Synthesis and antibacterial screening of some 2-[5-(aryloxymethyl)-1, 3, 4-oxadiazol-2-ylsulfanyl] acetic acids

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ABSTRACT

A series of 2-[5-(aryloxymethyl)-1, 3, 4-oxadiazol-2-ylsulfanyl] acetic acids were prepared and evaluated for their antibacterial activity. Title compounds were synthesized from aryloxyacetic acid hydrazides. Aryloxyacetic acid hydrazides 1 on refluxing with carbon disulfide in presence of methanolic potassium hydroxide and then on acidification with hydrochloric acid yielded 5-aryloxymethyl-1, 3, 4-oxadiazole-2-thiones 2. 2-Chloro acetic acid on reaction with 2 in basic media and then on acidification with dilute hydrochloric acid furnished the title compounds 3. Structures of these compounds were characterized by modern spectroscopic techniques. All the compounds were studied for their in vitro antibacterial activity against two Gram positive strains (Bacillus subtilis and Staphylococcus aureus) and two Gram negative strains (Escherichia coli and Pseudomonas aeruginosa) and their minimum inhibitory concentration (MIC) were determined.

Keywords: 1, 3, 4-Oxadiazoles, antibacterial activity, minimum inhibitory concentration (MIC).

INTRODUCTION

The significant consumption of chemotherapeutic agents as the medicine for infectious diseases leads to the growth of microbial resistance to existing drugs. The development of resistance to the main classes of antibacterial drugs is accepted as a major health concern of global population. This turn out to be the challenge for the researchers working in the field of medicinal chemistry for the development of new antimicrobial drugs having a different mechanism of action to fight against the problem of multi-drug resistance [1]. Heterocyclic compounds continue to fascinate great interest due to their diverse biological activities. Amongst them five membered heterocyclic compounds occupy a unique place in the field of natural and synthetic organic chemistry. In recent years, attention has progressively been given to the synthesis of the compounds containing 1, 3, 4-oxadiazole nucleus as a lead of developing new antibacterial agents. 1, 3, 4-Oxadiazole derivatives constitute an important class of heterocycles displaying various biological activities like antibacterial [2-6], antifungal [7, 8], insecticidal [9], herbicidal [10], anticancer [11], anti-inflammatory [12] etc. Further, [5-(aryl)-1, 3, 4-thiadiazole-2-thio] acetates and propionates have been found to possess antimycobacterialactivity [13]. These reports including our continuing research work in the field of synthesis and antimicrobial activity of medicinally important compounds [14, 15] encouraged us to undertake the synthesis of some 2-[5-(aryloxymethyl)-1, 3, 4-oxadiazol-2-ylsulfanyl] acetic acids. The synthesized compounds were characterized on the basis of modern analytical techniques. These compounds were studied for their in vitro antibacterial activity.
MATERIALS AND METHODS

The purity of the synthesized compounds were checked by thin layer chromatography on silica gel G in different solvent systems using iodine vapors as detecting agent. Melting points were determined by Toshniwal Melting Point Boiling Point Determination Apparatus in open capillary tubes and are uncorrected. Infra-red spectra were recorded on Shimadzu 8000-FTIR Spectrophotometer in KBr Phase. Proton NMR spectra were recorded in CDCl3 on Bruker Advance DRX-300 FT-NMR Spectrometer using trimethylsilane as internal standard. Aryloxyacetic acid hydrazides 1a-g were synthesized by the reaction of hydrazine hydrate with the corresponding methyl esters of aryloxy acetic acids as given in the literature [16]. Similarly, 5-aryloxyethyl-1, 3, 4-oxadiazole-2-thiones 2a-g were prepared according to the method reported earlier [17].

General Procedure for the Synthesis of 2-[5-(aryloxymethyl)-1, 3, 4-oxadiazol-2-ylsulfanyl] acetic acids: 5-Aryloxyethyl-1, 3, 4-oxadiazole-2-thione (2a-g, 0.01 M) was dissolved in sodium hydroxide solution (10%, 10 ml). This solution was added drop-wise into a solution of 2-chloro acetic acid (0.011 M) which was previously neutralized with saturated solution of sodium carbonate, and mixture was stirred for 6 to 8 h. After completion of reaction the product was obtained by precipitation with dilute hydrochloric acid. The title compound was filtered, washed, dried and re-crystallized from the rectified spirit. The physical and analytical data of the synthesized title compounds are given as follows.

2-(5-(phenoxymethyl)-1,3,4-oxadiazol-2-ylsulfanyl) acetic acid (3a): Yield: 89%; m.p.: 150-152 ºC; IR (KBr, cm⁻¹): 3200-2400 broad band (O-H), 1736 (C=O), 1607 (C=N–N=C), 783, 706 (monosubstituted benzene).1H NMR (CDCl3): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.37 (m, 5H, ArH), 4.12 (s, 2H, CH2).

2-(5-((4-chlorophenoxy)methyl)-1, 3, 4-oxadiazol-2-ylsulfanyl) acetic acid (3b): Yield: 86%; m.p.: 155-156 ºC; IR (KBr, cm⁻¹): 3200-2400 broad band (O-H), 1735 (C=O), 1608 (C=N–N=C), 1263, 1068 (C-O-C), 826 (p-disubstituted benzene).1H NMR (CDCl3): δ (ppm) 11.70 (s, 1H, COOH), 7.26-7.36 (m, 4H, ArH), 4.12 (s, 2H, CH2), 2.41-2.43 (3H, s, aromatic methyl protons).

2-(5-((4-methoxyphenoxy)methyl)-1,3,4-oxadiazol-2-ylsulfanyl) acetic acid (3c): Yield: 83%; m.p.: 150-151 ºC; IR (KBr, cm⁻¹): 3200-2400 broad band (O-H), 1732 (C=O), 1606 (C=N–N=C), 1256, 1036 (C-O-C), 835 (p-disubstituted benzene).1H NMR (CDCl3): δ (ppm) 11.71 (s, 1H, COOH), 7.28-7.38 (m, 4H, ArH), 4.11 (s, 2H, CH2), 3.86 (3H, s, aromatic methoxy protons).

2-(5-((4-bromophenoxy)methyl)-1, 3, 4-oxadiazol-2-ylsulfanyl) acetic acid (3d): Yield: 88%; m.p.: 144-146 ºC; IR (KBr, cm⁻¹): 3200-2400 broad band (O-H), 1736 (C=O), 1616 (C=N–N=C), 1252, 1065 (C-O-C), 797, 833 (p-disubstituted benzene).1H NMR (CDCl3): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.34 (m, 4H, ArH), 4.12 (s, 2H, CH2).

2-(5-((4-fluorophenoxy)methyl)-1, 3, 4-oxadiazol-2-ylsulfanyl) acetic acid (3f): Yield: 82%; m.p.: 148-149 ºC; IR (KBr, cm⁻¹): 3200-2400 broad band (O-H), 1735 (C=O), 1609 (C=N–N=C), 1251, 1032 (C-O-C), 838 (p-disubstituted benzene).1H NMR (CDCl3): δ (ppm) 11.73 (s, 1H, COOH), 7.29-7.39 (m, 4H, ArH), 4.13 (s, 2H, CH2).

2-(5-((4-nitrophenoxy)methyl)-1,3,4-oxadiazol-2-ylsulfanyl) acetic acid (3g): Yield: 85%; m.p.: 155-157 ºC; IR (KBr, cm⁻¹): 3200-2400 broad band (O-H), 1730 (C=O), 1606 (C=N–N=C), 1265, 1068 (C-O-C), 833 (p-disubstituted benzene).1H NMR (CDCl3): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.35 (m, 3H, ArH), 4.11 (s, 2H, CH2).

Antibacterial Activity: All the titled compounds were studied for their in vitro antibacterial activity against two Gram positive strains, i.e., Bacillus subtilis (MTCC 121) and Staphylococcus aureus (MTCC 96) and two Gram negative strains, i.e., Escherichia coli (MTCC 40) and Pseudomonas aeruginosa (MTCC 2453). Antibacterial activity was checked by serial two fold dilution technique [18]. Ciprofloxacin was used as a standard drug. Stock solutions of the compounds were prepared in dimethyl sulfoxide having acetoncentration of 10 µg ml⁻¹. Double strength nutrient brothwas used as a growth media. The stock solution was serially diluted to give concentrations of 5.0 -0.01 µg ml⁻¹ in nutrient broth. The inoculum size was approximately 10⁶ colony forming units (CFU/ml). The inoculated tubes were incubated for 24 h at 37±1 °C. After 24 h, the inoculated culture tubes were macroscopically examined for turbidity. The culture tubes showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound. The MIC for the title compounds and the standard drug, i.e., ciprofloxacin are given in Table 1.
Table 1: *In Vitro* Antibacterial Activity of the Title Compounds (3a-g)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimum Inhibitory Concentration µg ml⁻¹</th>
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<tr>
<td></td>
<td><em>S. aureus</em> (MTCC 121)</td>
<td><em>B. subtilis</em> (MTCC 96)</td>
<td><em>E. coli</em> (MTCC 40)</td>
<td><em>P. aeruginosa</em> (MTCC 2453)</td>
</tr>
<tr>
<td>3a</td>
<td>0.65</td>
<td>0.60</td>
<td>0.55</td>
<td>0.60</td>
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<tr>
<td>3b</td>
<td>0.60</td>
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<td>0.40</td>
<td>0.50</td>
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<tr>
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<td>0.45</td>
<td>0.35</td>
<td>0.45</td>
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<tr>
<td>3g</td>
<td>0.40</td>
<td>0.40</td>
<td>0.30</td>
<td>0.40</td>
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<tr>
<td>Standard Drug</td>
<td>0.15</td>
<td>0.12</td>
<td>0.01</td>
<td>0.25</td>
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</tbody>
</table>

RESULTS AND DISCUSSION

Chemistry

The syntheses of 2-[5-(aryloxymethyl)-1, 3, 4-oxadiazol-2-ylsulfanyl] acetic acids 3 were accomplished following the steps outlined in Scheme 1. Reaction of aromatic carboxylic acid hydrazides 1 with carbon disulfide and methanolic potassium hydroxide and then acidification with dilute hydrochloric acid yielded the corresponding 5-aryloxymethyl-1, 3, 4-oxadizole-2-thiones 2. The intermediates 2 on reaction with 2-chloro acetic acid in basic medium and then subsequent acidification with dilute hydrochloric acid afforded the title compounds 3 in good yield.

![Scheme 1: Synthesis of 2-[5-(aryloxymethyl)-1, 3, 4-oxadiazol-2-ylsulfanyl] acetic acids](image-url)

Ar
3a C₆H₅
3b CH₃-C₆H₄
3c CH₃O-C₆H₄
3d Cl-C₆H₄
3e Br-C₆H₄
3f F-C₆H₄
3g NO₂-C₆H₄

Ar
3a C₆H₅
3b CH₃-C₆H₄
3c CH₃O-C₆H₄
3d Cl-C₆H₄
3e Br-C₆H₄
3f F-C₆H₄
3g NO₂-C₆H₄
Infrared spectra of each compound showed a broad band for O-H stretching vibrations in the range of 3200-2400 cm\(^{-1}\). The C=O stretching vibrations for the carboxyl group were absorbed in the range of 1736-1730 cm\(^{-1}\). The absorption for aromatic C-H bending vibrations was observed below 900 cm\(^{-1}\). IR absorption bands due to C-O-C grouping of 1, 3, 4-oxadiazole nucleus were observed in the range of 1275-1200 cm\(^{-1}\) and 699-7.67 ppm. Methylene protons appeared as singlet at δ (ppm) 4.13-4.11.

Minimum Inhibitory Concentration (MIC)

The reference standard ciprofloxacin inhibited Gram positive bacteria *S. aureus* and *Bacillus subtilis* at MIC of 0.15 µg ml\(^{-1}\) and 0.12 µg ml\(^{-1}\) respectively whereas MIC against Gram negative bacteria *E. coli* and *P. aeruginosa* were found to be in the range of 0.01 µg ml\(^{-1}\) and 0.25 µg ml\(^{-1}\) respectively. All the synthesized compounds 3a-g showed significant antibacterial activity against *P. aeruginosa* (MIC 0.40-0.60µg ml\(^{-1}\)), *S. aureus* (MIC 0.40-0.65µg ml\(^{-1}\)) and *B. subtilis* (MIC 0.40-0.60µg ml\(^{-1}\)) whereas moderate antibacterial activity was found against *E. coli* (MIC 0.30-0.55µg ml\(^{-1}\)) as compared to the standard drug ciprofloxacin (Table 1). Compounds containing 4-nitro moiety (3g) was found to be most active. The results of the MIC for the standard drug, ciprofloxacin, against the bacterial strains used were found to be within the range as reported in literature [19-21].

CONCLUSION

Present work describes a straightforward synthesis of new 2-[5-(aryloxy methyl)-1, 3, 4-oxadiazol-2-ylsulfanyl]acetic acids. The structures of the synthesized title compounds were ascertained by the modern analytical techniques. The title compounds were screened* in vitro* antibacterial activity against two Gram positive strains, *i.e.*, *Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 96) and two Gram negative strains, *i.e.*, *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453). Compounds 3g exhibited significant activity against all the bacterial strains used in this study. These results suggest that some more compounds should be synthesized and checked for antibacterial activity to explore the possibility of 2-[5-(aryloxy methyl)-1, 3, 4-oxadiazol-2-ylsulfanyl]acetic acids as a novel series of antibacterial drugs.

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REFERENCES