Turk J Chem
32 (2008) , 147 – 155.

Synthesis and Antimicrobial Activities of 1,2,4-Oxadiazin-5-one, 6-one and 5-Thiones

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Received 14.06.2007

N-Substituted pyridine carboxamide oximes **(2)** were obtained from the reactions of pyridine hydroxamic acid chloride hydrochlorides **(1)** with primary amines. The reactions of carboxamide oximes with chloroacetyl chloride in the presence of triethylamine gave the corresponding 3,4-disubstituted-1,2,4-oxadiazin-5-ones **(3)**, which on treatment with P_2S_5 gave in moderate yielded the corresponding 3,4-disubstituted-1,2,4-oxadiazin-5-thiones **(4)**. The reaction of pyridine carboxamide oximes with α-aminoacid ester led to the formation of 3,5-disubstituted-1,2,4-oxadiazin-6-ones **(5)** in moderate yields. The structures of the prepared compound were evaluated by spectroscopy. Some of the representatives of 3,4-disubstituted-1,2,4-oxadiazin-5-ones, thiones, and 3,5-disubstituted-1,2,4-oxadiazin-6-ones were screened for antibacterial activity using disc diffusion. It was found that all the tested compounds have good antimicrobial activities.

Key Words: Amidoxime, oxadiazine, antimicrobial activity.

Introduction

The amidoximes are useful starting materials in the synthesis of many heterocyclic compounds.[1,2] Pyridine amidoximes were reported to give oxadiazoles and oxadiazin-5-ones on treatment with alkyl chloroformates and chloroacetyl chloride.[3,4] The above mentioned heterocyclics have a diversity of biological activities.[5−8]

Here we report the synthesis of a series of 3,4-disubstituted-1,2,4-oxadiazin-5-ones, 3,4-disubstituted-1,2,4-oxadiazin-5-thiones, and 3,5-disubstituted-1,2,4-oxadiazin-6-ones and their antibacterial activities against the organisms *Mycobacterium smegmatis* CCM 2067, *Staphylacoccus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 15313, *Escherichia coli* ATCC 11230, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, and the yeast cultures *Candida albicans* ATCC 10231, *Kluyveromyces fragilis*

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NRRL 2415, *Rhodotorula rubra* DSM 70403, *Debaryomyces hanseni* DSM 70238, and *Hanseniaspora guilliermondii* DSM 3432.

Experimental

Chemistry

Melting points were obtained on an Electrothermal Digital melting point apparatus. The ^{1}H-NMR spectra were recorded on Bruker-gmbh DPX-400 (400 MHz) in $CDCl_3$ and DMSO-d_6 using TMS as internal standard. The ^{13}C-NMR spectra were recorded on a Varian Mercury Plus 400 MHz spectrometer. The IR spectra were measured on a MATTSON 1000 FTIR spectrophotometer. Elemental analyses were performed at an instrumental analysis laboratory (TÜBİTAK, Ankara).

General procedure for N-substituted pyridinecarboxamide oximes[9] (2a-g). Pyridine hydroxamic acid chloride hydrochlorides[9] **1** (3.088 g, 16 mmol) in dry methanol (40 mL) were added dropwise to a solution of aniline derivative (5.912 g, 48 mmol) in ethanol (40 mL). The reaction mixture was refluxed for 3 h and the solvent was evaporated at 40 °C under reduced pressure. The residue was treated with hot chloroform (3 × 15 mL). The solvent was evaporated under reduced pressure and the residue crystallised from an appropriate solvent.

N-(4-Methoxyphenyl) pyridine-2-carboxamide oxime (2a). Compound was crystallised from ethanol; yield 64%; mp 186-188 °C; IR (KBr) $\upsilon_{NH,OH}$ 3367, 3100, and 3067; $\upsilon_{C=N}$ 1633, 1592, and 1567 cm^{-1}; ^{1}H-NMR $CDCl_3\delta$ 3.74 (s, 3H), 6.70-6.81 (m, 4H), 7.26 (m, 1H), 7.37(s, broad, 1H), 7.66 (m, 2H), 8.54 (m, 1H). MS m/z 243 (M^+)

N-(3,4-Dimethoxybenzyl) pyridine-2-carboxamide oxime (2b). Compound was crystallised from benzene-petroleum ether (40-60^o)(1:2); yield 59%; mp 107-108 °C; IR (KBr) $\upsilon_{NH,OH}$ 3358, 3283, and 3167, $\upsilon_{C=N}$ 1667 and 1583 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.80 (s, 3H), 3.82 (s, 3H), 4.58 (d, 2H, J=4.37), 5.99 (s, broad, 1H), 6.75-6.83 (m, 3H), 7.26 (m, 1H), 7.62-7.69 (m, 2H), 8.60 (m, 1H). MS m/z 287 (M^+).

N-(1-Adamantyl) pyridine-2-carboxamide oxime (2c). Compound was crystallised from benzene-petroleum ether (40-60^o)(1:2); yield 39%; mp 197-198 °C; IR (KBr) $\upsilon_{NH,OH}$ 3350, 3150, 3100, and 3067, $\upsilon_{C=N}$ 1617, 1600, and 1567 cm^{-1} ; ^{1}H-NMR ($CDCl_3$)δ 1.50-1.76 (m, 12H), 1.98 (s, broad, 3H), 5.36 (s, 1H,), 7.26-7.35 (m, 1H), 7.60-7.71 (m, 2H), 8.61(m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$)δ 29.7; 36.0; 44.4; 53.0; 123.8; 124.0; 136.4; 148.7; 153.1; 154.0. MS m/z 271 (M^+).

N-Benzylpyridine-4-carboxamide oxime (2d). Compound was crystallised from benzene; yield 68%; mp 132-133 °C; IR (KBr) $\upsilon_{NH,OH}$ 3233 and 3025, $\upsilon_{C=N}$ 1633 and 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 4.21 (d, 2H, J=5.71), 5.76 (s, broad, 1H), 7.15-7.37 (m, 7H), 8.63 (m, 2H); ^{1}H-NMR (DMSO-d_6) δ 4.16 (d, 2H, J=7.2), 6.44-6.47 (t, 1H), 7.07-7.31 (m, 7H), 8.53 (m, 2H), 10.2 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$)δ 47.5; 122.9; 126.6; 127.5; 128.8; 138.9; 139.3; 150.0; 154.5. MS m/z 227 (M^+).

N-(4-Methoxyphenyl) pyridine-4-carboxamide oxime (2e). Compound was crystallised from ethanol; yield 40%; mp 212-213 °C; IR (KBr) $\upsilon_{NH,OH}$ 3350, 3117, and 3050, $\upsilon_{C=N}$ 1642 and 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 3.73 (s, 3H), 6.70 (s, 4H), 7.15 (s, 1H), 7.26-7.30 (m, 2H), 8.54 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$)δ 55.4; 114.3; 122.7; 124.3; 132.1; 139.4; 149.9; 151.0; 156.4. MS m/z 243 (M^+).

N-(3,4-Dimethoxybenzyl) pyridine-4-carboxamide oxime (2f). Compound was crystallised from ethanol; yield 66%; mp 173-174 °C; IR (KBr) $\upsilon_{NH,OH}$ 3350, 3117, and 3050, $\upsilon_{C=N}$ 1642 and 1600

cm^{-1}; ^{1}H-NMR ($CDCl_3$-DMSO-d_6)δ 3.81 (s, 3H), 3.83 (s, 3H), 4.16 (d, 2H, J=6.90), 5.81 (t, 1H), 6.70-6.81 (m, 3H), 7.38 (m, 2H), 8.59 (m, 2H), 10.11 (s, 1H). MS m/z 288 (M^++1).

N-(1-Adamantyl) pyridine-4-carboxamide oxime (2g). Compound was crystallised from ethanol; yield 52%; mp 214-215 °C; IR (KBr) $\upsilon_{NH,OH}$ 3350, 3150, 3100, and 3067, $\upsilon_{C=N}$ 1617, 1600, and 1567 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 1.47-1.65 (m, 12H), 1.98 (s, broad, 3H), 5.04 (s, 1H), 7.47 (m, 2H), 8.65 (m, 2H). MS m/z 271 (M^+).

General Procedure for 3,4-disubstituted-1,2,4-oxadiazin-5-ones[4] (3a-k). A solution of chloroacetylchloride (0.21 g, 1.88 mol) in chloroform (3 mL) was added dropwise to an ice-cooled solution of N-substituted pyridinecarboxamide oxime **2** (0.40 g, 1.88 mmol) and triethylamine (0.38 g, 3.76 mmol) in chloroform (10 mL). The reaction mixture was stirred for 4 days at room temperature. Solvent was evaporated under reduced pressure. The remaining solid material was treated with ether. The solvent was evaporated and the crude product was crystallised from ether-petroleum ether (40-60 °C) (1:2) solvent mixture.

3-(2-Pyridyl)-4-phenyl-4H-1,2,4-oxadiazin-5(6H)-one (3a). Yield 48%; mp 160 °C; IR (KBr) $\upsilon_{C=O}$ 1717, $\upsilon_{C=N}$ 1583 and 1567 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 4.69 (s, 2H), 7.16-7.27 (m, 6H), 7.60-7.80 (m, 2H), 8.33 (m, 1H). MS m/z 254 (M^++1).

3-(2-Pyridyl)-4-benzyl-4H-1,2,4-oxadiazin-5(6H)-one (3b). Yield 59%; mp 77 °C; IR (KBr) $\upsilon_{C=O}$ 1717, $\upsilon_{C=N}$ 1600, 1575, and 1558 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 4.57 (s, 2H), 5.18 (s, 2H), 6.83 (m, 2H), 7.15 (m, 3H), 7.36-7.41 (m, 2H), 7.65 (m, 1H), 8.70 (m, 1H);^{13}C-NMR (100 MHz, $CDCl_3$)δ 44.7; 67.9; 124.9; 125.2; 127.2; 127.6; 128.6; 135.9; 137.2; 148.4; 149.0; 154.2; 164.5. MS m/z 268 (M^++1).

3-(2-Pyridyl)-4-(p-tolyl)-4H-1,2,4-oxadiazin-5(6H)-one (3c). Yield 45%; mp 115 °C; IR (KBr) $\upsilon_{C=O}$ 1717, $\upsilon_{C=N}$ 1667, 1617, and 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 2.25 (s, 3H), 4.67 (s, 2H), 6.70 (m, 2H), 6.95-7.04 (m, 3H), 7.67 (m, 2H), 8.34-8.55 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$)δ 55.3; 68.6; 113.9; 124.5; 124.6; 127.4; 129.2; 136.8; 148.4; 149.2; 154.2; 158.9; 164.0. MS m/z 268 (M^++1).

3-(2-Pyridyl)-4-(4-methoxyphenyl)-4H-1,2,4-oxadiazin-5(6H)-one (3d). Compound was purified by preparative TLC using Silica Gel HF_{254} as adsorbent and chloroform:n-hexane:methanol:acetone (9:8:2:1) solvent mixture as an eluent; R_f = 0.59; yield 27%; mp 111-112 °C; IR (KBr) $\upsilon_{C=O}$ 1725, $\upsilon_{C=N}$ 1617, 1592, and 1567 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 3.71 (s, 3H), 4.66 (s, 2H), 6.74 (m, 2H), 7.07 (m, 2H), 7.11-7.21 (m, 1H), 7.65 (m, 2H), 8.32-8.38 (m, 1H). MS m/z 283 (M^+).

3-(2-Pyridyl)-4-(3,4-dimethoxybenzyl)-4H-1,2,4-oxadiazin-5(6H)-one (3e). Yield 61%; mp 117 °C; IR (KBr) $\upsilon_{C=O}$ 1717, $\upsilon_{C=N}$ 1600, 1592, and 1567 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.71 (s, 3H), 3.79 (s, 3H), 4.55 (s, 2H), 5.11 (s, 2H), 6.31 (m, 2H), 6.61 (d, 1H, J=8.43), 7.36-7.68 (m, 3H), 8.70 (m, 1H). MS m/z 327 (M^+).

3-(3-Pyridyl)-4-(p-tolyl)-4H-1,2,4-oxadiazin-5(6H)-one (3f). Yield 35%; mp 134 °C; IR (KBr) $\upsilon_{C=O}$ 1717, $\upsilon_{C=N}$ 1600 and 1550 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 2.29 (s, 3H), 4.68 (s, 2H), 7.00-7.19 (m, 5H), 7.69 (m, 1H), 8.52 (m, 1H), 8.63 (d, 1H, J=1.97). MS m/z 267 (M^+).

3-(4-Pyridyl)-4-phenyl-4H-1,2,4-oxadiazin-5(6H)-one (3g). Yield 61%; mp 131 °C; IR (KBr) $\upsilon_{C=O}$ 1733, $\upsilon_{C=N}$ 1600, 1542, and 1567 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 4.68 (s, 2H), 7.13 (m, 2H), 7.25-7.32 (m, 5H), 8.52 (m, 2H). MS m/z 253 (M^+).

3-(4-Pyridyl)-4-benzyl-4H-1,2,4-oxadiazin-5(6H)-one (3h). Yield 64%; mp 97 °C; IR (KBr) $\upsilon_{C=O}$ 1717, $\upsilon_{C=N}$ 1600 and 1550 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 4.58 (s, 2H), 4.86 (s, 2H), 6.87 (m, 2H), 7.21-7.27 (m, 5H), 8.68 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$)δ 45.8; 68.0; 122.7; 127.0; 128.0; 128.9; 134.9; 136.6;

150.4; 154.0; 164.3. MS m/z 267 (M^+).

3-(4-Pyridyl)-4-(p-tolyl)-4H-1,2,4-oxadiazin-5(6H)-one (3i). Yield 42%; mp 162 °C; IR (KBr) $\upsilon_{C=O}$ 1725, $\upsilon_{C=N}$ 1600 and 1550 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 2.30 (s, 3H), 4.67 (s, 2H), 7.02-7.27 (m, 6H), 8.49-8.63 (m, 2H). MS m/z 267 (M^+).

3-(4-Pyridyl)-4-(4-methoxyphenyl)-4H-1,2,4-oxadiazin-5(6H)-one (3j). Yield 26%; mp 138 °C; IR (KBr) $\upsilon_{C=O}$ 1717, $\upsilon_{C=N}$ 1617 and 1550 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 3.75 (s, 3H), 4.66 (s, 2H), 6.80 (m, 2H), 7.03 (m, 2H), 7.25 (m, 2H), 8.52 (m, 2H). MS m/z 283 (M^+).

3-(4-Pyridyl)-4-(3,4-dimethoxybenzyl)-4H-1,2,4-oxadiazin-5(6H)-one (3k). Yield 50%; mp 137 °C; IR (KBr) $\upsilon_{C=O}$ 1716, $\upsilon_{C=N}$ 1600, 1542, and 1516 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.73 (s, 3H), 3.83 (s, 3H), 4.56 (s, 2H), 4.80 (s, 2H), 6.35 (m, 2H), 6.68 (m, 1H), 7.23 (m, 2H), 8.70 (m, 2H). MS m/z 327 (M^+)

3-Methyl-4H-1,2,4-oxadiazin-5(6H)-one (3l). Compound was reported in literature previously.[4]

General procedure for 3,4-disubstituted-1,2,4-oxadiazin-5(6H)-thiones[4] (4a-g). Compound **3** (0.1646 g, 65 mmol) was heated with excess of P_2S_5 (0.6 g, 0.27 mmol) in xylene (15 mL) for 9 h. The hot reaction mixture was filtered and xylene was evaporated under reduced pressure. The purification was performed by preparative TLC using Silica Gel HF_{254} as adsorbent and chloroform:n-hexane:methanol:acetone (9:8:2:1) solvent mixture as an eluent.

3-(2-Pyridyl)-4-phenyl-4H-1,2,4-oxadiazin-5(6H)-thione (4a). Compound was crystallised from petroleum ether; R_f = 0.74; yield 46%; mp 176-177 °C; IR (KBr) $\upsilon_{C=N}$ 1583 and 1567, $\upsilon_{C=S}$ 1433, 1283, and 1117 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.92 (s, 2H), 7.13-7.26 (m, 6H), 7.63 (m, 2H), 8.34 (m, 1H). MS m/z 269 (M^+).

3-(2-Pyridyl)-4-benzyl-4H-1,2,4-oxadiazin-5(6H)-thione (4b). R_f = 0.85; yield 69%; oil; IR (neat) $\upsilon_{C=N}$ 1583 and 1558, $\upsilon_{C=S}$ 1433, 1267, and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.81 (s, 2H), 5.71 (s, 2H), 6.82 (m, 2H), 7.13-7.31 (m, 5H), 7.58-7.63 (m, 1H), 8.68 (m, 1H). MS m/z 284 (M^++1).

3-(2-Pyridyl)-4-(p-tolyl)-4H-1,2,4-oxadiazin-5(6H)-thione (4c). Compound was crystallised from petroleum ether; R_f = 0.81; yield 52%; mp 138-139 °C; IR (KBr) $\upsilon_{C=N}$ 1583, 1575, 1567, $\upsilon_{C=S}$ 1433, 1283, 1117 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 2.27 (s, 3H), 4.93 (s, 2H), 7.07-7.10 (m, 5H), 7.64 (m, 2H), 8.38 (m, 1H). MS m/z 283 (M^+).

3-(2-Pyridyl)-4-(3,4-dimethoxybenzyl)-4H-1,2,4-oxadiazin-5(6H)-thione (4d). R_f = 0.45; yield 21%; oil; IR (neat) $\upsilon_{C=N}$ 1592 and 1567, $\upsilon_{C=S}$ 1450, 1267, and 1108 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 3.69 (s, 3H), 3.79 (s, 3H), 4.79 (s, 2H), 5.65 (s, 2H), 6.32 (m, 2H), 6.61 (m, 1H), 7.33-7.39 (m, 2H), 7.65 (m, 1H), 8.70 (m, 1H). MS m/z 343 (M^+).

3-(4-Pyridyl)-4-benzyl-4H-1,2,4-oxadiazin-5(6H)-thione (4e). Compound was crystallised from petroleum ether; R_f = 0.61; yield 59%; mp 110-111 °C; IR (KBr) $\upsilon_{C=N}$ 1608, 1592, and 1550, $\upsilon_{C=S}$ 1450, 1275, and 1125 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 4.82 (s, 2H), 5.41 (s, 2H), 6.87 (m, 2H), 7.19-7.27 (m, 5H), 8.68 (m, 2H). MS m/z 283 (M^+).

3-(4-Pyridyl)-4-(p-tolyl)-4H-1,2,4-oxadiazin-5(6H)-thione (4f). Compound was crystallised from petroleum ether; R_f = 0.48; yield 43%; mp 134-135 °C; IR (KBr) $\upsilon_{C=N}$ 1617, 1583, and 1542, $\upsilon_{C=S}$ 1408, 1283, and 1117 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 2.23 (s, 3H), 4.89 (s, 2H), 7.01-7.25 (m, 6H), 8.52 (m, 2H). MS m/z 283 (M^+).

3-(4-Pyridyl)-4-(3,4-dimethoxybenzyl)-4H-1,2,4-oxadiazin-5(6H)-thione (4g). R_f = 0.46; yield 18%; oil; IR (neat) $\upsilon_{C=N}$ 1608 and 1533, $\upsilon_{C=S}$ 1467, 1283, and 1133 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 3.72 (s, 3H), 3.83 (s, 3H), 4.79 (s, 2H), 5.35 (s, 2H), 6.35 (m, 2H), 6.68 (d, 1H, J=7.80), 7.20 (m, 2H), 8.69 (m,

2H). MS m/z 343 (M^+).

General procedure for 3,5-disubstituted-1,2,4-oxadiazin-6-ones[10](5a-b). A solution of the pyridine hydroxamic acid chloride hydrochloride (1.93 g, 10 mmol) and L-amino acid ester hydrochloride (2.76 g, 12 mmol) in absolute ethanol (40 mL) was dropwise added to stirred solution of triethylamine (3.54 g, 25 mmol) in ethanol (10 mL) at -15 °C. The temperature of the reaction mixture was then allowed to rise slowly to room temperature and stirring was continued for 2 h. The gelatinous precipitate of furoxan was removed by filtration, and the filtrate was concentrated in vacuo and then diluted with water (100 mL). The reaction mixture was extracted with chloroform (3 × 20 mL). The combined organic extracts were dried and the solvent removed under reduced pressure. The residue was crystallised from chloroform-petroleum ether (40-60 °C) (1:2) solvent mixture.

3-(2-Pyridyl)-5-benzyl-5H-1, 2, 4-oxadiazin-6-one (5a). Yield 40%; mp 127 °C; IR (KBr) υ_{NH} 3342, $\upsilon_{C=O}$ 1775 and 1750, $\upsilon_{C=N}$ 1633, 1583, and 1567 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 3.03 (dd, 1H, J=22.83, 9.59), 3.36 (dd, 1H, J=17.39, 3.64), 4.36 (m, 1H), 6.78 (s, broad, 1H), 7.20-7.44 (m, 6H), 7.79 (m, 1H), 8.05 (d, 1H, J=8.04), 8.52 (d, 1H, J=4.54). MS m/z 268 (M^++1).

3-(2-Pyridyl)-5-(izopropyl)-5H-1, 2, 4-oxadiazin-6-one (5b). Yield 47%; mp 150 °C; IR (KBr) υ_{NH} 3333, $\upsilon_{C=O}$ 1758, $\upsilon_{C=N}$ 1633, 1583, and 1567 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ 1.03 (d, 3H, J=6.79), 1.11 (d, 3H, J=6.97), 2.37 (m, 1H), 4.06 (dd, 1H, J=6.28, 1.69), 6.88 (s, broad, 1H), 7.43 (m, 1H), 7.81 (m, 1H), 8.08 (m, 1H), 8.60 (m, 1H). MS m/z 219 (M^+).

Antimicrobial test

Mycobacterium smegmatis CCM 2067, *Staphylacoccus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 15313, *Escherichia coli* ATCC 11230, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, *Kluyveromyces fragilis* NRRL 2415, *Rhodotorula rubra* DSM 70403, *Debaryomyces hanseni* DSM 70238, and *Hanseniaspora guilliermondii* DSM 3432 were used as bacteria and yeast cultures. The compounds were dissolved in DMSO to a final concentration of 30 μg/mL. Empty sterilised antibiotic discs having a diameter of 6 mm (Schleicher & Schull No 2668, Germany) were each impregnated with 20 μL of solution. All the above-mentioned bacteria were incubated at 30 ± 0.1 °C for 24 h by inoculation into Nutrient Broth (Difco), and the studied yeasts were incubated in Malt Extract Broth (Difco) for 48 h. An inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Mueller–Hinton Agar (Oxoid) plates (1 mL inoculum/plate). The discs injected with solutions were placed on the inoculated agar by pressing slightly and incubated at 35 °C (24 h) for the bacteria, and at 25 °C (72 h) for the yeasts.[11,12] At the end of the period, inhibition zones formed on the medium were evaluated in millimetres. Studies were performed in triplicate. On each plate, an appropriate reference antibiotic disc was applied, depending on the test micro-organism for comparison.

Results and Discussion

Chemistry

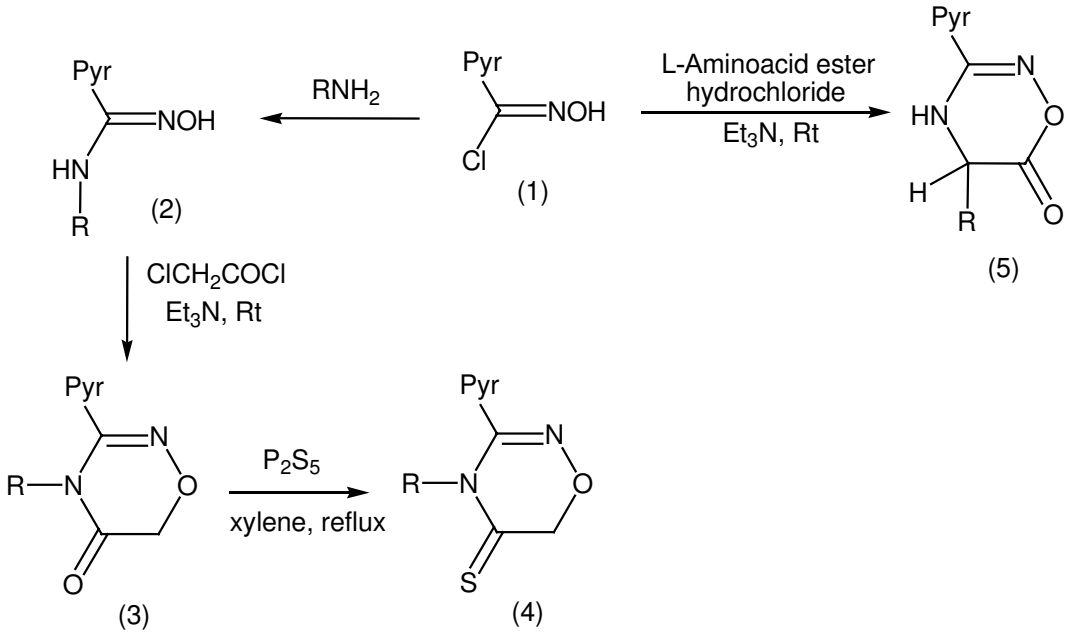
Pyridine hydroxamic acid chloride hydrochlorides **(1)** were reacted with primary amines to give N-substituted pyridine carboxamide oximes **(2a-g)** according to a literature procedure.[9] The IR and ^{1}H-NMR data of compounds **2** obtained are in accord with the literature values. In the ^{1}H-NMR spectrum of compound **2d**

in DMSO-d_6 a signal at 10.2 ppm (corresponds to the oxime OH) appears, but it does not in $CDCl_3$. This peak disappears upon D_2O exchange. The triplet or generally broad singlet at approximately 5.0-7.0 ppm is assigned to the NH proton. The characteristic ^{13}C-NMR assignments for the C=N bonds of compounds **2** are about 154 ppm. The reaction of pyridine carboxamide oximes **2** with chloroacetylchloride in the presence of triethylamine at room temperature give the corresponding 3,4-disubstituted 1,2,4-oxadiazin-5-ones[4] **(3a-k)** in good yields (Scheme). The IR spectra of compounds **3** show C=O absorption at 1715 cm^{-1} and C=N absorption at 1651 cm^{-1}. The singlet for the methylene proton is at ca. 4.67 ppm (2H, C_6-H_2) in the ^{1}H-NMR spectra. The signals at approximately 154, 164, and 68 ppm in the ^{13}C-NMR spectra corresponded to the C=N, C=O, and methylene carbons, respectively. 3,4-Disubstituted-1,2,4-oxadiazin-5-thiones[4] **(4a-g)** were prepared in moderate yields by the reaction of 3,4-disubstituted-1,2,4-oxadiazin-5-ones and excess phosphorus pentasulphide at reflux in xylene (Scheme). The disappearance of the C=O absorptions of compounds **3** and the arising of the N-C=S absorptions in the IR spectra around 1433, 1283, and 1117 cm^{-1} are regarded as proof of compound **4.**

Pyridine hydroxamic acid chloride hydrochlorides **1** were reacted with L-α-aminoacid ester hydrochlorides in the presence of triethyl amine at low temperature and gave pyridine substituted 1,2,4-oxadiazin-6-ones[10] (**5a-b)** (Scheme). These compounds exhibit infrared absorptions at about 3300, 1750, and 1633 cm^{-1} ascribed to N-H, C=O, and C=N bond stretching modes, respectively.

The IR and ^{1}H-NMR data of compounds **3**, **4**, and **5** obtained are in accord with the literature values reported previously for similar structures.[4,10]

The analytical data and physical properties of the synthesised compounds are summarised in Table 1.



Scheme. Synthesis of 1,2,4-oxadiazin-5-one, 1,2,4-oxadiazin-5-thione and 1,2,4-oxadiazin- 6-one.

Table 1. The analytical data and physical properties of the compounds.

Compound	Formula	Pry	R	Found (calcd.) %				Yield %	mp (°C)
				C	H	N	S		
2a	$C_{13}H_{13}N_3O_2$	2-Pyr	4-$(CH_3O)C_6H_4$	64.25 (64.20)	4.37 (5.35)	17.36 (17.28)	-	64	186-188
2b	$C_{15}H_{17}N_3O_3$	2-Pyr	3,4-$(CH_3O)_2$ $C_6H_3CH_2$	-	-	-	-	59	107-108
2c	$C_{16}H_{21}N_3O$	2-Pyr	$C_{10}H_{15}$	70.83 (70.85)	7.61 (7.75)	15.31 (15.50)	-	39	197-198
2d	$C_{13}H_{13}N_3O$	4- Pyr	$C_6H_5CH_2$	68.75 (68.72)	5.58 (5.73)	18.08 (18.50)	-	68	132-133
2e	$C_{13}H_{13}N_3O_2$	4- Pyr	4-$(CH_3O)C_6H_4$	64.15 (64.20)	4.88 (5.35)	17.38 (17.28)	-	40	212-213
2f	$C_{15}H_{17}N_3O_3$	4- Pyr	3,4-$(CH_3O)_2$ $C_6H_3CH_2$	61.91 (62.72)	6.21 (5.92)	14.12 (14.63)	-	66	173-174
2g	$C_{16}H_{21}N_3O$	4- Pyr	$C_{10}H_{15}$	70.75 (70.85)	7.92 (7.75)	15.44 (15.50)	-	52	214-215
3a	$C_{14}H_{11}N_3O_2$	2-Pyr	C_6H_5	66.32 (66.40)	3.68 (4.35)	16.46 (16.60)	-	48	160
3b	$C_{15}H_{13}N_3O_2$	2-Pyr	$C_6H_5CH_2$	66.99 (67.42)	4.56 (4.87)	15.67 (15.73)	-	59	77
3c	$C_{15}H_{13}N_3O_2$	2-Pyr	4-$CH_3C_6H_4$	-	-	-	-	45	115
3d	$C_{15}H_{13}N_3O_3$	2-Pyr	4-$(CH_3O)C_6H_4$	-	-	-	-	27	111-112
3e	$C_{17}H_{17}N_3O_4$	2-Pyr	3,4-$(CH_3O)_2$ $C_6H_3CH_2$	61.41 (62.38)	5.41 (5.20)	12.39 (12.84)	-	61	117
3f	$C_{15}H_{13}N_3O_2$	3-Pyr	4-$CH_3C_6H_4$	66.71 (67.42)	4.49 (4.87)	15.72 (15.73)	-	35	134
3g	$C_{14}H_{11}N_3O_2$	4- Pyr	C_6H_5	66.25 (66.40)	3.97 (4.35)	16.38 (16.60)	-	61	131
3h	$C_{15}H_{13}N_3O_2$	4- Pyr	$C_6H_5CH_2$	67.60 (67.42)	4.47 (4.87)	15.76 (15.73)	-	64	97
3i	$C_{15}H_{13}N_3O_2$	4- Pyr	4-$CH_3C_6H_4$	-	-	-	-	42	162
3j	$C_{15}H_{13}N_3O_3$	4- Pyr	4-$(CH_3O)C_6H_4$	63.18 (63.60)	4.50 (4.59)	14.87 (14.84)	-	26	138
3k	$C_{17}H_{17}N_3O_4$	4- Pyr	3,4-$(CH_3O)_2$ $C_6H_3CH_2$	63.25 (62.38)	5.52 (5.20)	13.03 (12.84)	-	50	137
4a	$C_{14}H_{11}N_3OS$	2-Pyr	C_6H_5	62.72 (62.45)	3.46 (4.09)	15.78 (15.61)	10.92 (11.90)	46	176-177
4b	$C_{15}H_{13}N_3OS$	2-Pyr	$C_6H_5CH_2$	63.20 (63.60)	4.76 (4.60)	14.76 (14.84)	11.15 (11.31)	69	oil
4c	$C_{15}H_{13}N_3OS$	2-Pyr	4-$CH_3C_6H_4$	63.85 (63.60)	4.04 (4.59)	14.91 (14.84)	10.78 (11.31)	52	138-139
4d	$C_{17}H_{17}N_3O_3S$	2-Pyr	3,4-$(CH_3O)_2$ $C_6H_3CH_2$	-	-	-	-	21	oil
4e	$C_{15}H_{13}N_3OS$	4- Pyr	$C_6H_5CH_2$	63.66 (63.60)	4.50 (4.59)	14.92 (14.84)	11.15 (11.31)	59	110-111
4f	$C_{15}H_{13}N_3OS$	4- Pyr	4-$CH_3C_6H_4$	-	-	-	-	43	134-135
4g	$C_{17}H_{17}N_3O_3S$	4- Pyr	3,4-$(CH_3O)_2$ $C_6H_3CH_2$	-	-	-	-	18	oil
5a	$C_{15}H_{13}N_3O_2$	2-Pyr	$C_6H_5CH_2$	-	-	-	-	40	127
5b	$C_{11}H_{13}N_3O_2$	2-Pyr	$(CH_3)_2CH$	60.47 (60.27)	5.57 (5.94)	19.33 (19.18)	-	47	150

mp, Melting point; Pyr, Pyridine.

Antimicrobial activity

Table 2 shows the antimicrobial activity of the compounds, and the inhibition zones formed by standard antibiotic discs are indicated in Table 3. As can clearly be seen from Table 2, both tested doses of all compounds have strong antimicrobial activities against all tested micro-organisms. In classifying the antibacterial activity as gram-positive or gram-negative, it would generally be expected that a much greater number would be active against gram-positive than gram-negative bacteria.[13] The compounds were active against both gram-positive and gram-negative bacteria in different levels. Notably, they have stronger antimicrobial activities against the yeast cultures than against the bacteria used in this study.

Table 2. Antimicrobial activity of the compounds tested.

Micro-organisms	Diameter of inhibition zones (mm)									
	3i		**3l**		**4f**		**5a**		**5b**	
	a	b	a	b	a	b	a	b	a	b
Staphylococcus aureus	25	36	21	26	25	29	18	27	22	27
Escherichia coli	27	34	22	31	28	32	21	28	26	33
Proteus vulgaris	32	40	28	34	33	41	21	29	29	34
Mycobacterium smegmatis	18	25	20	27	14	24	17	24	17	24
Listeria monocytogenes	22	31	17	21	18	22	16	24	19	24
Pseudomonas aureginosa	12	19	22	28	20	25	17	29	10	19
Candida albicans	-	-	-	-	-	-	-	12	-	-
Debaryomyces hansenii	-	-	-	-	-	-	-	-	-	-
Kluyveromyces fragilis	-	-	-	13	-	-	-	14	-	12
Rhodotorula rubra	-	-	12	18	-	-	12	16	-	12
Hanseniaspora guilliermondii	-	-	-	-	-	-	-	-	-	-

a: Low dose of compounds b: High dose of compounds

Table 3. Antimicrobial activities of some standard antibiotics.

Micro-organisms	Inhibition Zone (mm)								
	P10	SAM20	CTX30	VA 30	OFX5	TE30	NY100	KETO20	CLT10
Escherichia coli	18	12	10	22	30	28	-	-	-
Staphylococcus aureus	13	16	12	13	24	26	-	-	-
Pseudomonas aureginosa	8	10	54	10	44	34	-	-	-
Proteus vulgaris	10	16	18	20	28	26	-	-	-
Mycobacterium smegmatis	15	21	11	20	32	24	-	-	-
Listeria monocytogenes	10	12	16	26	30	28	-	-	-
Candida albicans	-	-	-	-	-	-	20	21	15
Kluyveromyces fragilis	-	-	-	-	-	-	18	16	18
Rhodotorula rubra	-	-	-	-	-	-	18	22	16
Hanseniaspora guilliermondii	-	-	-	-	-	-	21	24	22
Debaryomyces hansenii	-	-	-	-	-	-	16	14	18

All compounds have higher antimicrobial activities than those of P10, SAM20, CTX30, and VA30 against all tested micro-organisms. *Proteus vulgaris* is the most sensitive bacterium against all compounds,

having diameter zones of above 28 mm. These tested compounds have higher antimicrobial effects than all of the standard antibiotics against *Proteus vulgaris*. While compounds **3l**, **5a**, and **5b** have from moderately to high antimicrobial activity against the yeast cultures used in this study, as compared to the standard antifungal antibiotics Nystatin, Ketaconozole, and Clotrimizole, compounds **3i** and **4f** have no antiyeast activity. Moreover, *Debaryomyces hansenii* and *Hanseniaspora guilliermondii* are resistant to all compounds. The antiyeast activity of compound **5b** is weak, when compared to the standard antiyeast antibiotics.

The compounds differ significantly in their activity against the tested micro-organisms. These differences may be attributed to fact that the cell wall in gram-positive bacteria is single layered, whereas the gram-negative cell wall is a multi-layered structure and the yeast cell wall is quite complex.[14] In this study all compounds are highly active against both gram-positive and gram-negative bacteria and moderately active against yeasts. The activity against bacteria and the yeast cultures may be indicative of the presence of a broad spectrum.

The results of our study indicate that the compounds have the potential to generate novel metabolites. The compounds demonstrating especially antibacterial activity could result in the discovery of novel antibacterial agents showing broad spectrum activities, and this may help in the discovery of new chemical classes of antibiotics that could serve as selective agents against infectious diseases.

P10, Penicillin G (10 units); SAM20, Ampicillin 10 μg; CTX30, Cefotaxime 30 μg; VA30, Vancomycin 30 μg; OFX5, Oflaxacin 5 μg; TE30, Tetracyclin 30 μg; NY100, Nystatin 100 μg; KETO20, Ketaconazole 20 μg; CLT10, Clotrimazole 10 μg.

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