = 16.8, 0.9 Hz, 1H, H-3$_a$'''), 3.87 (dd, *J* = 8.7, 3.6 Hz, 1H, H$_e$-2'), 3.80 (d, *J* = 7.2 Hz, 2H, H-1'''), 3.61 (br.d, *J* = 11.7 Hz, 1H, H$_a$-2'), 3.09-3.01 (m, 1H, H-3'), 2.66 (br.t, *J* = 9.9 Hz, 1H, H$_e$-6'), 2.45 (td, *J* = 11.4, 3.0 Hz, 1H, H$_a$-6'), 2.08-2.04 (m, 1H, H$_a$-5'), 1.89-1.80 (m, 1H, H$_a$-4'), 1.79-1.71 (m, 1H, H$_e$-5'), 1.67-1.59 (m, 1H, H$_e$-4'); EIMS (*m/z*): 399 $[M]^+$, 359 $[C_{13}H_{14}ClN_3O_3S_2]^{\bullet+}$, 300 $[C_{12}H_{13}ClN_2O_3S]^{\bullet+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 224 $[M-C_6H_4ClSO_4]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$, 41 $[C_3H_5]^+$.

5-{1-[(4-Chlorophenyl) sulfonyl] -3-piperid`inyl}-1, 3, 4-oxadiazol-2-yl butyl sulfide (Compound 5d)

Greyish white solid; Yield: 79 %; M.P. 95 - 97 °C; Molecular formula: $C_{17}H_{22}ClN_3O_3S_2$; Molecular Mass: 415 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3016 (Ar-H), 1588 (C=N stretching), 1543 (Ar C=C stretching), 1329 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.68 (d, *J* = 8.4 Hz, 2H, H-2" & H-6"), 7.48 (d, *J* = 8.4 Hz, 2H, H-3" & H-5"), 3.88 (dd, *J* = 8.7, 3.6 Hz, 1H, H$_e$-2'), 3.64 (br.d, *J* = 11.7 Hz, 1H, H$_a$-2'), 3.19 (t, *J* = 7.8 Hz, 2H, H-1'''), 3.09-3.04 (m, 1H, H-3'), 2.67 (br.t, *J* = 10.2 Hz, 1H, H$_e$-6'), 2.51 (td, *J* = 11.4, 3.0 Hz, 1H, H$_a$-6'), 2.13-2.08 (m, 1H, H$_a$-5'), 1.93-1.87 (m, 1H, H$_a$-4'), 1.85-1.81 (m, 1H, H$_e$-5'), 1.78 (quint, *J* = 7.8 Hz, 2H, H-2'''), 1.69-1.55 (m, 1H, H$_e$-4'), 1.45 (sext, *J* = 7.8 Hz, 2H, H-3'''), 0.92 (t, *J* = 7.8 Hz, 3H, H-4'''); EIMS (*m/z*): 415 $[M]^+$, 359 $[C_{13}H_{14}ClN_3O_3S_2]^{\bullet+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 240 $[M-C_6H_4ClSO_4]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl) sulfonyl]-3-piperidinyl} -1, 3, 4-oxadiazol-2-yl pentyl sulfide (Compound 5e)

White solid; Yield: 82 %; M.P. 126 - 128 °C; Molecular formula: $C_{18}H_{24}ClN_3O_3S_2$; Molecular Mass: 429 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3018 (Ar-H), 1584 (C=N stretching), 1543 (Ar C=C stretching), 1331 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.66 (d, *J* = 8.4 Hz, 2H, H-2" & H-6"), 7.47 (d, *J* = 8.4 Hz, 2H, H-3" & H-5"), 3.89 (dd, *J* = 8.7, 3.6 Hz, 1H, H$_e$-2'), 3.63 (br.d, *J* = 11.7 Hz, 1H, H$_a$-2'), 3.18 (t, *J* = 7.8 Hz, 2H, H-1'''), 3.12-3.04 (m, 1H, H-3'), 2.67 (br.t, *J* = 10.2 Hz, 1H, H$_e$-6'), 2.51 (td, *J* = 11.4, 3.0 Hz, 1H, H$_a$-6'), 2.10-2.05 (m, 1H, H$_a$-5'), 1.93-1.85 (m, 1H, H$_a$-4'), 1.83-1.79 (m, 1H, H$_e$-5'), 1.76 (quint, *J* = 7.8 Hz, 2H, H-2'''), 1.74-1.60 (m, 1H, H$_e$-4'), 1.41-1.29 (m, 4H, H-3''' & H-4'''), 0.88 (t, *J* = 7.8 Hz, 3H, H-5'''); EIMS (*m/z*): 429 $[M]^+$, 359 $[C_{13}H_{14}ClN_3O_3S_2]^{\bullet+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 254 $[M-C_6H_4ClSO_4]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl) sulfonyl] -3-piperidinyl}-1, 3, 4-oxadiazol-2-yl heptyl sulfide (Compound 5f)

White solid; Yield: 80 %; M.P. 71 - 73 °C; Molecular formula: $C_{20}H_{28}ClN_3O_3S_2$; Molecular Mass: 457 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3013 (Ar-H), 1578 (C=N stretching), 1547 (Ar C=C stretching), 1337 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.70 (d, *J* = 8.4 Hz, 2H, H-2" & H-6"), 7.51 (d, *J* = 8.4 Hz, 2H, H-3" & H-5"), 3.99 (dd, *J* = 11.4, 3.3 Hz, 1H, H$_e$-2'), 3.72 (br.d, *J* = 11.4 Hz, 1H, H$_a$-2'), 3.22 (t, *J* = 7.2 Hz, 2H, H-1'''), 3.16-3.13 (m, 1H, H-3'), 2.61 (br.t, *J* = 11.7 Hz, 1H, H$_e$-6'), 2.41 (td, *J* = 11.4, 2.7 Hz, 1H, H$_a$-6'), 2.17-2.11 (m, 1H, H$_e$-5'), 1.90-1.71 (m, 3H, H$_a$-5' & H-4'), 1.57-1.26 (m, 10H, H-2''' to H-6'''), 0.84 (t, *J* = 6.9 Hz, 3H, H-7'''); EIMS (*m/z*): 457 $[M]^+$, 359 $[C_{13}H_{14}ClN_3O_3S_2]^{\bullet+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 282 $[M-C_6H_4ClSO_4]^+$, 175 $[C_6H_4ClO_2S]^+$, 111 $[C_6H_4Cl]^+$.

5-{1-[(4-Chlorophenyl) sulfonyl]-3-piperidinyl} -1, 3, 4-oxadiazol-2-yl octyl sulfide (Compound 5g)

Greyish white solid; Yield: 77 %; M.P. 76 - 78 °C; Molecular formula: $C_{21}H_{30}ClN_3O_3S_2$; Molecular Mass: 471 gmol^{-1}; IR (KBr, cm^{-1}) ν_{max}: 3015 (Ar-H), 1576 (C=N stretching), 1547 (Ar C=C stretching), 1338 (-SO_2 stretching); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.67 (d, *J* = 8.4 Hz, 2H, H-2" & H-6"), 7.52 (d, *J* = 8.4 Hz, 2H, H-3" & H-5"), 3.87 (dd, *J* = 8.7, 3.6 Hz, 1H, H$_e$-2'), 3.64 (br.d, *J* = 11.7 Hz, 1H, H$_a$-2'), 3.18 (t, *J* = 7.8 Hz, 2H, H-1'''), 3.10-3.02 (m, 1H, H-3'), 2.69 (br.t, *J* = 10.2 Hz, 1H, H$_e$-6'), 2.53 (td, *J* = 11.4, 3.0 Hz, 1H, H$_a$-6'), 2.13-2.09 (m, 1H, H$_a$-5'), 1.91-1.84 (m, 1H, H$_a$-4'), 1.82-1.79 (m, 1H, H$_e$-5'), 1.74 (qui, *J* = 7.8 Hz, 2H, H-2'''), 1.70-1.59 (m, 1H, H$_e$-4'),1.41-1.24

(m, 10H, H-3''' to H-7'''), 0.85 (t, *J* = 6.9 Hz, 3H, H-8'''); EIMS (*m/z*): 471 $[M]^+$, 359 $[C_{13}H_{14}ClN_3O_3S_2]^{\bullet+}$, 284 $[C_{12}H_{13}ClN_2O_2S]^{\bullet+}$, 286 $[C_{12}H_{13}ClNO_3S]^+$, 258 $[C_{11}H_{13}ClNO_2S]^+$, 296 $[M-C_6H_4ClSO_4]^+$, 175 $[C_6H_4ClO_2S]^+$, 113 $[C_8H_{17}]^+$, 111 $[C_6H_4Cl]^+$.

Biological activities

The lipoxygenase inhibitory and antimicrobial activities of the compounds are shown in Tables 2 and 3, respectively, while their MIC values are shown in Table 4. Compounds 5a, 5e, 5f and 5g showed very strong lipoxygenase inhibitory potential with 5e having % inhibition comparable to that of the standard, Baicalein^R.

Results for antimicrobial activities revealed that although none of the compounds exhibited % inhibition comparable to the standard drug Ciprofloxacin® most of them had good % inhibition values with some (5a, 5b 5d and 5e) having 70 % and above with respect to *S. typhi*, *E. coli* and *B. subtilis*.

Compound 5b consistently exhibited the lowest MIC values (relative to the other compounds) for all the bacterial strains used. The MIC values of 5b were closest to those of the standard drug, Ciprofloxacin® when compared with MIC values for 5a, 5c 5d, 5e 5f and 5g (Table 4).

Table 2: Lipoxygenase inhibitory activities of the compounds

Compound	Conc.	Inhibition (%)	IC_{50} (µM/L)
5a	0.5	70.26 ± 0.98	127.41 ± 0.13
5b	0.5	42.39 ± 1.65	>500
5c	0.5	58.41 ± 0.43	265.2 ± 0.34
5d	0.5	46.24 ± 0.92	>500
5e	**0.5**	**94.71 ± 0.45**	**20.72 ± 0.34**
5f	0.5	78.09 ± 0.56	201.31 ± 1.67
5g	0.5	81.41 ± 0.98	204.21 ± 1.56
Baicalein	**0.5**	**93.79 ± 1.27**	**22.41 ± 1.30**

Values are Mean ± S.E.M

Table 3: Antibacterial activity (% Inhibition) of the test compounds

Compound	Inhibition (%)				
	Gram negative bacterial strains			*Gram positive bacterial strains*	
	S. typhi (-)	*E. coli (-)*	*P. aeruginosa (-)*	*B. subtilis (+)*	*S. aureus(+)*
5a	71.61 ± 1.06	70.27 ± 2.55	68.85 ± 2.85	40.80 ± 5.00	63.47 ± 2.74
5b	78.32 ± 0.41	62.04 ± 2.78	69.49 ± 0.41	63.38 ± 1.97	59.70 ± 3.70
5c	64.22 ± 2.11	65.82 ± 1.45	61.25 ± 1.95	35.75 ± 4.85	65.47 ± 5.00
5d	74.27 ± 1.36	64.31 ± 2.55	52.24 ± 2.14	71.87 ± 3.48	56.20 ± 0.60
5e	76.95 ± 1.59	61.48 ± 0.83	67.45 ± 1.12	74.80 ± 2.07	60.90 ± 1.70
5f	55.32 ± 0.68	48.43 ± 4.26	48.16 ± 5.00	53.89 ± 3.99	50.35 ± 1.65
5g	68.09 ± 1.18	49.26 ± 1.19	52.76 ± 0.31	68.48 ± 0.71	52.65 ± 1.55
Ciprofloxacin	**91.79 ± 1.45**	**90.87 ± 0.56**	**92.13 ± 0.97**	**91.18 ± 1.22**	**90.45 ± 2.98**

Results are presented as mean ± SEM (n = 3)

Table 4: Antibacterial activity (MIC) of the test compounds

Compound	MIC (mg/ml)				
	Gram negative bacterial strains			*Gram positive bacterial strains*	
	S. typhi (-)	*E.coli (-)*	*P. aeruginosa (-)*	*B.subtilis (+)*	*S. aureus(+)*
5a	13.09 ± 1.90	12.95 ± 2.00	12.67 ± 1.14	-	16.92 ± 0.98
5b	8.18 ± 2.00	10.60 ± 1.83	10.84 ± 3.00	9.81 ± 1.86	11.73 ± 5.00
5c	13.40 ± 2.50	12.64 ± 1.87	15.38 ± 2.50	-	16.22 ± 1.20
5d	9.50 ± 1.03	12.72 ± 3.56	15.08 ± 1.50	9.25 ± 3.29	12.79 ± 1.42
5e	8.24 ± 3.36	14.68 ± 0.58	13.68 ± 2.92	8.55 ± 3.21	11.99 ± 2.84
5f	14.84 ± 0.69	-	-	13.62 ± 3.50	15.39 ± 1.41
5g	9.06 ± 1.26	-	18.37 ± 1.92	10.51 ± 2.00	16.12 ± 3.11
Ciprofloxacin	**7.15 ± 1.29**	**7.90 ± 1.87**	**8.21 ± 1.21**	**7.12 ± 2.11**	**8.00 ± 2.98**

Results are presented as mean ± SEM

DISCUSSION

The IR spectra of Compound 5b revealed the presence of C=N at 1589 cm^{-1}, aromatic C=C double bond was confirmed by bands at 1537 cm^{-1} and presence of sulfonyl group at about 1324 cm^{-1}. ^{1}H-NMR recorded at 400 MHz using $CDCl_3$ as solvent gave characteristic two doublets at 7.70 and 7.50 ppm for 4-chlorophenyl sulfonyl group while signals for piperidine ring have a broad range of chemical shift value varying for each axial and equatorial proton.

Signals around 3.99ppm and 3.71ppm were to equatorial and axial proton of second position of the piperidine ring relatively downfield due to the adjacent electronegative nitrogen atom while proton of third position appeared as multiplet in range of 3.18 - 3.16 as a result of neighboring two methylene groups. Signals around 2.62 and 2.44 belong to equatorial and axial proton of sixth position of piperidine ring. Four protons of fourth and fifth positions exhibited chemical shift at about 2.15 - 2.10 and 1.86 - 1.83 for equatorial and axial protons respectively. The propyl substituent on sulfur of 1, 3, 4-oxadiazole-2-thiol appeared in aliphatic region and was confirmed by two triplets, one relatively downfield at δ (ppm) 3.20 (t, J = 6.8 Hz, 2H, H-1‴) due to electronegative sulfur atom. The other triplet methyl group appeared in upfield region at δ (ppm) 1.03 (t, J = 7.2 Hz, 3H, H-3‴) while the central methylene appeared as sextet at δ (ppm) 1.80 (sext, J = 7.2 Hz, 2H, H-2‴). The characteristic peaks at *m/z* 286 and 284 corresponded to ({1-[(4-chlorophenyl) sulfonyl]-3-piperidinyl} methylidyne) oxonium and 1-[(4-chlorophenyl) sulfonyl]-3-piperidine cyanide cationic fragments respectively. Base peak appeared at *m/z* 175 corresponding to 4-chlorophenyl sulfonylcation and the molecular ion peak was at *m/z* 401. The structures of all the other compounds were arrived at on the basis of these spectral information.

Compounds **5e**, **5g** and **5f** exhibited% inhibition values 94.71 ± 0.45, 81.41 ± 0.98 and 78.09 ± 0.56, respectively, compared to standard Baicalein with % inhibition of 93.79 ± 1.27. Compound **5e** exhibited excellent IC_{50} value 20.72 ± 0.34 probably due to *n*-pentyl group substitution at 2-thiol position of oxadiazole ring which provides the molecule a more favorable geometry that probably facilitates its binding to active site of the enzyme, as compared to Baicalein^R with IC_{50} of 22.41 ± 1.30.

The different compounds showed different activities against the different bacterial strains. Some showed good % inhibition but their MIC values were not so appreciable. Compounds **5a, 5b, 5d,5e** and **5g** gave good % inhibition values against *S. typhi* bacterial strain but only compounds **5b,5d, 5e** and **5g** gave good MIC values (8.18 ± 2.00, 9.50 ± 1.03, 8.24 ± 3.36 and 9.06 ± 1.26, respectively). This is most probably due to aliphatic straight chain substitutions, as compared to standard Ciprofloxacin^R 7.15 ± 1.29.

The compounds 5a – 5g are S-substituted alkyl derivatives of 1, 3, 4 oxadiazole. The anti-microbial and anti-inflammatory properties of these compounds seen in this study are in agreement with results reported by other studies involving anti-microbial and anti-inflammatory potential of other synthetic 1, 3, 4- oxadiazole derivatives. Some novel 1, 3, 4- oxadiazole derivatives have been shown to exhibit antimicrobial activities against *S. aureus*, *B. subtilis, E. coli and Pseudomonas* [19]. In addition, potent antimicrobial and anti-inflammatory derivatives of 1, 3, 4 oxadiazole have been chemically synthesized by condensation of 4-methoxybenzo hydride with different aromatic acids [20]. New antimicrobial derivative of 1 3, 4-oxodiazole with 5 – chloro – 2 – methoxyphenyl moiety have also been reported by other investigators [21].

CONCLUSION

Our results indicate that, of the seven derivatives synthesized and studied, compound **5e** shows the best lipoxygenase inhibitory activity, probably due to the effect of the *n*-pentyl chain on its orientation, while compound **5b** was the most active antibacterial agent against all the bacterial strains tested.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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