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Synthesis and antibacterial activity of some 5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl substituted benzothioates

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ABSTRACT

*A series of 5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl substituted benzothioateswas synthesized and studied for their antibacterial activity. These compounds were prepared from 2, 4-dichloro benzoic acid hydrazide. 2, 4-dichloro benzoic acid hydrazide **1** on refluxing with carbon disulfide and methanolic potassium hydroxide and then on subsequent acidification with hydrochloric acid furnished 5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazole-2-thiones **2**. Aroyl chlorides reacted with **2** in Schotten-Baumann reaction conditions yielded the title compounds **3**. These compounds were characterized by modern spectroscopic techniques. All the compounds were evaluated for their in vitro antibacterial activity against two Gram negative strains (Escherichia coli and Pseudomonas aeruginosa) and two Gram positive strains (Bacillus subtilis and Staphylococcus aureus) and their minimum inhibitory concentration (MIC) were determined.*

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

INTRODUCTION

The huge consumption of chemotherapeutic agents as the medication for infectious diseases leads to the growth of microbial resistance to existing drugs. The advent of resistance to the foremost classes of antibacterial drugs is accepted as a major health concern of worldwide population. This turn out to be the challenge for the medicinal chemists for the discovery of novel antimicrobial drugs having a different mechanism of action to battle the problem of multi-drug resistance [1].Heterocyclic compounds continue to fascinate considerable 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 increasingly been given to the synthesis of 1, 3, 4-oxadiazole derivatives as a source of developing new antibacterial agents. 1, 3, 4-Oxadiazole derivatives constitute an important class of heterocycles possessing diverse biological activities like antibacterial [2-6], antifungal [7, 8], insecticidal [9], herbicidal [10], anticancer [11], anti-inflammatory [12] etc. These reports including our ongoing research program in the field of synthesis and antimicrobial activity of medicinally important compounds [13, 14] inspired us to undertake the synthesis of some 5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl substituted benzothioates. The synthesized compounds were characterized on the basis of modern analytical techniques. These compounds were evaluated for their in vitro antibacterial activity.

MATERIALS AND METHODS

The purity of the synthesized compounds were ascertained by thin layer chromatography on silica gel G in various 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 $CDCl_3$on Bruker

Avance DRX-300 FT-NMR Spectrometer using tetramethylsilane as internal standard. 2, 4-dichloro benzoic acid hydrazide **1** was prepared by the reaction of hydrazine hydrate with the corresponding methyl ester of 2, 4-dichloro benzoic acid as described in the literature [15]. Similarly, 5-(2, 4-dichloro phenyl)-1, 3, 4-oxadiazole-2-thione **2** was synthesized according to the method reported earlier [16].

General Procedure for the Synthesis of 5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl substituted benzothioates: 5-(2, 4-dichloro phenyl)-1, 3, 4-oxadiazole-2-thione (**2**, 0.01 M)was dissolved in sodium hydroxide solution (10%, 20 ml). Aroyl chloride (0.011 M) was added dropwise with stirring. After completion of the reaction precipitated compound was filtered, washed with cold water, dried and re-crystallized from rectified spirit. The physical and analytical data of the synthesized title compounds are given as follows.

5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl benzothioate (3a): Yield: 83%; m.p.: 141-142 ^{o}C; IR (KBr, cm^{-1}): 1685 (thioester), 1651, 1620, 1562, 1423 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1062 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 682 (mono substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.27-7.34 (m, 8H, ArH).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 2-methylbenzothioate (3b): Yield: 81%; m.p.: 132-133 ^{o}C; IR (KBr, cm^{-1}): 1684 (thioester), 1651, 1622, 1560, 1423 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1061 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 772 (*o*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.26-7.44 (m, 7H, ArH), 2.67 (3H, s, aromatic methyl protons).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 3-methylbenzothioate (3c): Yield: 80%; m.p.: 135-136 ^{o}C; IR (KBr, cm^{-1}): 1683 (thioester), 1652, 1620, 1563, 1423 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1064 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 845, 802 (*m*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.27-7.42 (m, 7H, ArH), 2.66 (3H, s, aromatic methyl protons).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 4-methylbenzothioate (3d): Yield: 85%; m.p.: 140-141 ^{o}C; IR (KBr, cm^{-1}): 1680 (thioester), 1650, 1620, 1563, 1420 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1062 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 825 (*p*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.27-7.44 (m, 7H, ArH), 2.66 (3H, s, aromatic methyl protons).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 2-methoxybenzothioate (3e): Yield: 82%; m.p.: 138-139 ^{o}C; IR (KBr, cm^{-1}): 1688 (thioester), 1651, 1622, 1560, 1420 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1250 (alkyl aryl ether), 1064 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 770 (*o*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.26-7.85 (m, 7H, ArH), 3.85 (3H, s, aromatic methoxy protons).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 4-methoxybenzothioate (3f): Yield: 86%; m.p.: 144-145 ^{o}C; IR (KBr, cm^{-1}): 1686 (thioester), 1650, 1622, 1560, 1422 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1251 (alkyl aryl ether), 1063 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 820 (*p*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.26-7.84 (m, 7H, ArH), 3.84 (3H, s, aromatic methoxy protons).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 2-chlorobenzothioate (3g): Yield: 83%; m.p.: 145-146 ^{o}C; IR (KBr, cm^{-1}): 1685 (thioester), 1652, 1622, 1564, 1423 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1065 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 772 (*o*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.26-7.36 (m, 7H, ArH).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 3-chlorobenzothioate (3h): Yield: 84%; m.p.: 143-144 ^{o}C; IR (KBr, cm^{-1}): 1684 (thioester), 1650, 1620, 1564, 1423 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1065 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 845, 805 (*m*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.25-7.36 (m, 7H, ArH).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 4-chlorobenzothioate (3i): Yield: 85%; m.p.: 146-147 ^{o}C; IR (KBr, cm^{-1}): 1683 (thioester), 1651, 1620, 1564, 1420 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1062 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 822 (*p*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.24-7.36 (m, 7H, ArH).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 2-bromobenzothioate (3j): Yield: 82%; m.p.: 148-149 ^{o}C; IR (KBr, cm^{-1}): 1681 (thioester), 1650, 1622, 1564, 1420 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1061 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 770 (*o*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.26-7.36 (m, 7H, ArH).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 3-bromobenzothioate (3k): Yield: 81%; m.p.: 142-143 ^{o}C; IR (KBr, cm^{-1}): 1682 (thioester), 1652, 1620, 1564, 1420 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1063 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 847, 803 (*m*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.25-7.38 (m, 7H, ArH).

5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl 4-bromobenzothioate (3l): Yield: 87%; m.p.: 148-149 ^{o}C; IR (KBr, cm^{-1}): 1684 (thioester), 1650, 1622, 1564, 1421 (ring *str* of 1, 3, 4-oxadiazole nucleus), 1062 (C–O *str* of 1, 3, 4-oxadiazole nucleus), 825 (*p*-di-substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 7.24-7.37 (m, 7H, ArH).

Antibacterial Activity: All the compounds were screened for their in vitro antibacterial activity against two Gram negative strains, *i.e., Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453), and two Gram positive strains, *i.e., Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 96). Antibacterial activity was assessed by serial two fold dilution technique [17]. Ciprofloxacin was used as a standard drug. All the compounds were dissolved in dimethyl sulfoxide to give a concentration of 10 μg ml^{-1}. Double strength nutrient broth was 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 10^6 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 tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higherconcentration) 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-l)

Compound	Minimum Inhibitory Concentration μg ml^{-1}			
	E. coli **(MTCC 40)**	***P. aeruginosa*** **(MTCC 2453)**	***S. aureus*** **(MTCC 96)**	***B. subtilis*** **(MTCC 121)**
3a	0.60	0.65	0.55	0.65
3b	0.50	0.65	0.50	0.55
3c	0.60	0.65	0.50	0.60
3d	0.50	0.65	0.50	0.55
3e	0.60	0.60	0.55	0.65
3f	0.55	0.60	0.55	0.65
3g	0.50	0.65	0.50	0.60
3h	0.45	0.60	0.45	0.50
3i	0.40	0.45	0.40	0.45
3j	0.50	0.55	0.50	0.55
3k	0.45	0.50	0.45	0.50
3l	0.40	0.45	0.40	0.45
Standard Drug	0.01	0.25	0.15	0.12

RESULTS AND DISCUSSION

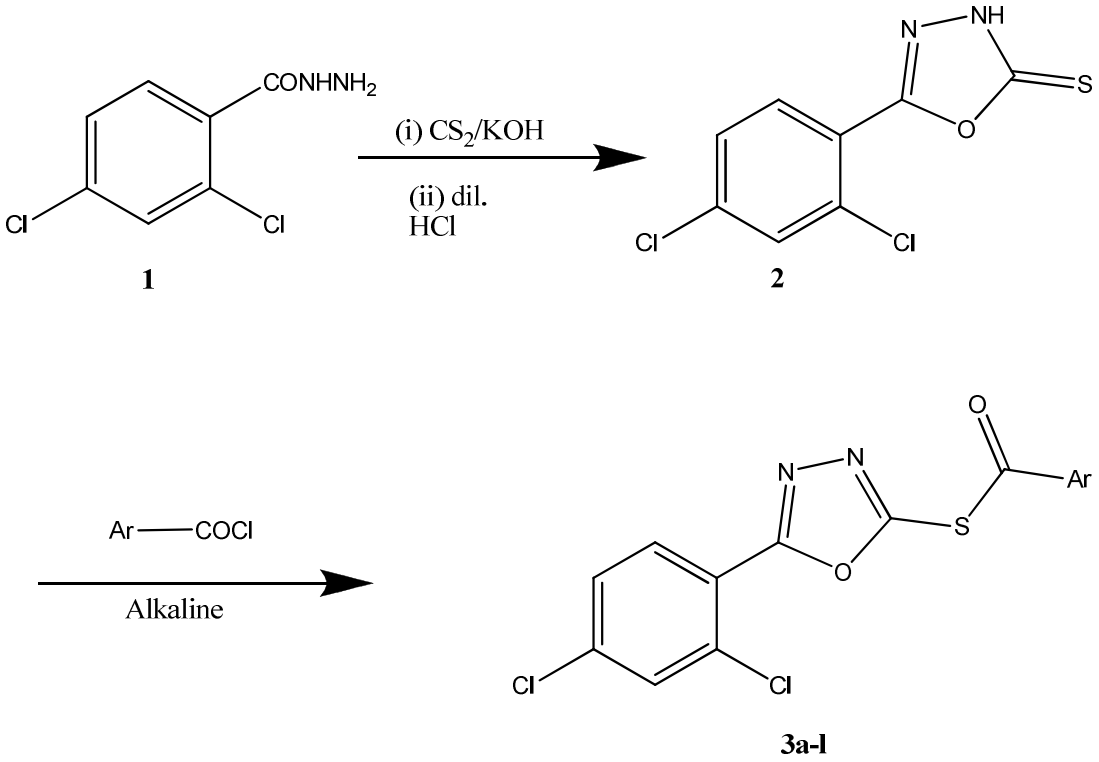
Chemistry

The syntheses of 5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl substituted benzothioates**3** were achieved following the steps outlined in Scheme **1**. Reaction of 2, 4-dichlorobenzoic acid hydrazide**1** with methanolic potassium hydroxide and carbon disulfide and then acidification with dilute hydrochloric acid afforded the corresponding 5-(2, 4-dichloro phenyl)-1, 3, 4-oxadiazole-2-thione **2**. The intermediate **2** on reaction with aroyl chlorides under the Schotten-Baumann reaction conditions furnished the title compounds **3** in good yield.

Infrared spectra of each compound showed a peak for thioester group in the range of 1680-1685 cm^{-1}. Ring stretching vibrations of 1, 3, 4-oxadiazole nucleus were observed at about 1650, 1620, 1565, 1420 cm^{-1}. The C–O stretching vibrations of 1, 3, 4-oxadiazole nucleus was also observed at about 1060cm^{-1}. The absorption for aromatic C–H bending vibrations was observed below 900 cm^{-1}. In case of ^{1}H NMR, the chemical shift value for methyl and methoxy groups were observed at 2.65 and 3.85 δ (ppm) respectively and appeared as singlet (s). Aromatic protons appeared as multiplet (m) in the assigned value of 7.24-7.38 δ (ppm).

Minimum Inhibitory Concentration (MIC)

The reference standard ciprofloxacin inhibited Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* at a MIC of 0.01 μg ml^{-1} and 0.25 μgml^{-1}, respectively whereas against Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* MIC was found to be 0.15 μgml^{-1} and 0.12 μgml^{-1}, respectively. All the synthesized compounds **3a-l** showed significant antibacterial activity against *P. aeruginosa* (MIC 0.45–0.65 μgml^{-1}), *S. aureus* (MIC 0.40–0.55 μg ml^{-1}) and *B. subtilis* (MIC 0.45–0.65 μgml^{-1}) whereas moderate antibacterial activity was found against *E. coli* (MIC 0.40–0.60 μgml^{-1}) as compared to the standard drug ciprofloxacin (Table 1). Compounds containing 4-chloro and 4-bromo moiety (**3i** and **3l**) were 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 [18-20].



Compound	Ar	Compound	Ar
3a	C_6H_5	**3b**	2-CH_3C6H4
3c	3-CH_3C6H4	**3d**	4-CH_3C6H4
3e	2-OCH_3C6H4	**3f**	2-OCH_3C6H4
3g	2-Cl6H4	**3h**	3-ClC6H4
3i	4-ClC6H4	**3j**	2-BrC6H4
3k	3-BrC6H4	**3l**	2-BrC6H4

Scheme 1: Synthesis of 5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl substituted benzothioates

CONCLUSION

Present study describes a straightforward synthesis of some 5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl substituted benzothioates. The structures of the synthesized compounds were ascertained by the modern analytical techniques. The title compounds were evaluated for *in vitro* antibacterial activity against two Gram negative strains, *i.e., Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453), and two Gram positive strains, *i.e., Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 96). 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 screened for antibacterial activity to explore the possibility of 5-(2, 4-dichlorophenyl)-1, 3, 4-oxadiazol-2-yl substituted benzothioates as a novel series of antibacterial drugs.

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