Res Chem Intermed
DOI 10.1007/s11164-013-1437-0

Synthesis and antibacterial activity of novel ethyl 2-alkoxyimino-2-benzimidazol-2-yl acetates bearing a morpholine group

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Received: 11 August 2013 / Accepted: 3 October 2013

Abstract In the search for new benzimidazole derivatives with high antibacterial activity and for which bacterial resistance is low, novel ethyl 2-(alkoxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1*H*-benzimidazol-2-yl]acetates have been synthesized by multi-step reactions and characterized by ^{1}H NMR, IR, and ESI–MS analysis. The compounds were evaluated for in-vitro antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The results revealed that four of the compounds had excellent bioactivity against *S. aureus*, with IC_{50} values of 12.72–26.58 μg/mL, even better than that of the control agent (levofloxacin hydrochloride).

Keywords Benzimidazole · *N*-Alkoxyimine · Morpholine · Synthesis · Antibacterial activity

Introduction

Antibacterial drugs are widely used clinically and are important in disease control and treatment. In recent years, however, increasing and unreasonable use of antibacterial drugs have resulted in the development of resistant pathogens [1–3], and overcoming drug resistance has become a crucial issue in antibacterial medicine research. The development of new antibacterial agents with novel structures is an important strategy for solving the problem of bacterial resistance.

Benzimidazole derivatives have attracted much interest because of their broad biological activity, for example antibacterial [4], antifungal [5], antitumor [6], anticancer [7], and anti-inflammatory [8] activity. As antimicrobial agents,

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Published online: 16 October 2013

especially, these derivatives have been extensively studied [9]. For example, dibenzyl ether bearing a benzimidazole group has high antibacterial activity which can effectively inhibit *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *Escherichia coli*, with MIC values of 3.12–6.25 μg/mL [10]. 4-Benzimidazol-2-yl benzoyl hydrazone has good inhibitory activity against *E. coli*, *Bacillus proteus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, with MIC values of 6.25–50 μg/mL [5]. *N*-Alkoxyimines have also attracted much attention, because of their remarkable biological activity, for example antibacterial [11], antifungal [12], insecticidal [13], and herbicidal [14] activity. Moreover, many *N*-alkoxyimine derivatives have the advantages of high efficiency and low toxicity, and are environmentally friendly. Many benzimidazole derivatives bearing a morpholine group have antimicrobial activity, e.g. antibacterial and antifungal activity [15–17]. Motivated by these findings, and in continuation of our interest in the synthesis and bioactivity of benzimidazole derivatives [18, 19], we have attached an *N*-alkoxyimine group to a benzimidazole ring. Also, to improve penetration of pathogen cells, we linked an ester moiety to the molecule. Thus, we designed and synthesized ten novel ethyl 2-(alkoxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1*H*-benzimidazol-2-yl]acetates, and evaluated their antibacterial activity against two types of bacteria. The synthetic pathway is shown in Scheme 1.

Experimental

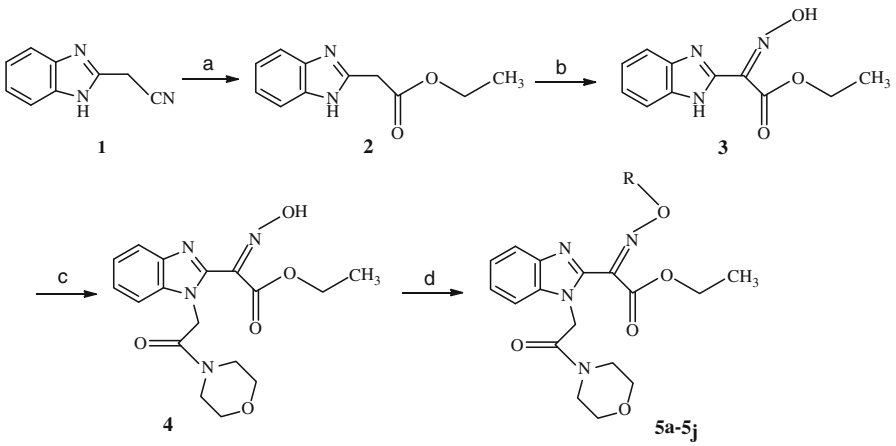
Chemicals and instruments

Commercial reagents were purchased from Aladdin Chemistry (Shanghai, China) and BASF Chemical (Tianjin, China). Melting points were determined on an X-5 microscope melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR-440 infrared spectrophotometer, as KBr discs or as films of solutions in $CHCl_3$. ^1H NMR spectra were acquired on a Bruker AM-400 spectrometer with $CDCl_3$ as solvent and TMS as internal standard. ESI mass spectra were acquired on an Agilent 6410 triple-quadrupole mass spectrometer. Microwave-assisted synthesis was performed in an XH-100B microwave reactor.

1*H*-Benzimidazol-2-yl acetonitrile (**1**) was synthesized in accordance with a procedure reported elsewhere [20]. The key intermediate ethyl 2-(1*H*-benzimidazol-2-yl)acetate (**2**) was prepared in accordance with Ref. [21].

Synthesis of ethyl 2-(1*H*-benzimidazol-2-yl)-2-hydroxyimino acetate **3**

A solution of 4.08 g (20 mmol) compound **2** in glacial acetic acid (30 mL) was added dropwise to 45 % aqueous sodium nitrite solution (10 mL, 65 mmol) at 0–5 °C under a nitrogen atmosphere. The reaction mixture was stirred at 20 °C for 2 h then poured into ice–water and neutralized with saturated sodium carbonate solution. The precipitate was isolated by filtration, washed with water, and vacuum dried. The solid obtained was recrystallized from methanol–diethyl ether (2:1 *v*/*v*) to give compound **3** (3.34 g; yield 72 %) as pale yellow crystals, m.p. 152.3–153.9 °C.



Scheme 1 Synthetic route to compounds **5a**–**j**. Reagents and conditions: **a** 95 % EtOH, H_2SO_4, reflux, 6 h; **b** $NaNO_2$, AcOH, 0–5 °C, 2 h, **c** 4-(chloroacetyl)morpholine, K_2CO_3, MeCN, microwave, 6 min; **d** RX or $ArCH_2X$, K_2CO_3, TABI, acetone, r.t., overnight

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44–1.49 (t, $J = 7.2$ Hz, 3H, CH_3), 4.47–4.53 (q, $J = 7.2$ Hz, 2H, CH_2), 7.26–7.69 (m, 4H, ArH), 11.06 (s, 1H, OH). IR (KBr) ν (cm^{-1}): 3416 (N–H), 3258 (O–H), 3070 (Ar–H), 1714 (C=O), 1622 (C=N), 1279, 1043 (C(O)–O–C). ESI–MS *m*/*z*: calcd for $C_{11}H_{11}N_3O_3$ $[M + Na]^+$ 256.2, obsd $[M + H]^+$ 256.4.

Synthesis of ethyl 2-(hydroxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***4***

Method A A mixture of compound **3** (3.3 g, 14 mmol), anhydrous potassium carbonate (2.4 g, 14 mmol), and 4-(chloroacetyl)morpholine (2.5 g, 15 mmol) in acetone (70 mL) was heated under reflux for 2 h. After cooling to room temperature, the precipitate was isolated by filtration and the solvent was removed from the filtrate by rotary evaporation, leaving an oily residue which was purified by silica gel chromatography with acetone–petroleum ether (1:5 *v*/*v*) to furnish compound **4** (1.48 g; yield 30 %) as a white solid.

Method B A well-ground mixture of compound **3** (0.47 g, 2 mmol), anhydrous potassium carbonate (0.28 g, 2 mmol), and dibenzo-18-crown-6 (0.10 g) was added to a solution of 4-(chloroacetyl)morpholine (0.33 g, 2 mmol) in acetonitrile (10 mL). The reaction mixture was placed in a microwave reactor and subjected to microwave irradiation (500 W) for 6 min. After cooling to room temperature, the mixture was filtered and the solvent was removed from the filtrate by rotary evaporation to afford a pale yellow solid. The solid was washed with an appropriate amount of water–acetonitrile (3:1 *v*/*v*) to give compound **4** (0.37 g; yield 51 %) as a white solid.

M.p. 176.8–178.3 °C (from acetone–H_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38–1.42 (t, 3H, J = 7.2 Hz, CH_3), 3.40–3.77 (m, 8H, morpholine-H), 4.45–4.51 (q, 2H, J = 7.2 Hz, $COOCH_2$), 5.12 (s, 2H, NCH_2), 7.26–7.89 (m, 4H, ArH), 12.36 (s, 1H, OH). IR (KBr) v (cm^{-1}): 3249 (O–H), 3078 (Ar–H), 1748 (C=O), 1672 (C=O), 1272, 1,064 (C(O)–O–C). ESI–MS m/z: calcd for $C_{17}H_{20}N_4O_5$ $[M + Na]^+$ 383.4, obsd 383.6.

General procedure for synthesis of ethyl 2-(alkoxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetates ***5a–5j***

A mixture of compound **4** (1.5 mmol), anhydrous potassium carbonate (2.8 g, 20 mmol), the corresponding halohydrocarbon (1.5 mmol), and tetrabutylammonium iodide (TBAI, 0.1 g) in anhydrous acetone (20 mL) was stirred at room temperature overnight. The solvent was removed by rotary evaporation, furnishing an oily residue which was purified by silica gel chromatography with dichloromethane–methanol (20:1 v/v) to give compounds **5a**–**5j**.

Ethyl 2-(methoxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5a***

White solid, yield 66 % (0.37 g), m.p. 142.2–144.8 °C (from ethanol–H_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.33 (t, 3H, J = 7.2 Hz, $COOCH_2C\mathbf{H}_3$), 3.32–3.67 (m, 8H, morpholine-H), 3.89 (s, 3H, $NOCH_3$), 4.37 (q, 2H, J = 7.2 Hz, $COOCH_2$), 4.95 (s, 2H, NCH_2), 7.26–7.83 (m, 4H, ArH). IR (KBr) v (cm^{-1}): 3053 (Ar–H), 1734 (C=O), 1660 (C=O), 1614 (C=N), 1239, 1029 (C(O)–O–C). ESI–MS m/z: $[M + Na]^+$ 397.2, $[M + H]^+$ 375.3, $[M - 101 - OR]^+$ 242.5, herein 101 means the weight of (methyl + morpholinyl). HRMS m/z: $[M + Na]^+$ calcd for $C_{18}H_{22}N_4O_5$ 397.3808, obsd 397.3802.

Ethyl 2-(ethoxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5b***

White solid, yield 49 % (0.29 g), m.p. 117.1–118.7 °C (from ethanol–H_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.31 (t, 3H, J = 7.2 Hz, $COOCH_2C\mathbf{H}_3$), 1.45 (t, 3H, J = 7.2 Hz, $NOCH_2C\mathbf{H}_3$), 3.31–3.67 (m, 8H, morpholine-H), 4.32–4.39 (m, 4H, $COOCH_2$- and $NOCH_2$), 4.94 (s, 2H, NCH_2), 7.27–7.83 (m, 4H, ArH). IR (KBr) v (cm^{-1}): 3062 (Ar–H), 1735 (C=O), 1,655 (C=O), 1618 (C=N), 1238, 1027 (C(O)–O–C). ESI–MS m/z: $[M + Na]^+$ 411.4, $[M + H]^+$ 389.4, $[M - 101 - OR]^+$ 242.7. HRMS m/z: $[M + Na]^+$ calcd for $C_{19}H_{24}N_4O_5$ 411.4074, obsd. 411.4069.

Ethyl 2-(n-propoxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5c***

White solid, yield 50 % (0.30 g), m.p. 153.5–154.8 °C (from ethanol–H_2O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (t, 3H, J = 7.6 Hz, $NO(CH_2)_2C\mathbf{H}_3$), 1.41 (t, 3H, J = 7.2 Hz, $COOCH_2C\mathbf{H}_3$), 1.82–1.88 (m, 2H, $NOCH_2C\mathbf{H}_2$), 3.32–3.69 (m, 8H,

morpholine-H), 4.39 (q, 2H, $J = 7.2$ Hz, $COOCH_2$), 4.51 (t, 2H, $J = 7.2$ Hz, $NOCH_2$), 4.94 (s, 2H, NCH_2), 7.27–7.79 (m, 4H, ArH). IR (KBr) ν (cm^{-1}): 3051 (Ar–H), 1740 (C=O), 1664 (C=O), 1617 (C=N), 1237, 1027 (C(O)–O–C). ESI–MS *m/z*: $[M + Na]^+$ 425.4, $[M + H]^+$ 403.5, $[M - 101 - OR]^+$ 242.8. HRMS *m/z*: $[M + Na]^+$ calcd for $C_{20}H_{26}N_4O_5$ 425.4339, obsd.425.4336.

Ethyl 2-(n-butoxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5d***

White solid, yield 46 % (0.29 g), m.p. 167.3–168.6 °C (from ethanol–H_2O). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.92 (t, 3H, $J = 7.6$ Hz, NO $(CH_2)_3C\mathbf{H}_3$), 1.31 (t, 3H, $J = 7.2$ Hz, $COOCH_2C\mathbf{H}_3$), 1.35 (m, 2H, $NO(CH_2)_2C\mathbf{H}_2$), 1.71 (m, 2H, $NOCH_2C\mathbf{H}_2$), 3.31–3.66 (m, 8H, morpholine-H), 4.29 (t, 2H, $J = 7.6$ Hz, $NOCH_2$), 4.36 (q, 2H, $J = 7.2$ Hz, $COOCH_2$), 4.93 (s, 2H, NCH_2), 7.27–7.82 (m, 4H, ArH). IR (KBr) ν (cm^{-1}): 3059 (Ar–H), 1727 (C=O), 1662 (C=O), 1610 (C=N), 1239, 1016 (C(O)–O–C). ESI–MS *m/z*: $[M + Na]^+$ 439.4, $[M + H]^+$ 417.4, $[M - 101 - OR]^+$ 242.6. HRMS *m/z*: $[M + Na]^+$ calcd for $C_{21}H_{28}N_4O_5$ 439.4605, obsd. 439.4609.

Ethyl 2-(benzyloxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5e***

Pale yellow solid, yield 60 % (0.41 g), m.p. 66.7–69.8 °C (from ethanol–H_2O). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.40 (t, 3H, $J = 7.2$ Hz, $COOCH_2C\mathbf{H}_3$), 3.31–3.72 (m, 8H, morpholine-H), 4.51 (q, 2H, $J = 7.2$ Hz, $COOCH_2$), 4.78 (s, 2H, NCH_2), 5.74 (s, 2H, OCH_2-Ar), 7.07–7.88 (m, 9H, ArH). IR (KBr) ν (cm^{-1}): 3058 (Ar–H), 1735 (C=O), 1666 (C=O), 1607 (C=N), 1243, 1028 (C(O)–O–C). ESI–MS *m/z*: $[M + Na]^+$ 473.5, $[M + H]^+$ 451.4, $[M - 101 - OR]^+$ 242.6. HRMS *m/z*: $[M + Na]^+$ calcd for $C_{24}H_{26}N_4O_5$ 473.4767, obsd. 473.4771.

Ethyl 2-(4-fluorobenzyloxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5f***

Yellow oil, yield 57 % (0.40 g). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.16 (t, 3H, $J = 7.2$ Hz, $COOCH_2C\mathbf{H}_3$), 3.32–3.69 (m, 8H, morpholine-H), 4.18 (q, 2H, $J = 7.2$ Hz, $COOCH_2$), 4.93 (s, 2H, NCH_2), 5.57 (s, 2H, OCH_2-Ar), 6.95–7.40 (m, 8H, ArH). IR ($CHCl_3$) ν (cm^{-1}): 3064 (Ar–H), 1735 (C=O), 1664 (C=O), 1605 (C=N), 1224, 1029 (C(O)–O–C). ESI–MS *m/z*: $[M + Na]^+$ 491.5, $[M + H]^+$ 469.4, $[M - 101 - OR]^+$ 242.5. HRMS *m/z*: $[M + Na]^+$ calcd for $C_{24}H_{25}FN_4O_5$ 491.4672, obsd. 491.4668.

Ethyl 2-(4-nitrobenzyloxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5g***

Yellow oil, yield 58 % (0.43 g). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.41 (t, 3H, $J = 7.2$ Hz, $COOCH_2C\mathbf{H}_3$), 3.41–3.76 (m, 8H, morpholine-H), 4.48 (q, 2H,

$J = 7.2$ Hz, $COOCH_2$), 5.13 (s, 2H, NCH_2), 5.77 (s, 2H, OCH_2-Ar), 7.21–8.17 (m, 8H, ArH). IR ($CHCl_3$) v (cm^{-1}): 3064 (Ar–H), 1740 (C=O), 1661 (C=O), 1601 (C=N), 1239, 1032 (C(O)–O–C). ESI–MS *m/z*: $[M + Na]^+$ 518.5, $[M + H]^+$ 496.3, $[M - 101 - OR]^+$ 242.6. HRMS *m/z*: $[M + Na]^+$ calcd for $C_{24}H_{25}N_5O_7$ 518.4743, obsd. 518.4749.

Ethyl 2-(4-cyanobenzyloxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5h***

Pale yellow solid, yield 45 % (0.32 g), m.p. 75.3–77.4 °C (from ethanol–H_2O). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.18 (t, 3H, $J = 7.2$ Hz, $COOCH_2C\mathbf{H}_3$), 3.32–3.71 (m, 8H, morpholine-H), 4.20 (q, 2H, $J = 7.2$ Hz, $COOCH_2$), 4.93 (s, 2H, NCH_2), 5.72 (s, 2H, OCH_2-Ar), 7.18–7.88 (m, 8H, ArH). IR (KBr) v (cm^{-1}): 3051 (Ar–H), 2228 (C≡N), 1739 (C=O), 1664 (C=O), 1609 (C=N), 1238, 1029 (C(O)–O–C). ESI–MS *m/z*: $[M + Na]^+$ 498.6, $[M + H]^+$ 476.7, $[M - 101 - OR]^+$ 242.4. HRMS *m/z*: $[M + Na]^+$ calcd for $C_{25}H_{25}N_5O_5$ 498.4862, obsd 498.4866.

Ethyl 2-(4-methoxylbenzyloxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5i***

Yellow oil, yield 53 % (0.38 g). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.15 (t, 3H, $J = 7.2$ Hz, $COOCH_2C\mathbf{H}_3$), 3.30–3.68 (m, 8H, morpholine-H), 3.75 (s, 3H, OCH_3), 4.17 (q, 2H, $J = 7.2$ Hz, $COOCH_2$), 4.93 (s, 2H, NCH_2), 5.50 (s, 2H, OCH_2-Ar), 6.78–7.84 (m, 8H, ArH). IR ($CHCl_3$) v (cm^{-1}): 3061 (Ar–H), 1728 (C=O), 1667 (C=O), 1612 (C=N), 1241, 1029 (C(O)–O–C). ESI–MS *m/z*: $[M + Na]^+$ 503.7, $[M + H]^+$ 481.6, $[M - 101 - OR]^+$ 242.7. HRMS *m/z*: $[M + Na]^+$ calcd for $C_{25}H_{28}N_4O_6$ 503.5027, obsd 503.5031.

Ethyl 2-((2,4-dichlorobenzyl)oxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1H-benzimidazol-2-yl]acetate ***5j***

Pale yellow solid, yield 54 % (0.42 g), m.p. 77.7–80.2 °C (from ethanol–H_2O). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.23 (t, 3H, $J = 7.2$ Hz, $COOCH_2C\mathbf{H}_3$), 3.29–3.77 (m, 8H, morpholine-H), 4.24 (q, 2H, $J = 7.2$ Hz, $COOCH_2$), 4.87 (s, 2H, NCH_2), 5.62 (s, 2H, CH_2-Ar), 6.92–7.86 (m, 7H, ArH). IR (KBr) v (cm^{-1}): 3058 (Ar–H), 1740 (C=O), 1664 (C=O), 1590 (C=N), 1237, 1030 (C(O)–O–C). ESI–MS *m/z*: $[M + Na+H]^+$ 543.3, $[M + H]^+$ 520.5, $[M - 101 - OR]^+$ 242.8. HRMS *m/z*: $[M + Na]^+$ calcd for $C_{24}H_{24}Cl_2N_4O_5$ 542.3669, obsd 542.3674.

Antibacterial activity assays

The antibacterial activity of compounds **5a**–**5j** against *E. coli* (ATCC 29522) and *S. aureus* (ATCC 29213) (obtained from the College of Animal Science and Technology, Shandong Agricultural University, Taian, China) was evaluated by use of the plate count method [22]. The culture medium was obtained by mixing a

solution of **5** with nutrient agar medium on which bacteria suspensions were coated uniformly.

Culture was performed at 37 ± 1 °C for 16 h. Levofloxacin hydrochloride, a commercial pharmaceutical, was used as positive control, and sterile water was used as blank. Three replicates were performed in antibacterial activity assays. Inhibition was expressed as the mean of values obtained from three independent experiments. Last, the concentration that inhibited growth by 50 % (IC_{50}) was calculated (Table 1). Percentage inhibition was calculated by use of the formula:

$$\text{Inhibition rate} = \frac{N_0 - N_1}{N_0} \times 100(\%)$$

where N_0 is the number of bacterial colonies in the blank test and N_1 is the number of bacterial colonies in the presence of the tested compounds.

Results and discussion

Chemistry

Compounds **5a**–**5j** were prepared by use of the reaction sequence shown in Scheme 1. The starting material 1*H*-benzimidazol-2-yl acetonitrile (**1**) was treated with 95 % alcohol and concentrated sulfuric acid to give ethyl 2-benzimidazol-2-yl acetate (**2**), which was reacted with sodium nitrite in glacial acetic acid to produce ethyl 2-(1*H*-benzimidazol-2-yl)-2-hydroxyimino acetate (**3**). Compound **3** was reacted with 4-(chloroacetyl)morpholine in acetone to yield intermediate **4**, followed by etherification to obtain compounds **5a**–**5j**.

To improve the yield and reduce reaction time we used a microwave-assisted synthetic method. For preparation of compound **4** the reaction was performed with a yield of 51 % in 6 min whereas the yield was low (30 %) under conventional reflux conditions for 2 h. However, compound **5** could not be obtained by use of the microwave-assisted method because the *N*-alkoxyimine readily decomposes at high temperature.

The identities of all the newly synthesized compounds were confirmed by ^{1}H NMR, IR, and MS analysis; results were in good agreement with the proposed structures.

Antibacterial activity

The antibacterial activity of the compounds against *E. coli* (ATCC 29522) and *S. aureus* (ATCC 29213) is presented in Table 1. All the compounds had weak antibacterial activity against *E. coli*; IC_{50} values were larger than that of the positive control, levofloxacin hydrochloride (IC_{50} = 28.38 μg/mL). However, most of the compounds, especially **5a**, **5f**, **5h**, and **5i**, had significant antibacterial activity against *S. aureus*; IC_{50} values of **5a**, **5f**, **5h**, and **5i** were 19.34, 12.72, 20.34, and 26.58 μg/mL, respectively, much higher than that of the control (IC_{50} = 48.51 μg/mL).

Table 1 The in vitro antibacterial activities of compounds **5a–5j**

Compound	*R*	IC_{50} (μg/mL)	
		E. coli	*S. aureus*
5a	CH_3	85.17	19.34
5b	CH_2CH_3	103.04	30.71
5c	*n*-C_3H_7	111.15	43.54
5d	*n*-C_4H_9	114.86	67.56
5e	$C_6H_5CH_2$	88.84	50.54
5f	4-$FC_6H_4CH_2$	116.28	12.72
5g	4-$O_2NC_6H_4CH_2$	106.99	47.24
5h	4-$NCC_6H_4CH_2$	83.29	20.34
5i	4-$CH_3OC_6H_4CH_2$	56.35	26.58
5j	2,4-$Cl_2C_6H_3CH_2$	84.80	48.51
Levofloxacin hydrochloride		28.38	41.37

Conclusion

In summary, ten novel ethyl 2-(alkoxyimino)-2-[1-(4-morpholinocarbonylmethyl)-1*H*-benzimidazol-2-yl] acetates were synthesized by multi-step reactions, and their in-vitro antibacterial activity against *E. coli* and *S. aureus* was evaluated. The result revealed that four of the compounds had excellent bioactivity against *S. aureus*, with IC_{50} values of 12.72–26.58 μg/mL, even better than that of levofloxacin hydrochloride. This study is a useful reference in the search for novel benzimidazole antibacterial agents.

Acknowledgments This project was supported by Shandong Province Natural Science Foundation (no. ZR2009BM044).

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