J. Iran. Chem. Soc., Vol. 6, No. 1, March 2009, pp. 77-81.

JOURNAL OF THE
Iranian
Chemical Society

Syntheses and Antibacterial Studies of Some 2-[5-(Aryl)-[1,3,4]oxadiazole-2-ylsulfanyl] alkanoic Acids

N. Jain[a,*], D.P. Pathak[b], P. Mishra[c] and S. Jain[d]

[a]*JCDM College of Pharmacy, Sirsa-125 055 (Haryana), India*

[b]*Delhi Institute of Pharmaceutical Science and Research, New Delhi, India*

[c]*Department of Pharmaceutical Sciences, Dr. H.S. Gour Vishwavidyalaya Sagar-470 003 (M.P.)*

[d]*Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology,Hisar-125 001 (Haryana), India*

(Received 15 February 2008, Accepted 9 March 2008)

Some new 2-[5-(aryl)-[1,3,4]oxadiazole-2-ylsulfanyl]alkanoic acids were synthesized and studied for their antibacterial activity. These compounds were prepared from aromatic carboxylic acid hydrazides. Aromatic carboxylic acid hydrazides **1** on refluxing with carbon disulfide and methanolic potassium hydroxide and then on subsequent acidification with hydrochloric acid furnish 5-aryl-1,3,4-oxadiazole-2-thiones **2**. 2-Chloro alkanoic acids react with **2** in alkaline media and on acidification yield the title compounds **3**. These compounds were characterised by CHN analyses, IR, mass and ^{1}H NMR spectral data. 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: Synthesis, Antibacterial activity, 1,3,4-Oxadiazole derivatives, Minimum inhibitory concentration

INTRODUCTION

1,3,4-Oxadiazole derivatives constitute an important class of heterocycles possessing diverse biological activities like antibacterial [1-4], fungitoxic [5,6], insecticidal [7], herbicidal [8], anticancer [9], anti-inflammatory [10] *etc.* Further, [5-(aryl)-1,3,4-thiadiazole-2-ylthio]acetates and propionates have been found to possess antimycobacterial activity [11-14]. These reports including our earlier work [15,16] prompted us to undertake the synthesis of some 2-[5-(aryl)-1,3,4-oxadiazole-2-ylsulfanyl]alkanoic acids. These compounds were evaluated for their *in vitro* antibacterial activity.

*Corresponding author. E-mail: drnjain2208@yahoo.co.in

EXPERIMENTAL

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. Elemental analyses were done using Carlo Erba 1106 CHN Analyzer. 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 tetramethyl silane as internal standard. Mass spectra (negative ion peak) were recorded on API-4000 Mass Spectrometer.

Aromatic carboxylic acid hydrazides **1a-d** were prepared by the reaction of hydrazine hydrate with the corresponding methyl esters of aromatic carboxylic acids as described in the literature[17]. Similarly, 5-aryl-1,3,4-oxadiazole-2-thiones **2a-d** were synthesized according to the method reported earlier [18].

General Procedure for the Synthesis of 2-[5-(Aryl)-[1,3,4]oxadiazole-2-ylsulfanyl]alkanoic Acids (3a-h)

5-Aryl-1,3,4-oxadiazole-2-thione (**2a-d**, 0.01 M) was dissolved in sodium hydroxide solution (10%, 10 ml). This solution was added drop-wise into a solution of 2-chloro alkanoic acid (2-chloro ethanoic acid/2-chloro propanoic acid, 0.015 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-(4-Chlorophenyl)-[1,3,4]oxadiazole-2-ylsulfanyl] propionic acid (3a). Yield: 86%; m.p.:120-122 ºC; IR (KBr, cm^{-1}): 3300-2400 broad band (O-H), 1732 (C=O), 1609 (C=N-N=C), 1225,1070 (C-O-C), 832 (*p*-disubstituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.37 (m, 4H, ArH), 4.46-4.53 (q, *J* = 7.2 Hz, 1H, CH), 1.75-1.78 (d, *J* = 7.2 Hz, 3H, CH_3); MS, m/z (%): 283 [M-H]-(100%), 285 [M+2-H]- (35%). Anal.: Calcd. for $C_{11}H_9N_2O_3SCl$: C, 46.40; H, 3.19; N, 9.84. Found: C, 46.46; H, 3.23; N, 9.78.

2-[5-(4-Chlorophenyl)-[1,3,4]oxadiazole-2-ylsulfanyl] acetic acid (3b). 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), 1265, 1069 (C-O-C), 833 (*p*-disubstituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.37 (m, 4H, ArH), 4.12 (s, 2H, CH_2); MS, m/z (%): 269 [M-H]-(100%), 271 [M+2-H]- (35%). Anal.: Calcd. for $C_{10}H_7N_2O_3SCl$: C, 44.37; H, 2.61; N, 10.35. Found: C, 44.33; H, 2.65; N, 10.31.

2-[5-(3-Chlorophenyl)-[1,3,4]oxadiazole-2-ylsulfanyl] propionic acid (3c). Yield: 85%; m.p.: 126-128 ºC; IR (KBr, cm^{-1}): 3200-2400 broad band (O-H), 1734 (C=O), 1620 (C=N-N=C), 1256, 1072 (C-O-C), 773, 881 (*m*-disubstituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.34 (m, 4H, ArH), 4.47-4.54 (q, *J* = 7.2 Hz, 1H, CH), 1.76-1.78 (d, *J* = 7.2 Hz, 3H, CH_3). MS, m/z (%): 283 [M-H]-(100%), 285 [M+2-H]-, (35%). Anal.: Calcd. for $C_{11}H_9N_2O_3SCl$: C, 46.40; H, 3.19; N, 9.84. Found: C, 46.35; H, 3.14; N, 9.80.

2-[5-(3-Chlorophenyl)-[1,3,4]oxadiazole-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, 883 (*m*-disubstituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.34 (m, 4H, ArH), 4.12 (s, 2H, CH_2). MS, m/z (%): 269 [M-H]- (100%), 271 [M+2-H]- (35%). Anal.: Calcd. for $C_{10}H_7N_2O_3SCl$: C, 44.37; H, 2.61; N, 10.35. Found: C, 44.42; H, 2.62; N, 10.41.

2-[5-(2-Chlorophenyl)-[1,3,4]oxadiazole-2-ylsulfanyl] propionic acid (3e). Yield: 83%; m.p.: 150-152 ºC; IR (KBr, cm^{-1}): 3200-2400 broad band (O-H), 1732 (C=O), 1601 (C=N-N=C), 1256, 1036 (C-O-C), 756 (*o*-disubstituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 11.75 (s, 1H, COOH), 7.27-7.37 (m, 4H, ArH), 4.47-4.54 (q, *J* = 7.2 Hz, 1H, CH), 1.76-1.78 (d, *J* = 7.2 Hz, 3H, CH_3). MS, m/z (%): 283 [M-H]- (100%), 285 [M+2-H]- (35%). Anal.: Calcd. for $C_{11}H_9N_2O_3SCl$: C, 46.40; H, 3.19; N, 9.84. Found: C, 46.44; H, 3.25; N, 9.89.

2-[5-(2-Chlorophenyl)-[1,3,4]oxadiazole-2-ylsulfanyl] acetic acid (3f). Yield: 86%; m.p.: 160-162 ºC; IR (KBr, cm^{-1}): 3200-2400 broad band (O-H), 1732 (C=O), 1601 (C=N-N=C), 1256, 1036 (C-O-C), 756 (*o-di*substituted benzene); ^{1}H NMR ($CDCl_3$): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.37 (m, 4H, ArH), 4.12 (s, 2H, CH_2). MS, m/z (%): 269 [M-H]-(100%), 271 [M+2-H]- (35%). Anal.: Calcd. for $C_{10}H_7N_2O_3SCl$: C, 44.37; H, 2.61; N, 10.35. Found: C, 44.31; H, 2.68; N, 10.39.

2-[5-(2,4-Dichlorophenyl)-[1,3,4]oxadiazole-2-ylsulfanyl]propionic acid (3g). Yield: 86%; m.p.: 161-163 ºC; IR (KBr, cm^{-1}): 3300-2400 broad band (O-H), 1732 (C=O), 1608 (C=N-N=C), 1224, 1070 (C-O-C), 834 (*p*-disubstituted benzene), ^{1}H NMR ($CDCl_3$): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.35 (m, 3H, ArH), 4.46-4.53 (q, *J* = 7.2 Hz, 1H, CH), 1.75-1.78 (d, *J* = 7.2 Hz, 3H, CH_3); MS, m/z (%): 317 [M-H]- (100%), 319 [M+2-H]-(70%), 321 [M+4-H]-(11%). Anal.: Calcd. for C, 41.40; H, 2.53; N, 8.78. Found: C, 41.35; H, 2.57; N, 8.72.

2-[5-(2,4-Dichlorophenyl)-[1,3,4]oxadiazole-2-ylsulfanyl]

acetic acid (3h). 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); ^{1}H NMR ($CDCl_3$): δ (ppm) 11.72 (s, 1H, COOH), 7.27-7.35 (m, 3H, ArH), 4.11 (s, 2H, CH_2); MS, m/z (%): 303 [M-H]- (100%), 305 [M+2-H]- (70%), 307 (M+4-H)- (11%). Anal.: Calcd. for C, 39.36; H, 1.98; N, 9.18. Found: C, 39.31; H, 1.92; N, 9.23.

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 [19]. 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 (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.

RESULTS AND DISCUSSION

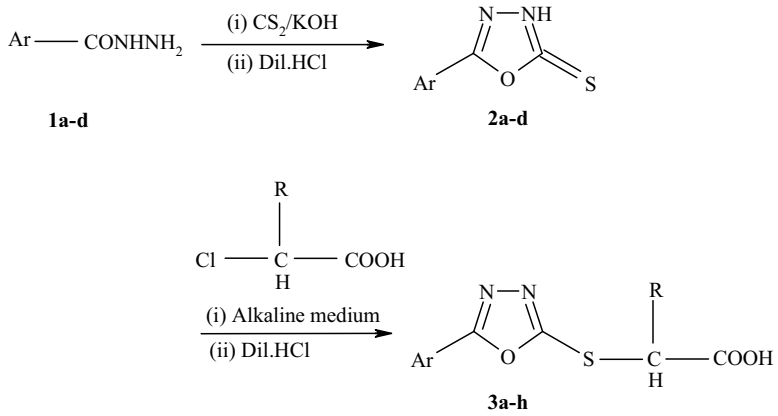
Chemistry

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

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 1075-1020 cm^{-1}. Similarly, the grouping C=N-N=C of 1,3,4-oxadiazole nucleus also showed the IR absorption in

Table 1. *In Vitro* Antibacterial Activity of the Title Compounds (**3a-h**)

Compound	Minimum inhibitory concentration (μg ml^{-1})			
	E. coli (MTCC 40)	*P. aeruginosa* (MTCC 2453)	*S. aureus* (MTCC 121)	*B. subtilis* (MTCC 96)
3a	0.40	0.45	0.40	0.40
3b	0.40	0.45	0.40	0.40
3c	0.40	0.55	0.40	0.45
3d	0.40	0.55	0.40	0.45
3e	0.45	0.60	0.45	0.55
3f	0.45	0.60	0.45	0.55
3g	0.35	0.40	0.35	0.35
3h	0.35	0.40	0.35	0.35
Ciprofloxacin (Standard drug)	0.01	0.25	0.15	0.12



	Ar		Ar	R
2a	4-ClC_6H_4	**3a**	4-ClC_6H_4	CH_3
2b	3-ClC_6H_4	**3b**	4-ClC_6H_4	H
2c	2-ClC_6H_4	**3c**	3-ClC_6H_4	CH_3
2d	2,4-ClC_6H_3	**3d**	3-ClC_6H_4	H
		3e	2-ClC_6H_4	CH_3
		3f	2-ClC_6H_4	H
		3g	2,4-ClC_6H_3	CH_3
		3h	2,4-ClC_6H_3	H

Scheme 1

the assigned range of 1670-1600 cm^{-1}. In case of ^1H NMR, the chemical shift value for carboxyl group was observed in the range of 12.41-11.72 δ (ppm) and appeared as singlet (s). Aromatic protons appeared as multiplet (m) in the assigned value of 6.99-7.67 δ (ppm). Methylene protons appeared as singlet at δ (ppm) 4.12 whereas methine and aliphatic methyl protons absorbed at δ (ppm) 4.47-4.54 and δ (ppm) 1.76-1.78, respectively. Both of them also showed splitting of signals and appeared as quartet (q) and doublet (d), respectively. The coupling constant, *J*, was found to be 7.2 Hz for them. All the title compounds showed [M-H]- of 100% intensity as the molecular ion peak along with isotopic peak at [M+2-H]- of about 35% intensity of the parent ion peak due to the presence of chlorine and sulfur atoms. The dichloro compounds showed [M+4-H]- peak in addition to [M+2-H]- due to presence of two atoms of chlorine. The results of elemental analyses were found in good agreement with the calculated values.

Minimum Inhibitory Concentration (MIC)

The reference standard ciprofloxacin inhibited Gram negative bacteria *E. coli* and *P. aeruginosa* at a MIC of 0.01 μg ml^{-1} and 0.25 μg ml^{-1}, respectively whereas against Gram positive bacteria *S. aureus* and *Bacillus subtilis* MIC was found to be 0.15 μg ml^{-1} and 0.12 μg ml^{-1}, respectively. All the synthesized compounds **3a-h** showed significant antibacterial activity against *P. aeruginosa* (MIC 0.40-0.60 μg ml^{-1}), *S. aureus* (MIC 0.35-0.45 μg ml^{-1}) and *B. subtilis* (MIC 0.35-0.55 μg ml^{-1}) whereas moderate antibacterial activity was found against *E. coli* (MIC 0.35-0.45 μg ml^{-1}) as compared to the standard drug ciprofloxacin (Table 1). Compounds containing 2,4-dichloro moiety (**3g** and **3h**) 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 [20-22].

In conclusion, we have described a straightforward

synthesis of new 2-[5-(aryl)-[1,3,4]oxadiazole-2-ylsulfanyl] alkanoic acids and studied their *in vitro* antibacterial activity. Compounds **3g** and **3h** exhibited significant activity against all the bacterial strains used in this study.

ACKNOWLEDGEMENTS

The authors are thankful to Chairman, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar (Haryana) for providing necessary facilities to carry out this work. Our sincere thanks to Chairman, Department of Applied Chemistry, G.J.U.S.&T., Hisar for IR spectra. Thanks are also due to RSIC, CDRI, Lucknow, for providing C, H, N analyses and ^{1}H NMR spectra. The Director, IMTECH, Chandigarh is also duly acknowledged for providing bacterial strains.

REFERENCES

[1] M.G. Mamolo, D. Zampieri, L. Vio, M. Fermeglia, M. Ferrone, S. Pricl, G. Scialino, E. Banfi, Bioorg. Med. Chem.13 (2005) 3797.

[2] Y.A. Al-Soud, N.A. Al-masoudi, J. Braz. Chem. Soc. 14 (2003) 790.

[3] S. Jain, N. Jain, P. Mishra, Indian J. Heterocyclic Chem. 14 (2005) 359.

[4] H. El-Masry, H.H. Fahmy, S.H. Ali Abdel Wahed, Molecules. 5 (2000) 1429.

[5] X.J. Zou, L.H. Lai, G.Y. Jin, Z.X. Zhang, J. Agric Food Chem. 50 (2002) 3757.

[6] A. Shehata, Saudi Pharmaceutical Journal. 11 (2003) 87.

[7] S. Holla, C.S. Prasanna, B. Poojary, K.S. Rao, K. Shridhara, U.G. Bhat, Indian J. Chem. 43 (2004) 864.

[8] M.A. Shaban, A.Z. Nasr, S.M. El-badry, J. Islamic Acad. Sci. 4 (1991) 184.

[9] S. Aboraia, H.M. Abdel-Rahman, N.M. Mahfouz, M.A. El-Gendy, Bioorg. Med. Chem.14 (2006) 1236.

[10] M.M. Burbuliene, V. Jakubkiene, G. Mekuskiene, E. Udrenaite, R. Smicius, P. Vainilavicius, Farmaco. 59 (2004) 767.

[11] A. Foroumadi, Z. Kargar, A. Sakhteman, Z. Sharifzadeh, R. Feyzmohammadi, M. Kazemi, A. Shafiee, Bioorg. Med. Chem. Lett. 16 (2006) 1164.

[12] A. Foroumadi, F. Soltani, H. Moallemzadeh-Haghighia, A. Shafiee, Arch. Pharm. Chem. Life Sci. 338 (2005) 112.

[13] A. Foroumadi, Z. Kiani, F. Soltani, Farmaco 58 (2003) 1073.

[14] A. Foroumadi, M. Mirzaei, A. Shafiee, Pharmazie 56 (2001) 610.

[15] D.P. Pathak, N. Jain, P. Mishra, S. Jain, Indian J. Heterocyclic Chem.14 (2005) 373.

[16] D.P. Pathak, N. Jain, P. Mishra, S. Jain, Indian J. Heterocyclic Chem. 15 (2005) 177.

[17] H.L. Yale, K. Loose, J. Martins, M. Holsing, F.M. Perry, J. Bernstein, J. Am. Chem. Soc. 75 (1953) 1933.

[18] S. Jain, P. Mishra., Indian J. Heterocyclic Chem. 13 (2004) 307.

[19] J.G. Cappucino, N. Sherman, Microbiology- A Laboratory Manual, Addison Weslay Longman, Inc., Harlow, 1999.

[20] Bauernfeind, J. Antimicrob. Chemother. 40 (1997) 639.

[21] A.A. Hoogkamp-Korstanje, J. Antimicrob. Chemother. 40 (1997) 427.

[22] D.J. Weber, S.M. Saviteer, W.A. Rutala, C.A. Thomann, Antimicrob. Agents Chemother. 32 (1988) 642.