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Original article

Design, synthesis and screening of some novel benzoxazole based 1,3,4-oxadiazoles as potential antimicrobial agents

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ABSTRACT

A series of novel 2-(5-substituted-[1,3,4]oxadiazol-2-yl)-benzoxazoles (**7a**–**h**) were synthesized in good yields in two different directions by involving benzoxazole-2-carboxylic acid (**1**) as raw material and benzoxazole-2-carbonyl chloride (**2**), benzoxazole-2-carboxylic acid methyl ester (**3**), benzoxazole-2-carboxylic acid hydrazide (**4**), benzoxazole-2-carboxylic acid *N*′-acetyl hydrazide (**5a**–**d**) and benzoxazole-2-carboxylic acid-ethylidene-hydrazides (**6a**–**d**) as reactive intermediates. The chemical structures of all the synthesized compounds were elucidated by their IR, ^{1}H NMR and ^{13}C NMR and mass spectral data. Further, the target compounds were screened for their antimicrobial activity against various Gram-positive and Gram-negative bacteria.

1. Introduction

1,3,4-Oxadiazoles are biologically active, synthetically useful and important heterocyclic compounds and investigation of their chemical and biological behaviors have gained more importance in recent decades. Different classes of oxadiazoles possess an extensive spectrum of pharmacological activities such as antimalarial [1], anti-inflammatory [2], anticonvulsant [3], analgesic [4], antimicrobial [5], antimycobacterial [6], antitumor [7], herbicidal [8], vasodialatory [9], cytotoxic [10], hypolipidemic [11], and antiedema [12].

Recent observations suggest that substituted benzoxazoles possess potential activity with lower toxicities in the chemotherapeutic approach in man [13]. Careful literature survey revealed that targets containing benzoxazole moiety have remarkable biological activities like antibacterial [14], antihistaminic [15], antiparasitics [16], antiviral [17] and antifungal [18] activity.

2. Experimental

The general procedures are showed in Scheme 1 and the details are listed below:

*Benzoxazole-2-carbonyl chloride (**2**)*: To a solution of benzoxazole-2-carboxylic acid **1** (0.01 mol) in ethanol (20 mL), thinly chloride (0.01 mol) was added. The mixture was stirred at room temperature for 4 h. After completion of the reaction (monitored by TLC, EtOAc:petroleum-ether, 2:1) then the mixture was poured in water (30 mL) and extracted with Et_2O (3× 20 mL). The organic phase was separated, and dried over Na_2SO_4. Evaporation of the solvent gave **2** in pure form.

*Benzoxazole-2-carboxylic acid methyl ester (**3**)*: A mixture of compound **2** (0.01 mol) and triethyl amine (0.5 mL) in methanol (5 mL) was refluxed for 3 h. After cooling, the mixture was poured into crushed ice, and neutralized with 5% aq. HCl solution. The precipitated solid was filtered and purified using column chromatography (petroleum ether:ethyl acetate, 9:1) to yield pure **3**.

*Benzoxazole-2-carboxylic acid hydrazide (**4**)*: To a mixture of **3** (0.01 mol) in 10 mL of absolute ethanol and hydrazine hydrate (0.04 mol) was added. Then the reaction mixture was refluxed for 8 h. After completion of the reaction (monitored by TLC), it was then diluted with ice-cold water (20 mL) and the solid obtained was purified by crystallization from ethanol to afford pure product benzoxazole-2-carboxylic acid hydrazide **4**.

*Benzoxazole-2-carboxylic acid N′-acetyl hydrazide (**5a**–**d**)*: To a solution of **4** (0.01 mol) in dioxane (10 mL) corresponding benzoyl chloride (0.01 mol) was added. The reaction mixture was refluxed for 4–5 h, then the solvent was removed under reduced pressure

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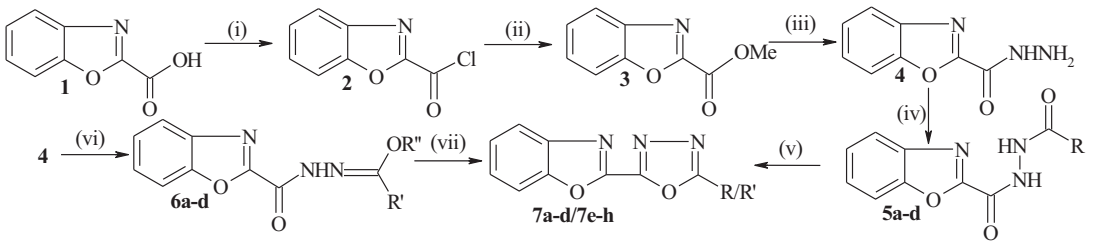

Scheme 1. Synthesis of benzoxazolyl-1,3,4-oxadiazoles. (i) $SOCl_2$, EtOH, r.t., 4 h; (ii) CH_3OH, TEA, reflux, 3 h; (iii) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 8 h; (iv) RCOCl, dioxane, reflux, 4–5 h; (v) $POCl_3$, reflux, 4–5 h; (vi) R′–C–(OR″)$_3$, reflux, 10–12 h; (vii) pyridine, reflux, 4–5 h; **5**(**a**) R=4-$CH_3C_6H_4$; (**b**) R=4-ClC_6H_4; (**c**) R=4-BrC_6H_4; (**d**) R=4-$NO_2C_6H_4$; **6**(**a**) R′=H, R″=CH_2CH_3; (**b**) R′=CH_3, R″=CH_2CH_3; (**c**) R′=CH_2CH_3, R″=CH_2CH_3; (**d**) R′=Ph, R″=CH_2CH_3; **7**(**a**) R=4-$CH_3C_6H_4$; (**b**) R=4-ClC_6H_4; (**c**) R=4-BrC_6H_4; (**d**) R=4-$NO_2C_6H_4$; (**e**) R′=H; (**f**) R′=CH_3; (**g**) R′=CH_2CH_3; (**h**) R′=Ph.

and the residue obtained was triturated with an ice-water mixture. The solid product obtained was filtered off and recrystallized from ethanol to give **5a–d**.

*Benzoxazole-2-carboxylic acid ethylidene-hydrazides (**6a–d**)*: A mixture of benzoxazole-2-carboxylic acid hydrazide **4** (0.01 mol) and suitable orthoformate (5 mL) was boiled under reflux for 10–12 h. After cooling, the solvent was removed under reduced pressure and the residue obtained was triturated with ethanol. The solid product obtained was collected by filtration and recrystallized from ethanol to give compounds **6a–d**.

*2-Substituted-[1,3,4]-oxadiazol-2-yl-benzoxazoles (**7a–d**)*: A solution of **5a–d** (0.01 mol) in phosphorous oxychloride (5 mL) was heated under reflux at 100 °C for 4–5 h. After cooling, the solvent was removed *in vacuo* and the residue was poured into an ice-water and neutralized with ammonium hydroxide (20%). The solid product obtained was collected by filtration and recrystallized from ethanol to give **7a–d**.

*2-Substituted-[1,3,4]-oxadiazol-2-yl-benzoxazoles (**7e–h**)*: To a solution **6a–d** (0.01 mol) in pyridine (10 mL) was refluxed for 4–5 h, then the solvent was removed under reduced pressure and the residue was triturated with an ice-water. The solid product obtained was filtered off and recrystallized from ethanol to give **7e–h**.

*Benzoxazole-2-carbonyl chloride (**2**)*: Brown solid, yield: 70%, mp: 125–127 °C; IR (KBr, cm^{-1}): ν 3035 (C–H, Ar), 1672 (C=O), 1579, 1552, 1498 (C=N), 1147 (C–O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12–7.46 (m, 4H, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.5, 151.4, 148.2, 142.0, 123.6, 121.4, 118.7, 112.4; MS: *m*/*z* 181 (M^+).

*Benzoxazole-2-carboxylic acid methyl ester (**3**)*: Brown solid, yield: 76%, mp: 146–148 °C; IR (KBr, cm^{-1}): ν 3028 (C–H, Ar), 1678 (C=O), 1565, 1555, 1484 (C=N), 1142 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.98–7.54 (m, 4H, Ar–H), 1.23 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.3, 153.2, 148.6, 142.5, 127.6, 123.0, 119.7, 116.5, 48.5; MS: *m*/*z* 181(M^+).

*Benzoxazole-2-carboxylic acid hydrazide (**4**)*: Pale yellow solid, yield: 74%, mp: 137–139 °C; IR (KBr, cm^{-1}): ν 3356 (N–H), 3045 (C–H, Ar), 1664 (C=O), 1578 (C=C), 1458 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.87–7.32 (m, 4H, Ar–H), 7.65 (s, 1H, CONH), 5.54 (s, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.2, 150.4, 147.3, 142.0, 127.8, 122.5, 117.6, 108.4; MS: *m*/*z* 177 (M^+).

*4-Methyl benzoic acid N′-(benzoxazole-2-carbonyl)hydrazide (**5a**)*: Pink solid, yield: 74%, mp: 150–152 °C; IR (KBr, cm^{-1}): ν 3123 (N–H), 3024 (C–H, Ar), 2962 (C–H, CH_3), 1680 (C=O), 1560 (C=C, aromatic), 1430 (C=N), 1235 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.52 (s, 1H, NH), 10.42 (s, 1H, NH), 7.75–7.25 (m, 8H, Ar), 2.14 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.5, 166.7, 153.4, 151.2, 142.1, 139.0, 132.8, 128.5, 128.3, 127.5, 127.0, 126.6, 124.2, 121.3, 112.6, 24.1; MS: *m*/*z* 295 (M^+).

*4-Chloro benzoic acid N′-(benzooxazole-2-carbonyl)hydrazide (**5b**)*: Pale yellow solid, yield: 76%, mp: 142–144 °C; IR (KBr, cm^{-1}): ν 3128 (N–H), 3030 (C–H, Ar), 2965 (C–H, CH_3), 1678 (C=O), 1565 (C=C, aromatic), 1425 (C=N), 1232 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.48 (s, 1H, NH), 10.36 (s, 1H, NH), 7.70–7.12 (m, 8H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 164.3, 155.4, 153.2, 144.7, 141.0, 137.9, 132.0, 129.6, 127.2, 125.6, 123.4, 121.0, 120.8, 117.5; MS: *m*/*z* 315 (M^+).

*4-Bromo benzoic acid N′-(benzoxazole-2-carbonyl)hydrazide (**5c**)*: Yellow solid, yield: 75%, mp: 184–186 °C; IR (KBr, cm^{-1}): ν 3118 (N–H), 3018 (C–H, Ar), 2970 (C–H, CH_3), 1684 (C=O), 1574 (C=C, aromatic), 1434 (C=N), 1224 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.54 (s, 1H, NH), 10.50 (s, 1H, NH), 7.78–7.29 (m, 8H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.4, 165.2, 153.8, 151.7, 147.8, 144.6, 139.7, 134.5, 131.0, 127.8, 126.7, 122.0, 120.7, 118.7, 115.7; MS: *m*/*z* 360 (M^+).

*4-Nitro-benzoic acid N′-(benzoxazole-2-carbonyl)hydrazide (**5d**)*: Brown solid, yield: 72%, mp: 172–174 °C; IR (KBr, cm^{-1}): ν 3135 (N–H), 3032 (C–H, Ar), 2974 (C–H, CH_3), 1672 (C=O), 1574 (C=C, aromatic), 1442 (C=N), 1238 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.45 (s, 1H, NH), 10.36 (s, 1H, NH), 7.71–7.38 (m, 8H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.2, 163.8, 157.8, 154.2, 148.7, 144.3, 139.6, 134.7, 131.2, 129.8, 126.3, 121.7, 119.8, 118.7, 115.2; MS: *m*/*z* 326 (M^+).

*Benzoxazole-2-carboxylic acid ethoxymethylene-hydrazide (**6a**)*: Yellow solid, yield: 70%, mp: 160–162 °C; IR (KBr, cm^{-1}): ν 3112 (N–H), 3024 (C–H, aromatic), 2962 (C–H, aliphatic), 1680 (C=O), 1560 (C=C, aromatic), 1425 (C=N), 1145 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.12 (s, 1H, NH), 7.85–7.37 (m, 4H, Ar), 4.25 (s, 1H, CH), 4.00 (q, 2H, CH_2), 1.24 (t, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.6, 155.3, 151.0, 149.6, 143.2, 127.6, 123.5, 121.0, 112.0, 63.5, 17.3; MS: *m*/*z* 233 (M^+).

*Benzoxazole-2-carboxylic acid (1-ethoxyethylidene)hydrazide (**6b**)*: Yellish green solid, yield: 72%, mp: 158–160 °C; IR (KBr, cm^{-1}): ν 3120 (N–H), 3026 (C–H, aromatic), 2974 (C–H, aliphatic), 1674 (C=O), 1568 (C=C, aromatic), 1435 (C=N), 1138 (C–O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.58 (s, 1H, NH), 7.92–7.45 (m, 4H, Ar), 4.36 (s, 3H, CH_3), 3.78 (q, 2H, CH_2), 1.28 (t, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.3, 157.2, 150.6, 148.2, 144.5, 128.3, 125.6, 122.6, 115.2, 68.5, 18.4, 14.6; MS: *m*/*z* 247 (M^+).

*Benzoxazole-2-carboxylic acid (1-ethoxypropylidene)hydrazide (**6c**)*: Pale yellow solid, yield: 74%, mp: 142–144 °C; IR (KBr, cm^{-1}): ν 3124 (N–H), 3028 (C–H, aromatic), 2965 (C–H, aliphatic), 1678 (C=O), 1570 (C=C, aromatic), 1435 (C=N), 1140 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.79 (s, 1H, NH), 7.84–7.26 (m, 4H, Ar), 3.65 (q, 2H, CH_2), 3.25 (q, 2H, CH_2), 1.24 (t, 3H, CH_3), 1.19 (t, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 156.3, 151.0, 148.6, 142.2, 126.4, 124.5, 121.0, 117.6, 58.6, 25.4, 17.8, 14.6; MS: *m*/*z* 261 (M^+).

*Benzoxazole-2-carboxylic acid (ethoxyphenylmethylene) hydrazide (**6d**)*: Brown solid, yield: 72%, mp: 170–172 °C; IR (KBr, cm^{-1}): ν 3130 (N–H), 3032 (C–H, aromatic), 2965 (C–H, aliphatic), 1674 (C=O), 1574 (C=C, aromatic), 1432 (C=N), 1138 (C–O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.69 (s, 1H, NH), 7.83–7.19 (m, 9H, Ar), 4.02 (q, 2H, CH_2), 1.31 (t, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 156.3, 153.6, 151.0, 143.2, 133.5, 132.0, 129.6, 129.2, 127.4, 127.2, 125.3, 123.2, 120.1, 112.0, 56.3, 17.0; MS: *m*/*z* 293 (M^+).

2-(5-p-Tolyl-[1,3,4]oxadiazol-2-yl)benzoxazole (***7a***): Yellish green solid, yield: 73%, mp: 163–165 °C; IR (KBr, cm^{-1}): ν 3040 (C–H, Ar), 1565 (C=C), 1435 (C=N), 1135 (C–O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.62–7.45 (m, 4H, Ar–H), 3.45 (s, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.3, 154.3, 152.0, 150.1, 141.5, 137.6, 134.2, 129.3, 129.0, 126.7, 126.5, 125.4, 123.0, 120.4, 110.5, 24.6; MS: *m*/*z* 187 (M^+).

2-[5-(4-Chloro phenyl)-[1,3,4]oxadiazol-2-yl)benzoxazole (***7b***): Pink solid, yield: 75%, mp: 125–127 °C; IR (KBr, cm^{-1}): ν 3035 (C–H, Ar), 2965 (C–H, CH_3), 1560 (C=C), 1440 (C=N), 1130 (C–O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.58–7.23 (m, 4H, Ar–H), 3.45 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.4, 155.3, 154.3, 151.0, 144.6, 139.8, 134.6, 131.0, 127.8, 127.6, 126.5, 126.2, 122.5, 121.5, 112.7; MS: *m*/*z* 201 (M^+).

2-[5-(4-Bromo phenyl)-[1,3,4]oxadiazol-2-yl)benzoxazole (***7c***): Yellow solid, yield: 71%, mp: 136–138 °C; IR (KBr, cm^{-1}): ν 3042 (C–H, Ar), 1570 (C=C), 1448 (C=N), 1146 (C–O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.78–7.25 (m, 4H, Ar–H), 4.05 (q, 2H, CH_2), 1.31 (t, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.6, 154.4, 153.8, 152.5, 145.4, 138.6, 135.3, 132.5, 128.6, 128.2, 125.6, 125.1, 123.4, 120.8, 114.6; MS: *m*/*z* 215 (M^+).

2-[5-(4-Nitro phenyl)-[1,3,4]oxadiazol-2-yl)benzoxazole (***7d***): Pale yellow solid, yield: 70%, mp: 147–149 °C; IR (KBr, cm^{-1}): ν 3045 (C–H, Ar), 1562 (C=C), 1442 (C=N), 1140 (C–O); ^1H NMR (300 MHz, DMSO-d_6): δ 8.45 (dd, 1H, *J* = 7.7, 1.5 Hz, Ar–H), 7.89 (d, 1H, *J* = 8.0 Hz, Ar–H), 7.80 (dd, 1H, *J* = 7.7, 1.5 Hz, Ar–H), 7.53 (d, 1H, *J* = 7.8 Hz, Ar–H), 7.42–7.25 (m, 5H, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.6, 155.4, 154.6, 150.2, 147.8, 136.7, 135.5, 133.2, 129.4, 129.3, 126.7, 126.0, 125.2, 122.4, 118.3; MS: *m*/*z* 263 (M^+).

2-[1,3,4]Oxadiazol-2-yl-benzoxazole (***7e***): Brown solid, yield: 71%, mp: 128–130 °C; IR (KBr, cm^{-1}): ν 3055 (C–H, Ar), 1570 (C=C), 1445 (C=N), 1138 (C–O); ^1H NMR (300 MHz, DMSO-d_6): δ 8.42 (dd, 1H, *J* = 7.7, 1.4 Hz, Ar–H), 7.85 (d, 1H, *J* = 8.2 Hz, Ar–H), 7.74 (d, 2H, *J* = 7.8 Hz, Ar–H), 7.65 (dd, 1H, *J* = 7.7, 1.4 Hz, Ar–H), 7.54 (d, 2H, *J* = 7.8 Hz, Ar–H), 7.52 (d, 1H, *J* = 7.8 Hz, Ar–H), 2.80 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.6, 155.6, 152.4, 148.7, 142.0, 127.6, 122.0, 118.6, 112.0; MS: *m*/*z* 277 (M^+).

2-(5-Methyl-[1,3,4]oxadiazol-2-yl)benzoxazole (***7f***): Pale yellow solid, yield: 73%, mp: 131–133 °C; IR (KBr, cm^{-1}): ν 3060 (C–H, Ar), 1562 (C=C), 1440 (C=N), 1128 (C–O) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.32 (dd, 1H, *J* = 7.3, 1.7 Hz, Ar–H), 8.21 (d, 1H, *J* = 8.2 Hz, Ar–H), 7.84 (d, 2H, *J* = 7.4 Hz, Ar–H), 7.78 (dd, 1H, *J* = 7.3, 1.7 Hz, Ar–H), 7.65 (d, 2H, *J* = 7.4 Hz, Ar–H), 7.42 (d, 1H, *J* = 7.8 Hz, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.4, 153.2, 150.3, 147.5, 144.5, 129.4, 125.3, 121.1, 116.5, 18.6; MS: *m*/*z* 297 (M^+).

2-(5-Ethyl-[1,3,4]oxadiazol-2-yl)benzoxazole (***7g***): Yellowish green solid, yield: 75%, mp: 143–145 °C; IR (KBr, cm^{-1}): ν 3038 (C–H, Ar), 1568 (C=C), 1446 (C=N), 1140 (C–O); ^1H NMR (300 MHz, DMSO-d_6): δ 8.43 (dd, 1H, *J* = 8.0, 1.5 Hz, Ar–H), 8.05 (d, 1H, *J* = 8.1 Hz, Ar–H), 7.81 (dd, 1H, *J* = 8.0, 1.5 Hz, Ar–H), 7.74 (d, 2H, *J* = 7.0 Hz, Ar–H), 7.65 (d, 2H, *J* = 7.0 Hz, Ar–H), 7.36 (d, 1H, *J* = 7.8 Hz, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.3, 152.5, 151.7, 146.4, 145.6, 131.5, 127.4, 126.3, 119.8, 21.2, 16.3; MS: *m*/*z* 340 (M^+).

2-(5-Phenyl-[1,3,4]oxadiazol-2-yl)benzoxazole (***7h***): Yellow solid, yield: 70%, mp: 150–152 °C; IR (KBr, cm^{-1}): ν 3028, (C–H, Ar), 1572 (C=C), 1442 (C=N), 1143 (C–O); ^1H NMR (300 MHz, DMSO-d_6): δ 8.32 (dd, 1H, *J* = 8.2, 1.6 Hz, Ar–H), 8.12 (d, 1H, *J* = 8.2 Hz, Ar–H), 7.89 (dd, 1H, *J* = 8.2, 1.6 Hz, Ar–H), 7.69 (d, 2H, *J* = 7.4 Hz, Ar–H), 7.58 (d, 2H, *J* = 7.4 Hz, Ar–H), 7.42 (d, 1H, *J* = 8.2 Hz, Ar–H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.3, 156.7, 154.0, 150.2, 141.7, 136.8, 129.7, 129.4, 128.4, 127.6, 127.2, 125.4, 124.0, 120.8, 110.5; MS: *m*/*z* 308 (M^+).

3. Results and discussion

Based on these observations, inspired by the biological profile of benzoxazoles and oxadiazoles, their increasing importance in pharmaceutical and biological fields, and in continuation of our research on biologically active heterocycles, we have introduced oxadiazole moiety into the benzoxazole ring which leads to both active pharmacophores in a single molecular frame work for the intensified biological activities. Thus we have designed and synthesized a series of novel 2-(5-substituted-[1,3,4]oxadiazol-2-yl)-benzoxazoles (**7a**–**h**) from commercially available benzoxazole-2-carboxylic acid (**1**). Benzoxazole-2-carbonyl chloride (**2**) has been synthesized from compound **1** on reaction with thionyl chloride in presence of ethanol solvent on constant stirring at room temperature for 4 h. The intermediate benzoxazole-2-carboxylic acid methyl ester (**3**) was achieved form compound **2** on esterification with methanol in presence of triethyl amine under reflux for 3 h. Compound **3** on reaction with hydrazine hydrates in presence of ethanol solvent under reflux for 8 h was turned into the key intermediate benzoxazole-2-carboxylic acid hydrazide (**4**). Benzoxazole-2-carboxylic acid *N*′-acetyl hydrazides (**5a**–**d**) have been prepared form compound **4** and different acid chlorides in dioxane solvent at reflux temperature for 4–5 h. The subsequent ring closure reaction of compounds **5a**–**d** with $POCl_3$ under reflux for 4–5 h yielded the title compounds, 2-[1,3,4]oxadiazol-2-yl-benzoxazoles (**7a**–**d**). Benzoxazole-2-carboxylic acid-ethylidene-hydrazides (**6a**–**d**) were prepared form the reaction of same intermediate **4** with suitable orthoforamtes under reflux for 10–12 h. Finally 2-[1,3,4]-oxadiazol-2-yl-benzoxazoles (**7e**–**h**) have been achieved from the reaction of **6** in refluxing pyridine for 4–5 h. The chemical structures of all the newly synthesized compounds were confirmed by their IR, ^1H NMR, ^{13}C NMR and Mass spectral data and further the compounds **7a**–**h** were used to evaluate their antimicrobial activity.

The disc diffusion method [19] was used for the screening of anti microbial activity. The *in vitro* antibacterial activity of the synthesized compounds **7a**–**h** was tested against three gram-positive bacteria *i.e. Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus faecalis* and against five Gram-negative bacteria *i.e.*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeuroginosa*, *Proteus mirabilis*, *Salmonella typhi* using a nutrient agar medium. The antifungal activity of the compounds was screened against *Candia albicans* and *Aspergillus fumigatus* using Sabouraded dextrose agar medium. The sterilized medium (autoclaved at 121 °C for 15 min) was inoculated with the suspension of the micro organisms and poured into a Petri dish to give a depth of 3–4 mm. The paper impregnated with the synthesized compounds **7a**–**h** (300 μg/mL in DMF) was placed on the solidified medium. The plates were preincubated for 1 h at room temperature and incubated at 37° for 24 h and 48 h for antibacterial and antifungal activity respectively. Amicacin (300 μg/mL) was used in antibacterial activity studies, whereas fluconazole (300 μg/mL) was used in antifungal activity studies as reference compounds. After incubation, the relative susceptibility of the micro organisms to the potential antimicrobial agent is demonstrated by a clear zone of growth inhibition around the disc. The lowest concentration (highest dilution) of the compounds at which there was no visually detectable bacterial growth was taken as minimum inhibitory concentration (MIC) and it was determined for the compounds **7a**–**h**. The inhibition zone caused by the various compounds on the micro organisms was measured and the activity rated on the basis of the size of the inhibition zone. The observed zone of inhibition in mm is presented in Table 1.

The results of the antimicrobial screening of the tested compounds revealed that, all the tested compounds exhibited antimicrobial activity comparable with that of reference compounds. Most of the compounds showed significant activity against both bacteria and fungi. Some of the compounds showed high activity against both the bacteria and fungi. Most of the compounds showed highly to moderate activity against bacteria

Table 1
Antimicrobial activity of compounds **7a–h** in zone of inhibition (activity index) in mm.[a]

Compound	Antibacterial activity								Antifungal activity	
	S. aureus	*S. albus*	*S. faecalis*	*K. pneumoniae*	*E. coli*	*P. aeuroginosa*	*P. mirabilis*	*S. Typhi*	*C. albicans*	*A. fumigatus*
7a	14 (0.58)	12 (0.54)	19 (0.73)	14 (0.60)	02 (0.10)	15 (0.60)	10 (0.50)	08 (0.44)	14 (0.60)	16 (0.64)
7b	16 (0.66)	14 (0.63)	12 (0.46)	15 (0.65)	11 (0.55)	16 (0.64)	12 (0.57)	09 (0.50)	16 (0.69)	15 (0.60)
7c	15 (0.62)	16 (0.72)	16 (0.61)	17 (0.73)	– (0.00)	17 (0.65)	14 (0.66)	10 (0.55)	15 (0.65)	18 (0.72)
7d	18 (0.75)	15 (0.68)	18 (0.69)	19 (0.82)	14 (0.70)	18 (0.65)	15 (0.71)	12 (0.66)	17 (0.74)	19 (0.76)
7e	20 (0.83)	16 (0.72)	19 (0.73)	20 (0.87)	13 (0.65)	20 (0.65)	17 (0.80)	14 (0.77)	15 (0.65)	17 (0.68)
7f	21 (0.87)	15 (0.68)	18 (0.69)	19 (0.82)	17 (0.85)	21 (0.65)	18 (0.85)	15 (0.83)	21 (0.91)	23 (0.92)
7g	20 (0.83)	16 (0.72)	20 (0.77)	20 (0.87)	16 (0.80)	22 (0.65)	19 (0.90)	16 (0.88)	20 (0.87)	22 (0.88)
7h	22 (0.91)	20 (0.90)	24 (0.92)	21 (0.91)	11 (0.55)	23 (0.65)	12 (0.57)	12 (0.66)	20 (0.87)	22 (0.88)
Amicacin	24	22	26	23	20	25	21	18	–	–
Fluconazole	–	–	–	–	–	–	–	–	23	25

[a] Activity index – zone of inhibition of the sample/zone of inhibition of the standard.

and moderate to low activity on fungi. Compound **7a** was good active only against *S. faecalis* and almost inactive toward *E. coli*. This compound exhibited moderate activity against the rest of organisms. Compound **7b** showed mild to moderate activity against the tested Gram-positive and Gram-negative organisms. In contrast, surprisingly the compound **7c** with ethyl substituent is compare with other molecules was found to be totally inactive against *E. coli*. Highest antimicrobial activity was observed in the product **7h** with *para* nitro phenyl derivative against *S. aureus*, *S. albus*, *S. faecalis*, *K. pneumoniae* and *P. aeuroginosa* as compared to the standard, but shows only moderate activity against *E. coli* and *P. mirabilis*. This compound also performed high activity against two fungal organisms with marked activity index. In antimicrobial activity studies, it is clear that, an introduction of nitro group reflected better activity against different organisms. Both compounds **7f** and **7g** with relative substituents exhibit highest antifungal activity against *C. albicans* and *A. fumigatus* as compared to the standard drug used. It can be concluded that the antimicrobial activity of such compounds may change by introduction or elimination of a specific group. The remaining compounds exhibit moderate to good antimicrobial activity against all organisms employed.

4. Conclusion

The outstanding properties of this new class of antibacterial and antifungal substances deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed. More extensive study is also warranted to determine additional physicochemical and biological parameters to have a deeper insight into structure-activity relationship and to optimize the effectiveness of this series of molecules.

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