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RESEARCH ARTICLE

Synthesis, antimicrobial activity and cytotoxicity of novel oxadiazole derivatives

Zafer Asim Kaplancikli[1], Mehlika Dilek Altintop[1], Gulhan Turan-Zitouni[1], Ahmet Ozdemir[1], Rasime Ozic[2], and Gülşen Akalın[3]

[1]Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Anadolu University, Eskişehir, Turkey, [2]Faculty of Science, Department of Biology, Anadolu University, Eskişehir, Turkey, and [3]Faculty of Pharmacy, Department of Biochemistry, Anadolu University, Eskişehir, Turkey

Abstract

In the present study, 5-substituted-1,3,4-oxadiazolin-2-thiones (**1a**–**b**) were synthesized *via* the ring closure reactions of appropriate acid hydrazides with carbon disulphide. *N*-(Benzothiazol-2-yl)-2-[[5-substituted-1,3,4-oxadiazol-2-yl] sulfanyl]acetamide derivatives (**3a**–**j**) were obtained by the nucleophilic substitution reactions of 5-substituted-1,3,4-oxadiazolin-2-thiones (**1a**–**b**) with *N*-(benzothiazol-2-yl)-2-chloroacetamides. The chemical structures of the compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR and FAB^{+}-MS spectral data and elemental analyses. The synthesized compounds were screened for their antimicrobial activities against *Micrococcus luteus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Candida albicans*. All compounds except compound **3h** exhibited the highest antibacterial activity against *P. aeruginosa*. Among all compounds (**3a**–**j**), the compounds bearing 4-methoxyphenoxymethyl moiety on oxadiazole ring (**3a**–**e**) exhibited the highest inhibitory activity against *C. albicans*. Although compound **3j** did not possess 4-methoxyphenoxymethyl moiety on oxadiazole ring, this derivative also exhibited the same level of anti-candidal activity. The compounds were also investigated for their cytotoxic effects using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Compound **3a** exhibited the highest cytotoxic activity, whereas compound **3g** possessed the lowest cytotoxic activity against NIH/3T3 cells.

Keywords: Oxadiazole, benzothiazole, amide, antimicrobial activity, cytotoxicity

Introduction

Nosocomial infections have emerged as important causes of morbidity and mortality due to the fact that hospitalized patients tend to be more susceptible to infections because of their severe underlying disease conditions. If the patient is immunocompromised, micro-organisms that are not normally pathogenic are also capable of causing disease. Furthermore, hospital conditions contribute to the acquisition of drug resistance, which complicates the treatment of infections due to drug-resistant pathogens[1].

The development of resistance to antimicrobial agents has led to the failure of the treatment of bacterial and fungal infections, which are responsible for the bulk of nosocomial infections. In order to overcome this serious problem, which is considered as the inevitable consequence of the widespread use of these drugs, the development of new effective antimicrobial agents has gained great importance[1–7].

Oxadiazoles are widely used and studied pharmacophores in medicinal chemistry due to their broad spectrum and metabolic profile. Some studies have confirmed that oxadiazole derivatives possess antimicrobial activity. Furamizole, which is a nitrofuran derivative bearing oxadiazole nucleus, is an example of promising antibacterial agents[8–17].

Among oxadiazole derivatives, 1,3,4-oxadiazolin-2-thiones, which are synthesized by the ring closure reactions of various acid hydrazides with carbon disulphide, are versatile reaction intermediates in synthetic chemistry

Address for Correspondence: Dr. Zafer Asim Kaplancikli, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Anadolu University, 26470 Eskişehir, Turkey. Tel.: 90 222 3350580/3776. Fax: 90 222 3350750. E-mail: zakaplan@anadolu.edu.tr

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owing to the fact that the thiol group on oxadiazole ring acts as a strong nucleophile[18,19].

Medicinal chemists have carried out considerable research for novel antimicrobial agents that possess amide moiety. The prominent drugs bearing amide group are penicillins and cephalosporins, all of which are widely used as antibiotics for the treatment of systemic infections. Penicillins and cephalosporins, which are also classified as β-lactam antibiotics, possess cyclic amide as the main scaffold and acetamide moiety as the side chain[20–24].

Compounds bearing benzothiazole moiety have also been reported to exhibit a wide spectrum of biological effects including antimicrobial activity[25–27].

In this study, we described the synthesis of some new oxadiazole derivatives, which possess two important functional moieties, namely amide and benzothiazole and focused on their potential antimicrobial and cytotoxic effects.

Methods

Chemistry

All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined using an Electrothermal 9100 digital melting point apparatus and were uncorrected (Electrothermal, Essex, UK). The compounds were checked for purity by thin-layer chromatography on silica gel 60 F_{254}. Spectroscopic data were recorded on the following instruments: IR, Shimadzu 435 IR spectrophotometer (Shimadzu, Tokyo, Japan); ^{1}H NMR, Bruker 400 MHz NMR spectrometer (Bruker Bioscience, Billerica, MA) and ^{13}C NMR, Bruker Avance II 100 MHz NMR spectrometer (Bruker Bioscience, Billerica, MA) in DMSO-d_6 using tetramethylsilane as internal standard; MS-FAB, VG Quattro mass spectrometer (Fisons Instruments Vertriebs GmbH, Mainz, Germany), elemental analyses were performed on a Perkin-Elmer EAL 240 elemental analyser (Perkin-Elmer, Norwalk, CT).

General procedure for the synthesis of the compounds

5-Substituted-1,3,4-oxadiazolin-2-thiones (1a–b)

The acid hydrazide (5 mmol) was dissolved in a solution of potassium hydroxide (5 mmol) in ethanol (50 mL). Carbon disulfide (5 mL) was then added while stirring and the reaction mixture was heated under reflux for 10 h. The solution was cooled and acidified to pH 5–6 with hydrochloric acid solution and crystallised from ethanol[28].

N-(benzothiazol-2-yl)-2-chloroacetamides (2a–e)

Chloroacetyl chloride (5 mmol) was added dropwise with stirring to a mixture of 2-aminobenzothiazole (5 mmol) and triethylamine (2 mL) in toluene (50 mL) at 0–5°C. The solvent was evaporated under reduced pressure. The residue was washed with water and crystallized from ethanol[29].

N-(benzothiazol-2-yl)-2-[[5-substituted-1,3,4-oxadiazol-2-yl] sulfanyl]acetamides (3a–j)

A mixture of 1,3,4-oxadiazolin-2-thione (**1**) (2 mmol) and appropriate *N*-(benzothiazol-2-yl)-2-chloroacetamide (**2**) (2 mmol) in acetone was stirred at room temperature for 8 h in the presence of potassium carbonate and filtered. The residue was washed with water and crystallized from ethanol.

N-(benzothiazol-2-yl)-2-[[5-(4-methoxyphenoxymethyl)-1,3,4-oxadiazol-2-yl]sulfanyl]acetamide (3a)

IR (KBr) ν_{max} (cm^{-1}): 3206 (amide N–H), 1681 (amide C=O), 1605, 1551, 1441 (C=N and C=C), 1206 (C–O).

^{1}H NMR (400 MHz, DMSO-d_6): 3.69 (3H, s), 4.41 (2H, s), 5.32 (2H, s), 6.86 (2H, d, J = 8.9 Hz), 6.95 (2H, d, J = 8.9 Hz), 7.30 (1H, t, J = 7.1 Hz), 7.43 (1H, t, J = 7.1 Hz), 7.75 (1H, d, J = 7.9 Hz), 7.99 (1H, d, J = 7.8 Hz), 12.71 (1H, br).

^{13}C NMR (100 MHz, DMSO-d_6): 35.57 (CH_2), 55.59 (CH_3), 60.15 (CH_2), 104.99 (CH), 113.95 (2CH), 115.25 (CH), 116.18 (2CH), 121.14 (CH), 131.31 (CH), 151.22 (C), 154.31 (C), 155.79 (C), 156.45 (C), 164.19 (C), 164.20 (C), 166.04 (C), 180.06 (C).

For $C_{19}H_{16}N_4O_4S_2$ calculated: 53.26% C, 3.76% H, 13.08% N; found: 53.30% C, 3.82% H, 13.10% N.

MS (FAB) $[M+1]^+$: m/z 429.

N-(6-chlorobenzothiazol-2-yl)-2-[[5-(4-methoxyphenoxymethyl)-1,3,4-oxadiazol-2-yl]sulfanyl] acetamide (3b)

IR (KBr) ν_{max} (cm^{-1}): 3210 (amide N–H), 1685 (amide C=O), 1615, 1560, 1445 (C=N and C=C), 1211 (C–O).

^{1}H NMR (400 MHz, DMSO-d_6): 3.70 (3H, s), 4.40 (2H, s), 5.35 (2H, s), 6.88 (2H, d, J = 9.0 Hz), 6.99 (2H, d, J = 9.0 Hz), 7.45 (1H, dd, J = 8.6, 2.4 Hz), 7.76 (1H, d, J = 8.6 Hz), 8.13 (1H, d, J = 2.4 Hz), 12.79 (1H, br).

^{13}C NMR (100 MHz, DMSO-d_6): 35.56 (CH_2), 55.69 (CH_3), 60.05 (CH_2), 104.86 (CH), 114.55 (2CH), 115.15 (CH), 116.10 (2CH), 121.04 (CH), 127.62 (C), 151.13 (C), 154.20 (C), 155.70 (C), 156.21 (C), 164.15 (C), 163.91 (C), 166.02 (C), 180.02 (C).

For $C_{19}H_{15}ClN_4O_4S_2$ calculated: 49.30% C, 3.27% H, 12.10% N; found: 49.35% C, 3.29% H, 12.14% N.

MS (FAB) $[M+1]^+$: m/z 463.

N-(6-Methylbenzothiazol-2-yl)-2-[[5-(4-methoxyphenoxymethyl)-1,3,4-oxadiazol-2-yl]sulfanyl] acetamide (3c)

IR (KBr) ν_{max} (cm^{-1}): 3201 (amide N–H), 1676 (amide C=O), 1601, 1566, 1435 (C=N and C=C), 1228 (C–O).

^{1}H NMR (400 MHz, DMSO-d_6): 2.41 (3H, m), 3.71 (3H, s), 4.42 (2H, s), 5.33 (2H, s), 6.87 (2H, d, J = 9.1 Hz), 6.97 (2H, d, J = 9.1 Hz), 7.25 (1H, d, J = 8.2 Hz), 7.68 (1H, d, J = 8.2 Hz), 7.75 (1H, m), 12.64 (1H, br).

^{13}C NMR (100 MHz, DMSO-d_6): 20.95 (CH_3), 35.56 (CH_2), 55.60 (CH_3), 60.17 (CH_2), 104.75 (CH), 114.60 (2CH), 115.05 (CH), 116.18 (2CH), 121.20 (CH), 132.71 (C), 151.22 (C), 154.29 (C), 155.72 (C), 156.29 (C), 164.05 (C), 164.09 (C), 165.87 (C), 179.89 (C).

For $C_{20}H_{18}N_4O_4S_2$ calculated: 54.29% C, 4.10% H, 12.66% N; found: 54.32% C, 4.11% H, 12.69% N.

MS (FAB) $[M+1]^+$: m/z 443.

N-(6-methoxybenzothiazol-2-yl)-2-[[5-(4-methoxyphenoxymethyl)-1,3,4-oxadiazol-2-yl]sulfanyl] acetamide (3d)

IR (KBr) ν_{max} (cm^{-1}): 3221 (amide N–H), 1685 (amide C=O), 1621, 1552, 1415 (C=N and C=C), 1230 (C–O).

^{1}H NMR (400 MHz, DMSO-d_6): 3.69 (3H, s), 3.81 (3H, s), 4.44 (2H, s), 5.30 (2H, s), 6.84 (2H, d, J = 9.0 Hz), 6.97 (2H, d, J = 9.0 Hz), 7.05 (1H, dd, J = 8.8, 2.6 Hz), 7.59 (1H, d, J = 2.6 Hz), 7.67 (1H, d, J = 8.8 Hz), 12.66 (1H, br).

^{13}C NMR (100 MHz, DMSO-d_6): 35.57 (CH_2), 55.32 (CH_3), 55.62 (CH_3), 60.18 (CH_2), 104.75 (CH), 114.62 (2CH), 115.04 (CH), 116.15 (2CH), 121.29 (CH), 132.77 (C), 151.15 (C), 154.25 (C), 155.51 (C), 156.27 (C), 164.01 (C), 164.04 (C), 165.86 (C), 179.88 (C).

For $C_{20}H_{18}N_4O_5S_2$ calculated: 52.39% C, 3.96% H, 12.22% N; found: 52.41% C, 3.91% H, 12.15% N.

MS (FAB) $[M+1]^+$: m/z 459.

N-(6-ethoxybenzothiazol-2-yl)-2-[[5-(4-methoxyphenoxymethyl)-1,3,4-oxadiazol-2-yl]sulfanyl] acetamide (3e):

IR (KBr) ν_{max} (cm^{-1}): 3218 (amide N–H), 1682 (amide C=O), 1619, 1541, 1401 (C=N and C=C), 1218 (C–O).

^{1}H NMR (400 MHz, DMSO-d_6): 1.35 (3H, t, J = 7.0 Hz), 3.68 (3H, s), 4.07 (2H, q, J = 7.0 Hz), 4.44 (2H, s), 5.30 (2H, s), 6.84 (2H, d, J = 9.1 Hz), 6.96 (2H, d, J = 9.1 Hz), 7.03 (1H, dd, J = 8.8, 2.5 Hz), 7.56 (1H, d, J = 2.5 Hz), 7.66 (1H, d, J = 8.8 Hz), 12.63 (1H, br).

^{13}C NMR (100 MHz, DMSO-d_6): 14.63 (CH_3), 35.55 (CH_2), 55.31 (CH_3), 60.17 (CH_2), 63.62 (CH_2), 105.39 (CH), 114.62 (2CH), 115.41 (CH), 116.15 (2CH), 121.28 (CH), 132.75 (C), 148.94 (C), 151.13 (C), 154.25 (C), 155.49 (C), 164.01 (C), 164.04 (C), 165.86 (C), 179.02 (C).

For $C_{21}H_{20}N_4O_5S_2$ calculated: 53.38% C, 4.27% H, 11.86% N; found: 53.43% C, 4.35% H, 11.90% N.

MS (FAB) $[M+1]^+$: m/z 473.

N-(benzothiazol-2-yl)-2-[[5-(2-cyclohexylethyl)-1,3,4-oxadiazol-2-yl]sulfanyl]acetamide (3f):

IR (KBr) ν_{max} (cm^{-1}): 3217 (amide N–H), 2922, 2848 (aliphatic C–H), 1683 (amide C=O), 1600, 1554, 1446 (C=N and C=C).

^{1}H NMR (400 MHz, DMSO-d_6): 0.73–0.89 (2H, m), 0.97–1.26 (4H, m), 1.46–1.74 (7H, m), 2.81 (2H, t, J = 7.5 Hz), 4.38 (2H, s), 7.32 (1H, t, J = 7.0 Hz), 7.45 (1H, t, J = 7.0 Hz), 7.77 (1H, d, J = 8.0 Hz), 7.98 (1H, d, J = 7.8 Hz), 12.71 (1H, bs).

^{13}C NMR (100 MHz, DMSO-d_6): 21.90 (CH_2), 24.60 ($2CH_2$), 27.10 (CH_2), 32.05 ($2CH_2$), 33.61 (CH_2), 35.18 (CH_2), 38.27 (CH), 122.06 (CH), 132.61 (CH), 152.21 (CH), 154.68 (CH), 155.96 (C), 157.12 (C), 163.98 (C), 164.11 (C), 166.54 (C), 182.11 (C).

For $C_{19}H_{22}N_4O_2S_2$ calculated: 56.69% C, 5.51% H, 13.92% N; found: 56.73% C, 5.52% H, 13.96% N.

MS (FAB) $[M+1]^+$: m/z 403.

N-(6-chlorobenzothiazol-2-yl)-2-[[5-(2-cyclohexylethyl)-1,3,4-oxadiazol-2-yl]sulfanyl]acetamide (3g)

IR (KBr) ν_{max} (cm^{-1}): 3201 (amide N–H), 2922, 2850 (aliphatic C–H), 1683 (amide C=O), 1604, 1556, 1442 (C=N and C=C).

^{1}H NMR (400 MHz, DMSO-d_6): 0.73–0.89 (2H, m), 0.97–1.26 (4H, m), 1.46–1.74 (7H, m), 2.81 (2H, t, J = 7.5 Hz), 4.37 (2H, s), 7.47 (1H, dd, J = 8.7, 2.3 Hz), 7.76 (1H, d, J = 8.7 Hz), 8.13 (1H, d, J = 2.3 Hz), 12.79 (1H, bs).

^{13}C NMR (100 MHz, DMSO-d_6): 21.95 (CH_2), 24.43 ($2CH_2$), 26.92 (CH_2), 32.13 ($2CH_2$), 34.01 (CH_2), 35.22 (CH_2), 36.92 (CH), 121.18 (CH), 126.91 (CH), 150.02 (CH), 153.99 (C), 155.63 (C), 156.91 (C), 163.15 (C), 163.91 (C), 165.90 (C), 181.04 (C).

For $C_{19}H_{21}ClN_4O_2S_2$ calculated: 52.22% C, 4.84% H, 12.82% N; found: 52.25% C, 4.80% H, 12.83% N.

MS (FAB) $[M+1]^+$: m/z 437.

N-(6-methylbenzothiazol-2-yl)-2-[[5-(2-cyclohexylethyl)-1,3,4-oxadiazol-2-yl]sulfanyl]acetamide (3h)

IR (KBr) ν_{max} (cm^{-1}): 3200 (amide N–H), 2920, 2850 (aliphatic C–H), 1683 (amide C=O), 1612, 1564, 1460 (C=N and C=C).

^{1}H NMR (400 MHz, DMSO-d_6): 0.73–0.89 (2H, m), 0.97–1.26 (4H, m), 1.46-1.74 (7H, m), 2.41 (3H, m), 2.81 (2H, t, J = 7.5 Hz), 4.36 (2H, s), 7.27 (1H, d, J = 8.3 Hz), 7.65 (1H, d, J = 8.3 Hz), 7.77 (1H, m), 12.64 (1H, bs).

^{13}C NMR (100 MHz, DMSO-d_6): 21.51 (CH_3), 22.32 (CH_2), 23.93 ($2CH_2$), 26.81 (CH_2), 31.99 ($2CH_2$), 33.96 (CH_2), 35.41 (CH_2), 37.10 (CH), 120.96 (CH), 132.11 (CH), 152.02 (CH), 153.91 (C), 155.42 (C), 156.26 (C), 163.88 (C), 164.11 (C), 164.99 (C), 180.12 (C).

For $C_{20}H_{24}N_4O_2S_2$ calculated: 57.67% C, 5.81% H, 13.45% N; found: 57.70% C, 5.85% H, 13.49% N.

MS (FAB) $[M+1]^+$: m/z 417.

N-(6-methoxybenzothiazol-2-yl)-2-[[5-(2-cyclohexylethyl)-1,3,4-oxadiazol-2-yl]sulfanyl]acetamide (3i)

IR (KBr) ν_{max} (cm^{-1}): 3200 (amide N–H), 2920, 2847 (aliphatic C–H), 1681 (amide C=O), 1610, 1564, 1465 (C=N and C=C).

^{1}H NMR (400 MHz, DMSO-d_6): 0.73–0.89 (2H, m), 0.97–1.26 (4H, m), 1.46–1.74 (7H, m), 2.81 (2H, t, J = 7.5 Hz), 3.81 (3H, s), 4.36 (2H, s), 7.27 (1H, d, J = 8.3 Hz), 7.65 (1H, d, J = 8.3 Hz), 7.77 (1H, m), 12.64 (1H, bs).

^{13}C NMR (100 MHz, DMSO-d_6): 22.06 (CH_2), 23.72 ($2CH_2$), 26.65 (CH_2), 32.41 ($2CH_2$), 34.11 (CH_2), 35.82 (CH_2), 37.20 (CH), 55.22 (CH_3), 121.11 (CH), 131.99 (CH), 152.12 (CH), 153.88 (C), 156.10 (C), 156.38 (C), 163.48 (C), 164.05 (C), 165.16 (C), 181.01 (C).

For $C_{20}H_{24}N_4O_3S_2$ calculated: 55.53% C, 5.59% H, 12.95% N; found: 55.57% C, 5.61% H, 12.96% N.

MS (FAB) $[M+1]^+$: m/z 433.

N-(6-ethoxybenzothiazol-2-yl)-2-[[5-(2-cyclohexylethyl)-1,3,4-oxadiazol-2-yl]sulfanyl]acetamide (3j)

IR (KBr) ν_{max} (cm^{-1}): 3221 (amide N–H), 2922, 2850 (aliphatic C–H), 1676 (amide C=O), 1602, 1581, 1458 (C=N and C=C).

^{1}H NMR (400 MHz, DMSO-d_6): 0.73–0.89 (2H, m), 0.97–1.26 (4H, m), 1.38 (3H, t, J = 7.1 Hz), 1.46–1.74 (7H, m), 2.81 (2H, t, J = 7.5 Hz), 4.02 (2H, q, J = 7.1 Hz), 4.36 (2H, s), 7.27 (1H, d, J = 8.3 Hz), 7.65 (1H, d, J = 8.3 Hz), 7.77 (1H, m), 12.64 (1H, bs).

^{13}C NMR (100 MHz, DMSO-d_6): 14.55 (CH_3), 21.88 (CH_2), 23.46 ($2CH_2$), 26.12 (CH_2), 32.15 ($2CH_2$), 33.98 (CH_2), 35.61 (CH_2), 36.95 (CH), 62.51 (CH_2), 121.21 (CH), 132.09 (CH), 152.28 (CH), 153.68 (C), 155.81 (C), 156.63 (C), 162.11 (C), 164.78 (C), 165.06 (C), 182.15 (C).

For $C_{21}H_{26}N_4O_3S_2$ calculated: 56.48% C, 5.87% H, 12.55% N; found: 56.51% C, 5.90% H, 12.56% N.

MS (FAB) $[M+1]^+$: m/z 447.

Microbiology

Antimicrobial activity

The *in vitro* antimicrobial activities of the compounds (**3a**–**j**) were tested using the microbroth dilution method[30]. The tested micro-organism strains were *Micrococcus luteus* (NRLL B-4375), *Bacillus subtilis* (NRS-744), *Pseudomonas aeruginosa* (ATCC-254992), *Staphylococcus aureus* (NRRL B-767), *Escherichia coli* (ATCC-25922), *Listeria monocytogenes* (ATCC-7644) and *Candida albicans* (ATCC-22019). Microbroth dilution-susceptibility assay was used for antimicrobial evaluation of the compounds. Stock solutions of the samples were prepared in dimethyl sulfoxide. Dilution series using sterile distilled water were prepared from 1–4 mg/mL to 0.001–0.007 mg/mL in micro-test tubes that were transferred to 96-well microtitre plates. Overnight-grown bacterial and *C. albicans* suspensions in double-strength Mueller–Hinton broth were standardized to 10^8 colony-forming unit/mL using McFarland No: 0.5 standard solutions. Hundred microlitre of each micro-organism suspension was then added into the wells. The last well-chain without a micro-organism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37°C for 18–24 h, the first well without turbidity was determined as the minimum inhibitory concentration. Streptomycin was used as an antibacterial agent, whereas ketoconazole was used as an antifungal agent.

Toxicity

The level of cellular MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma) reduction was quantified as previously described in the literature with small modifications[31,32].

Cell culture and drug treatment

NIH/3T3 cells were obtained from the American Type Culture Collection (ATCC). The cells were incubated in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum (Life Technologies, UK), 100 IU/mL penicillin (Gibco, Paisley, Scotland) and 100 mg/mL streptomycin (Gibco) at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Exponentially growing cells were plated at 2×10^4 cells/mL into 96-well microtitre tissue culture plates (Nunc, Denmark) and incubated for 24 h before the addition of the drugs (the optimum cell number for cytotoxicity assays was determined in preliminary experiments). Stock solutions of compounds were prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Poole, UK) and further dilutions were made with fresh culture medium (the concentration of DMSO in the final culture medium was <0.1% which had no effect on the cell viability).

MTT assay for cytotoxicity of the compounds

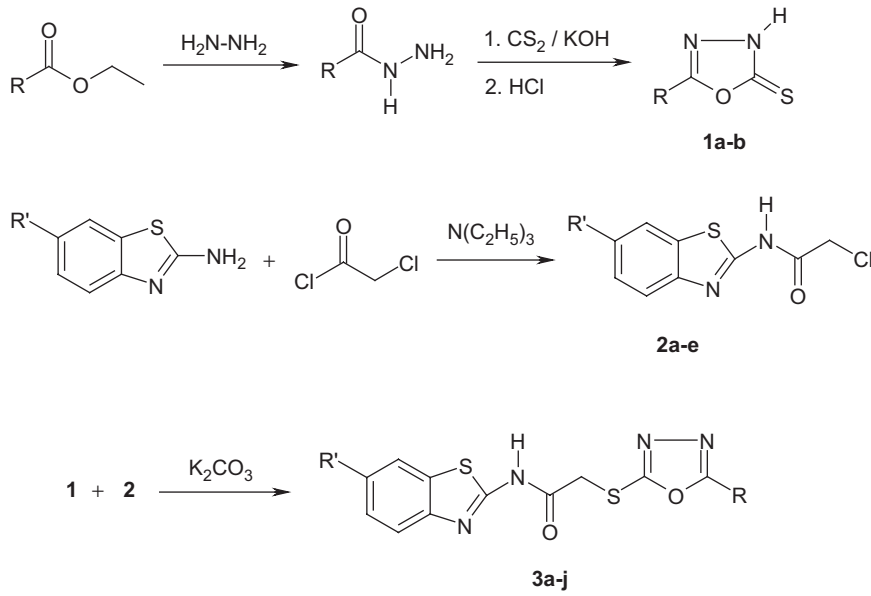
It is widely used as a measure of cytotoxicity. After 24 h of preincubation, the tested compounds were added to give final concentration in the range 0.5–500 μg/mL and the cells were incubated for 24 h. At the end of this period, MTT was added to a final concentration of 0.5 mg/mL and the cells were incubated for 4 h at 37 °C. After the medium was removed, the formazan crystals formed by MTT metabolism were solubilised by addition of 200 μl DMSO to each well and absorbance was read at 540 nm with a microtitre plate spectrophotometer (Bio-Tek plate reader). Every concentration was repeated in three wells and IC_{50} values were defined as the drug concentrations that reduced absorbance to 50% of control values.

Results and discussion

Initially, the compounds which have oxadiazoline-2-thione structure (**1a**–**b**) were obtained by the ring closure reactions of acid hydrazides with carbon disulphide. 2-Chloro-*N*-(benzothiazol-2-yl)acetamides (**2a**–**e**) were synthesized *via* the nucleophilic acyl substitution reactions of 2-aminobenzothiazoles with chloroacetyl chloride in the presence of triethylamine. The desired compounds (**3a**–**j**) were prepared by reacting 2-chloro-*N*-(benzothiazol-2-yl)acetamides (**2a**–**e**) with oxadiazoline-2-thiones (**1a**–**b**) under basic conditions. The presence of a base favours the thiol form, which is prone to nucleophilic substitution reactions. These reactions are summarized in Scheme 1 and some properties of the compounds are given in Table 1.

The structures of the compounds (**3a**–**j**) were confirmed by IR, ^{1}H NMR, ^{13}C NMR and FAB$^+$-MS spectral data and elemental analyses.

In the IR spectra of all compounds (**3a**–**j**), all derivatives have a strong, characteristic band in the region 1685–1676 cm^{-1} due to the amide C=O stretching vibration. The amide N–H stretching vibration of the compounds (**3a**–**j**) gives rise to a band at 3221–3200 cm^{-1}. The bands due to the C=C and C=N stretching vibrations are



Scheme 1. The synthetic protocol of the compounds (**3a**–**j**).

observed in the region 1621–1401 cm^{-1}. In the IR spectra of the compounds (**3a**–**e**), the stretching bands for C–O group occur at 1230–1206 cm^{-1}.

In the ^{1}H NMR spectra of the compounds (**3a**–**j**), the signal due to the amide proton appears at 12–13 ppm. The signal due to the–S–CH_2– protons gives rise to a singlet peak at 4.0–5.5 ppm. In the ^{1}H NMR spectra of the compounds **3a**–**e**, the O–CH_2 protons are observed at 4.42–4.44 ppm as a singlet peak. The benzothiazole protons of all derivatives are observed in the region 6.8–8.2 ppm. All other aromatic and aliphatic protons were observed at expected regions.

In their ^{13}C NMR spectra, the signal due to the–S–CH_2–carbon appears at 35–36 ppm. The other aromatic and aliphatic carbons were observed at expected regions.

In the mass spectra of all compounds (**3a**–**j**), the M+1 peak is observed. All compounds gave satisfactory elemental analysis.

All compounds were tested *in vitro* against *S. aureus*, *L. monocytogenes*, *E. coli*, *P. aeruginosa*, *M. luteus*, *B. subtilis* and *C. albicans* and compared with streptomycin and ketoconazole, respectively (Table 2).

All compounds (**3a**–**j**) showed less antimicrobial potency than streptomycin and ketoconazole. The results indicate that the compounds (**3a**–**j**) exhibit the highest antimicrobial effect on *P. aeruginosa* and *C. albicans*.

Among these compounds (**3a**–**j**), the compounds bearing 2-cyclohexylethyl moiety on oxadiazole ring (**3f**–**j**) exhibit the highest inhibitory activity against *S. aureus*. It is clear that there is a positive correlation between antibacterial activity against *S. aureus* and aliphatic group on oxadiazole ring.

The compounds that possess 4-methoxyphenoxymethyl moiety on oxadiazole ring (**3a**–**e**) are the most effective derivatives against *L. monocytogenes* and *E. coli*. It is apparent that 4-methoxyphenoxymethyl moiety on oxadiazole ring has an important impact on antibacterial activity against *L. monocytogenes* and *E. coli*.

All compounds except compound **3h** are equally active against *P. aeruginosa*. Compounds **3b** and **3c** exhibit the lowest antibacterial activity against *M. luteus*.

The compounds possessing 2-cyclohexylethyl moiety on oxadiazole ring (**3f**–**j**) except **3j** are more effective than the compounds possessing 4-methoxyphenoxymethyl moiety on oxadiazole ring (**3a**–**e**) against *B. subtilis*.

The compounds that possess 4-methoxyphenoxymethyl moiety on oxadiazole ring (**3a**–**e**) exhibit the highest inhibitory activity against *C. albicans*. This outcome confirms that the 4-methoxyphenoxymethyl group on oxadiazole ring may have a considerable influence on antifungal activity. Although compound **3j** does not possess 4-methoxyphenoxymethyl moiety on oxadiazole ring, this derivative also exhibits the same level of antifungal activity.

All compounds were also evaluated for their cytotoxic properties using MTT assay. The biological study indicated that compound **3a** possessed the highest cytotoxicity, whereas compound **3g** exhibited the lowest cytotoxicity against NIH/3T3 cells among the title compounds (Table 3).

Conclusions

In conclusion, we described the synthesis of new oxadiazole derivatives and evaluated their *in vitro* antimicrobial activities against various pathogenic bacteria and *C. albicans*.

5-Substituted-1,3,4-oxadiazolin-2-thiones(**1a**–**b**)were synthesized *via* the ring closure reactions of appropriate acid hydrazides with carbon disulphide. N-(Benzothiazol-2-yl)-2-[[5-Substituted-1,3,4-oxadiazol-2-yl]sulfanyl] acetamide derivatives (**3a–j**) were prepared by the

Table 1. Some properties of the synthesized compounds (**3a–j**).

Compound	R	R′	Yield (%)	M.p. (°C)	Molecular formula	Molecular weight
3a	4-Methoxyphenoxymethyl	H	80	206–207	$C_{19}H_{16}N_4O_4S_2$	428
3b	4-Methoxyphenoxymethyl	Cl	72	224–226	$C_{19}H_{15}ClN_4O_4S_2$	462,5
3c	4-Methoxyphenoxymethyl	CH_3	75	215–217	$C_{20}H_{18}N_4O_4S_2$	442
3d	4-Methoxyphenoxymethyl	OCH_3	78	207–208	$C_{20}H_{18}N_4O_5S_2$	458
3e	4-Methoxyphenoxymethyl	OC_2H_5	81	196–200	$C_{21}H_{20}N_4O_5S_2$	472
3f	2-Cyclohexylethyl	H	75	202–204	$C_{19}H_{22}N_4O_2S_2$	402
3g	2-Cyclohexylethyl	Cl	85	237–238	$C_{19}H_{21}ClN_4O_2S_2$	436,5
3h	2-Cyclohexylethyl	CH_3	80	219–220	$C_{20}H_{24}N_4O_2S_2$	416
3i	2-Cyclohexylethyl	OCH_3	82	184	$C_{20}H_{24}N_4O_3S_2$	432
3j	2-Cyclohexylethyl	OC_2H_5	84	194–197	$C_{21}H_{26}N_4O_3S_2$	446

Table 2. Antimicrobial activities of the compounds (**3a–j**) (μg/mL).

Compound	A	B	C	D	E	F	G
3a	500	250	250	250	250	500	500
3b	500	250	250	250	500	500	500
3c	500	250	250	250	500	500	500
3d	500	250	250	250	250	500	500
3e	500	250	250	250	250	500	500
3f	250	500	500	250	250	250	1000
3g	250	500	500	250	250	250	1000
3h	250	500	500	500	250	250	1000
3i	250	500	500	250	250	250	1000
3j	250	500	500	250	250	500	500
Reference 1	31.25	7.81	31.25	125	15.625	15.625	—
Reference 2	—	—	—	—	—	—	250

Reference 1, streptomycin; reference 2, ketoconazole.
A: *Staphylococcus aureus* (NRRL B-767), B, *Listeria monocytogenes* (ATCC-7644), C: *Escherichia coli* (ATCC-25922), D: *Pseudomonas aeruginosa* (ATCC-254992), E: *Micrococcus luteus* (NRLL B-4375), F: *Bacillus subtilis* (NRS-744), G: *Candida albicans* (ATCC-22019).

Table 3. *In vitro* cytotoxicity of the compounds (**3a–j**)

Compound	IC_{50} (μg/mL)[a]
3a	25.4 ± 0.8
3b	101.6 ± 16.1
3c	116.7 ± 15.3
3d	56.8 ± 7.6
3e	103.3 ± 15.3
3f	126.7 ± 25.2
3g	203.3 ± 32.1
3h	125 ± 25
3i	106 ± 10.4
3j	95 ± 13.3

[a]Cytotoxicity of the compounds to mouse fibroblast (NIH/3T3) cell line. Incubation for 24 h. IC_{50} is the drug concentration required to inhibit 50% of the cell growth. The values represent mean ± standard deviation of triplicate determinations.

nucleophilic substitution reactions of 5-substituted-1-,3,4-oxadiazolin-2-thiones (**1a–b**) with N-(benzothiazol-2-yl)-2-chloroacetamides in the presence of potassium carbonate.

The biological results indicate that *P. aeruginosa* and *C. albicans* are more susceptible to the synthesized compounds. All compounds except compound **3h** are equally active against *P. aeruginosa*. The compounds which possess 4-methoxyphenoxymethyl moiety on oxadiazole ring (**3a–e**) exhibit the highest inhibitory activity against *C. albicans*. This outcome confirms that 4-methoxyphenoxymethyl group on oxadiazole ring may have a considerable influence on anti-candidal activity. Compound **3j**, which does not possess 4-methoxyphenoxymethyl moiety on oxadiazole ring, also exhibits the same level of antifungal activity.

The cytotoxic effects of the compounds were also investigated and compound **3a** possessed the highest cytotoxicity, whereas compound **3g** exhibited the lowest cytotoxicity against NIH/3T3 cells.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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