Article

Synthesis and Antibacterial Evaluation of 2-Ethyl-1-(4-substituted)phenyl-1*H*-imidazole Derivatives as Open-Chain Analogues of 7-Alkoxyl-4,5-dihydro-imidazo[1,2-*a*]quinolines

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A novel series of 2-ethyl-1-(4-substituted)phenyl-1*H*-imidazole derivatives was designed, synthesized, and tested for its antibacterial activity against various bacterial strains. Most of the synthesized compounds showed potent inhibition of several Gram-positive and Gram-negative bacterial strains with minimum inhibitory concentration (MIC) values in the range of 4-256 nmol mL^{-1} *in vitro*. The compound [2-ethyl-1-(4-pentoxy)phenyl-1*H*-imidazole] exhibited the most potent inhibitory activity. The MIC of this compound against *S. aureus* was found to be 8 nmol mL^{-1}, smaller than that of the reference agents, ciprofloxacin and amoxicillin. Furthermore, the compound exhibited modest activity against several bacterial strains in a dose range of 8-256 nmol mL^{-1}.

Keywords: 2-ethyl-1-(4-substituted)phenyl-1*H*-imidazole, drug design, MIC, antibacterial activity

Introduction

The quinoline analogues have been very successful both medically and commercially over the decades. It could be easily synthesized, allowing stepwise improvements in essentially all critical drug-related categories (potency, antimicrobial spectrum, evolving resistance issues, pharmacokinetic profile, etc.). Structure modification of quinoline led to new efficient antibacterial candidate agents.[1-4] However, due to clinically significant parasite resistance to antibacterial drugs, there is an urgent need to promote the quest for new efficacious drugs to fight against resistant pathogens.[5-7]

In the course of searching for newer antibacterial candidates, we focused our attention on modifying the structure of quinoline and its analogues. Our research group has recently demonstrated that introduction of an imidazole ring to quinoline moiety caused an increase in its activity *in vitro*.[8] Compound **I** (Figure 1) was found to possess outstanding antibacterial activity, especially against Gram-negative (G⁻) bacterial strains. The minimum inhibitory concentration (MIC) of compound **I** against *Escherichia coli* (*E. coli*) was 0.5 µg mL^{-1}, lower than that

of the reference agents, ciprofloxacin and amoxicillin. Compound **I** was fused with an imidazole ring at positions 1 and 2 of the quinoline ring. The inhibitory activity of compound **I** against Gram-positive (G⁺) bacterial strains was found to be weaker than that against the Gram-negative strains. We hypothesized that the rigid quinoline ring was probably causing this difference in activity. Hence, it was designed a series of 2-ethyl-1-(4-substituted)phenyl-1*H*-imidazoles as open-chain analogues of compound **I**. Compared with compound **I**, the new scaffold had a more flexible construction, therefore, it was expected that these compounds could yield exciting pharmacological results.

Herein, we report the synthesis and activity evaluation of novel 2-ethyl-1-(4-substituted)phenyl-1*H*-imidazole derivatives as potent anti-bacterial agents. The strategy is intended to obtain potent broad-spectrum antibacterial activity using traditional medicinal chemistry techniques. All synthesized compounds were evaluated by the *Staphylococcus aureus* (*S. aureus*) and *E. coli* inhibition test. Further, compounds with outstanding activity were tested against several other bacterial strains, containing both G⁺/G⁻ bacteria. In addition, their structure-activity relationship would also be discussed.
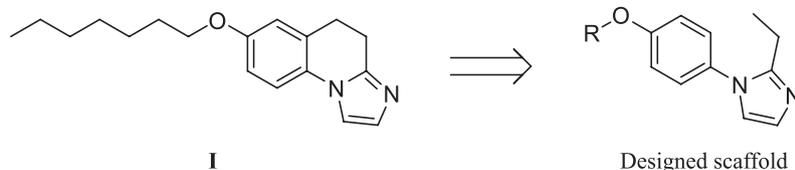
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Figure 1. Structure of leading compound **I** and open-chain analogue 2-ethyl-1-(4-substituted)phenyl-1*H*-imidazoles.

Experimental

Material and methods

Analytical thin-layer chromatography (TLC) was conducted on pre-coated silica gel 60 F254 plates (Qingdao Haiyang, China). Visualization of the plates was accomplished by using UV light and/or iodine. Chromatography was conducted on silica gel 60, 200 mesh (Qingdao Haiyang, China), under low pressure. Melting points were determined in open capillary tubes and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using AV-300 FT-NMR spectrometer (Bruker BioSpin AG, Fallanden, Switzerland). Chemical shifts (δ) are reported in ppm relative to tetramethylsilane. Mass spectra were recorded on an HP1100LC/MSD electrospray ionization mass spectrometer (ESI-MS) (Agilent Technologies, Santa Clara, CA, USA). All starting materials were obtained from commercial sources (Ouhechem, Beijing) and used as received unless otherwise indicated. All other chemicals were of analytical grade. The detailed procedures for preparing compounds **5a-5q** are described below.

Procedure for synthesis of 2-ethyl-1-(4-nitro)phenyl-1*H*-imidazole (2)

A mixture of **1** (9.61 g, 100 mmol), *p*-fluoro nitrobenzene (15.51 g, 110 mmol) and NaOH (4.40 g, 110 mmol) in dimethyl sulfoxide (DMSO, 100 mL) was heated to 90 °C with stirring. After 5 h, the end of the reaction was confirmed by TLC. After cooling to room temperature, the precipitates was filtered and washed with appropriate water. It was obtained a light yellow solid, 18.6 g, yield 84.9%.

Procedure for synthesis of 2-ethyl-1-(4-amino)phenyl-1*H*-imidazole (3)

A mixture of **2** (4.34 g, 20 mmol), and Pd/C (1 g, 10%, 60% H$_2$O) in ethanol (80 mL) was stirred under an atmosphere of hydrogen for 4 h at 60 °C. After the solution was cooled, the reaction mixture was filtered and evaporated to give a moderate yield of compound **3**. The compound was used directly in the next step without further purification.

Procedure for synthesis of 2-ethyl-1-(4-hydroxy)phenyl-1*H*-imidazole (4)

Under ice-salt bath, to a solution of compound **3** (3.74 g, 20 mmol) in sulfuric acid solution (25%, 50 mL), it was added dropwise sodium nitrite solution (1.52 g, 22 mmol, in 22 mL of H$_2$O), the reaction temperature was maintained at 0 °C or below. When the addition of the sodium nitrite was complete, it was stirred at 5 °C for 1 h to complete the reaction.

The diazonium intermediate was poured into a 70 °C of sulfuric acid solution (25%, 50 mL). The resulting mixture was stirred at 70 °C for 2 h. While the solution was cooled, the reaction mixture was adjusted to pH = 8 with saturated NaHCO$_3$ solution and extracted with ethyl acetate (3 × 200 mL). The combined organic layer was dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure. The residue obtained was purified by chromatography on a silica gel column (ethyl acetate) to give **4** as a light yellow solid. Yield 50.8%, mp 209-211 °C. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.22-1.27 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 2.67-2.74 (q, 2H, *J* 6.0, 9.0 Hz, –CH$_2$–), 6.99-7.26 (m, 5H, Ar-H). ESI-MS *m/z* 189 [M + 1]$^+$.

General synthetic procedure for target compounds 5a-5q

To a solution of compound **4** (0.19 g, 1 mmol) and sodium hydroxide (0.044 g, 1.1 mmol) in dimethylformamide (DMF, 10 mL), it was added the relevant alkyl halide (1.1 mmol), and the mixture was stirred at 80 °C until the completion of the reaction, which was as confirmed by TLC. The reaction was cooled and evaporated to dryness in vacuum. Target compounds **5a-5q** were obtained after purification of the crude residues on a silica gel column (ethyl acetate:petroleum ether, 2:1). The yields, melting points, and spectral data for each compound are provided below.

2-Ethyl-1-(4-benzyloxy)phenyl-1*H*-imidazole (5a)

Yield 81%; mp 66-68 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.22-1.27 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 1.97 (s, H, –CH–), 2.59-2.66 (q, 2H, *J* 6.0, 6.0 Hz, –CH$_2$–), 5.11 (s, 2H, –CH$_2$O–), 6.93-7.47 (m, 9H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 20.48, 70.38, 115.44, 120.92, 127.14, 127.39, 127.46, 128.20, 128.69, 136.43, 149.87, 158.56. ESI-MS *m/z* 279 [M + 1]$^+$.

2-Ethyl-1-[4-(2-chloro)benzyloxy]phenyl-1*H*-imidazole (5b)

Yield 84%; mp 98-100 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.22-1.27 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 2.60-2.67 (q, 2H, *J* 6.0, 6.0 Hz, –CH$_2$–), 5.22 (s, 2H, –CH$_2$O–), 6.95-7.58 (m, 8H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 20.48, 67.53, 115.48, 120.90, 127.04, 127.19, 127.41, 128.82, 129.25, 129.51, 131.25, 132.71, 134.15, 149.85, 158.29. ESI-MS *m/z* 313 [M + 1]$^+$.

2-Ethyl-1-[4-(3-chloro)benzyloxy]phenyl-1*H*-imidazole (5c)

Yield 77%; oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.22-1.27 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 2.88-2.96 (t, 2H, *J* 24.0 Hz, –CH–), 5.09 (s, 2H, –CH$_2$O–), 5.94-7.46 (m, 9H, Ar–H), 8.02 (s, H, –CH–); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.34, 20.49, 69.50, 115.43, 120.90, 125.32, 127.21, 127.41, 127.44, 128.32, 129.97, 131.27, 134.65, 138.50, 149.84, 158.22. ESI-MS *m/z* 313 [M + 1]$^+$.

2-Ethyl-1-[4-(4-chloro)benzyloxy]phenyl-1*H*-imidazole (5d)

Yield 88%; mp 95-94 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.22-1.27 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 5.08 (s, 2H, –CH$_2$O–), 6.94-7.39 (m, 8H, Ar–H). ESI-MS *m/z* 313 [M + 1]$^+$.

2-Ethyl-1-[4-(4-fluoro)benzyloxy]phenyl-1*H*-imidazole (5e)

Yield 85%; mp 80-82 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.23-1.28 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 2.61-2.68 (q, 2H, *J* 6.0, 6.0 Hz, –CH$_2$–), 5.07 (s, 2H, –CH$_2$O–), 6.95-7.45 (m, 10H, Ar–H). ESI-MS *m/z* 297 [M + 1]$^+$.

2-Ethyl-1-[4-(2-fluoro)benzyloxy]phenyl-1*H*-imidazole (5f)

Yield 83%; mp 75-77 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.22-1.27 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 5.07 (s, 2H, –CH$_2$O–), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 5.18 (s, 2H, –CH$_2$O–), 6.94-7.54 (m, 10H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 20.48, 64.05, 64.11, 115.33, 115.41, 115.61, 120.91, 123.50, 124.32, 124.37, 127.18, 127.40, 129.70, 129.75, 129.95, 130.05, 131.20, 149.86, 158.32, 158.85, 162.13. ESI-MS *m/z* 297 [M + 1]$^+$.

2-Ethyl-1-[4-(4-bromo)benzyloxy]phenyl-1*H*-imidazole (5g)

Yield 80%; mp 97-99 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.23-1.28 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 2.61-2.68 (q, 2H, *J* 6.0, 9.0 Hz, –CH$_2$–), 5.08 (s, 2H, –CH$_2$O–), 6.95-7.57 (m, 10H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.34, 20.46, 69.60, 115.44, 120.91, 122.14, 127.19, 127.31, 129.03, 131.16, 131.84, 135.44, 149.82, 158.28. ESI-MS *m/z* 357 [M + 1]$^+$, 359 [M + 3]$^+$.

2-Ethyl-1-[4-(2-bromo)benzyloxy]phenyl-1*H*-imidazole (5h)

Yield 80%; mp 102-104 °C; ^{1}H NMR (300 MHz,

CDCl$_3$) δ 1.23-1.28 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 2.61-2.69 (q, 2H, *J* 6.0, 9.0 Hz, –CH$_2$–), 5.18 (s, 2H, –CH$_2$O–), 6.95-7.63 (m, 10H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.34, 20.41, 69.82, 115.54, 120.96, 122.41, 127.06, 127.20, 127.66, 128.92, 129.52, 131.09, 132.77, 135.69, 149.83, 158.32. ESI-MS *m/z* 357 [M + 1]$^+$, 359 [M + 3]$^+$.

2-Ethyl-1-[4-(4-methyl)benzyloxy]phenyl-1*H*-imidazole (5i)

Yield 78%; mp 68-70 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.22-1.27 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 2.38 (s, 3H, –CH–), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 5.07 (s, 2H, –CH$_2$O–), 6.94-7.35 (m, 10H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.36, 20.43, 21.21, 70.32, 115.44, 120.95, 127.11, 127.16, 127.60, 129.37, 130.80, 133.35, 138.06, 149.86, 158.67. ESI-MS *m/z* 293 [M + 1]$^+$.

2-Ethyl-1-(4-propoxy)phenyl-1*H*-imidazole (5j)

Yield 75%; oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.04-1.09 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 1.21-1.26 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 1.81-1.88 (q, 2H, *J* 6.0, 6.0 Hz, –CH$_2$–), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 3.94-3.99 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$O–), 6.93-7.27 (m, 4H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 10.47, 12.35, 20.40, 22.51, 69.89, 115.04, 120.96, 127.07, 127.09, 130.46, 149.88, 159.03. ESI-MS *m/z* 231 [M + 1]$^+$.

2-Ethyl-1-(4-butoxy)phenyl-1*H*-imidazole (5k)

Yield 82%; oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.97-1.02 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 1.21-1.26 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 1.51-1.56 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$–), 1.78-1.83 (q, 2H, *J* 6.0, 6.0 Hz, –CH$_2$–), 2.60-2.67 (q, 2H, *J* 6.0, 6.0 Hz, –CH$_2$–), 3.98-4.03 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$O–), 6.94-7.26 (m, 4H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 13.79, 19.21, 20.41, 31.22, 68.09, 115.03, 120.97, 127.06, 127.15, 130.44, 149.88, 159.04. ESI-MS *m/z* 245 [M + 1]$^+$.

2-Ethyl-1-(4-pentoxy)phenyl-1*H*-imidazole (5l)

Yield 83%; oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.93-0.97 (t, 3H, *J* 6.0, 6.0 Hz, –CH$_3$), 1.21-1.26 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 1.43-1.45 (m, 4H, *J* 3.0, 3.0 Hz, –CH$_2$CH$_2$–), 1.80-1.85 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 3.97-4.02 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$O–), 6.93-7.26 (m, 4H, –CH$_2$–); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 13.98, 20.42, 22.42, 28.17, 28.88, 68.40, 99.98, 115.03, 120.96, 127.07, 127.14, 130.48, 149.88, 159.03. ESI-MS *m/z* 259 [M + 1]$^+$.

2-Ethyl-1-(4-hexyloxy)phenyl-1*H*-imidazole (5m)

Yield 81%; oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.90-0.94

(t, 3H, *J* 6.0, 6.0 Hz, –CH$_3$), 1.21-1.26 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 1.34-1.38 (m, 6H, *J* 3.0, 3.0 Hz, –(CH$_2$)$_3$–), 1.79-1.84 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$–), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 3.97-4.02 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$O–), 6.93-7.26 (m, 4H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 13.99, 20.42, 22.57, 25.68, 29.15, 31.54, 68.42, 77.41, 115.03, 120.95, 127.06, 127.15, 130.47, 149.88, 159.02. ESI-MS *m/z* 273 [M + 1]$^+$.

2-Ethyl-1-(4-heptyloxy)phenyl-1*H*-imidazole (5n)

Yield 81%; oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.88-0.93 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 1.21-1.35 (q, 11H, –CH$_2$–), 1.79-1.84 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$–), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 3.97-4.02 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$O–), 6.93-7.27 (m, 4H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 14.04, 20.42, 22.58, 25.98, 29.01, 29.18, 31.75, 68.42, 115.02, 120.95, 127.06, 127.17, 130.48, 149.88, 159.02. ESI-MS *m/z* 287 [M + 1]$^+$.

2-Ethyl-1-(4-octyloxy)phenyl-1*H*-imidazole (5o)

Yield 79%; oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.87-0.92 (t, 3H, *J* 6.0, 9.0 Hz, –CH$_3$), 1.21-1.35 (q, 14H, –CH$_2$–), 1.79-1.84 (d, 2H, *J* 9.0 Hz, –CH$_2$–), 2.60-2.67 (q, 2H, *J* 6.0, 6.0 Hz, –CH$_2$–), 3.97-4.02 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$O–), 6.93-7.26 (m, 4H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 14.05, 20.40, 22.63, 26.01, 29.18, 29.20, 29.31, 31.79, 68.43, 99.96, 115.04, 120.97, 127.06, 130.43, 149.88, 159.04. ESI-MS *m/z* 301 [M + 1]$^+$.

2-Ethyl-1-(4-nonoxy)phenyl-1*H*-imidazole (5p)

Yield 80%; oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.87-0.91 (t, 3H, *J* 6.0, 6.0 Hz, –CH$_3$), 1.21-1.29 (q, H, *J* 9.0, 9.0 Hz, –CH–), 1.45-1.50 (t, 14H, *J* 6.0, 9.0 Hz, –CH$_2$–), 1.79-1.84 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$–), 2.20 (s, H, –CH–), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 3.97-4.02 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$O–), 6.93-7.26 (m, 6H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 14.06, 20.41, 22.64, 26.01, 29.18, 29.23, 29.35, 29.50, 31.85, 68.42, 99.98, 115.04, 120.96, 127.06, 130.44, 149.88, 159.04. ESI-MS *m/z* 315 [M + 1]$^+$.

2-Ethyl-1-(4-decoxy)phenyl-1*H*-imidazole (5q)

Yield 82%; oil; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.86-0.91 (t, 3H, *J* 6.0, 6.0 Hz, –CH$_3$), 1.21-1.28 (q, 15H, *J* 6.0, 9.0 Hz, –CH$_2$–), 1.48 (s, 2H, –CH$_2$–), 1.79-1.84 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$–), 2.59-2.67 (q, 2H, *J* 9.0, 9.0 Hz, –CH$_2$–), 3.97-4.02 (t, 2H, *J* 6.0, 9.0 Hz, –CH$_2$O–), 6.93-7.26 (m, 4H, Ar–H); ^{13}C NMR (300 MHz, CDCl$_3$) δ 12.35, 14.07, 20.40, 22.65, 26.01, 29.18, 29.28, 29.35, 29.52, 31.87, 68.42, 99.98, 115.04, 120.96, 127.06, 130.43, 149.88, 159.04. ESI-MS *m/z* 329 [M + 1]$^+$.

Evaluation of antibacterial activity *in vitro*

MIC values of compounds **4** and **5a-5q** against several bacterial strains were measured using the broth microdilution method in 96-well plates.[9] The microorganisms used in the present study were as follows: *S. aureus* (ATCC 29213), *E. coli* (ATCC 8739), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 27853), *Klebsiella pneumoniae* (*K. pneumoniae*, ATCC 10031), *Proteus vulgaris* (*P. vulgaris*, ATCC 35659), *Salmonella typhimurium* (*S. typhimurium*, ATCC 14028), *Pasteurella multocida* (*P. multocida*, ATCC 6529), *Streptococcus mutans* (*S. mutans*, ATCC 25175), *Micrococcus luteus* (*M. luteus*, ATCC 21102), and *Streptococcus pneumoniae* (*S. pneumoniae*, ATCC 6305). Test compounds dissolved in DMSO were added to the culture medium (brain heart infusion for *S. mutans* and Müller-Hinton agar for other bacteria) to obtain final concentrations of 1-256 nmol mL^{-1}. The final bacterial concentration was approximately 10^6 CFU mL^{-1}. MIC values were measured after incubation for 20 h at 37 °C. MIC was defined as the lowest concentration of test sequences that completely inhibited the growth. Ciprofloxacin and amoxicillin were used as controls and assayed under identical conditions. All experiments were performed in triplicates.

Results and Discussion

We designed a series of 2-ethyl-1-(4-substituted) phenyl-1*H*-imidazole derivatives (**5a-5q**) and synthesized them according to Scheme 1. The compound **1**, i.e., 2-ethyl imidazole, was used as the starting material, which was reacted with *p*-fluoro nitrobenzene to give compound **2**.[10,11] The nitro group of compound **2** was converted to an amino via Pd/C hydrogenation in the presence of H$_2$. Subsequently, compound **3** was suspended in 25% sulfuric acid solution and treated with NaNO$_2$ solution to yield the scaffold 2-ethyl-1-(4-hydroxyl)phenyl-1*H*-imidazole (**4**).[12] Compound **4** was isolated and further reacted with appropriate alkyl halides, giving a series of 1-substituted phenyl-1*H*-imidazole derivatives. The structures of all the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

All the synthesized compounds were screened for their *in vitro* antibacterial activity against Gram-positive bacteria, *S. aureus*, and the Gram-negative bacteria, *E. coli*. MIC were determined using the serial dilution technique. We used ciprofloxacin and amoxicillin as reference compounds in the evaluation of antibacterial activity and measured the MIC in nmol mL^{-1}.
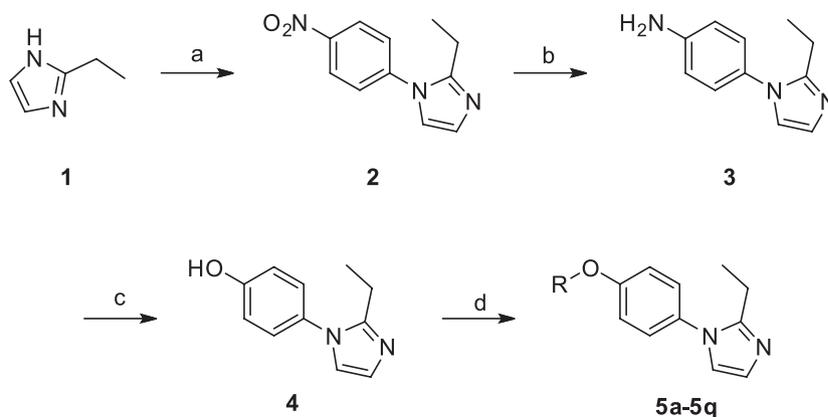
Scheme 1. Synthesis of target compounds **5a-5q**. (a) *p*-Fluoro nitrobenzene, NaOH, DMSO, 90 °C; (b) H$_2$, Pd/C, ethanol, 60 °C; (c) NaNO$_2$, 30% H$_2$SO$_4$; (d) RX, NaOH, DMF, 80 °C.

As shown in Table 1, 17 derivatives containing a benzyl and an alkyl group were designed and synthesized, providing different space effects and lipid water partition coefficients. Most of the synthesized compounds showed modest antibacterial activity *in vitro* against both

Table 1. Inhibitory activity[a] of compounds, **4**, **5a-5q**, and controls against *S. aureus* and *E. coli*

Compound	R	MIC[b] / (nmol mL^{-1}) vs. S. aureus	vs. E. coli	ClogP[c]
4	H	> 256	> 256	1.9
5a	C$_6$H$_5$CH$_2$	> 256	128	3.89
5b	(*o*-Cl)C$_6$H$_4$CH$_2$	64	32	4.43
5c	(*m*-Cl)C$_6$H$_4$CH$_2$	> 256	64	4.43
5d	(*p*-Cl)C$_6$H$_4$CH$_2$	64	16	4.43
5e	(*p*-F)C$_6$H$_4$CH$_2$	128	128	3.92
5f	(*o*-F)C$_6$H$_4$CH$_2$	> 256	256	3.92
5g	(*p*-Br)C$_6$H$_4$CH$_2$	128	64	4.32
5h	(*o*-Br)C$_6$H$_4$CH$_2$	> 256	256	4.32
5i	(*p*-CH$_3$)C$_6$H$_4$CH$_2$	> 256	> 256	4.61
5j	*n*-C$_3$H$_7$	64	8	3.53
5k	*n*-C$_4$H$_9$	64	8	3.87
5l	*n*-C$_5$H$_{11}$	8	4	4.89
5m	*n*-C$_6$H$_{13}$	8	16	5.29
5n	*n*-C$_7$H$_{15}$	16	16	5.87
5o	*n*-C$_8$H$_{17}$	128	32	6.45
5p	*n*-C$_9$H$_{19}$	128	32	6.75
5q	*n*-C$_{10}$H$_{21}$	> 256	32	7.43
Amoxicillin		32	128	
Ciprofloxacin	–	32	4	

[a]Anti-bacterial testing was carried out in triplicate; [b]MIC (minimum inhibitory concentration) values represent the average of three readings; [c]it was calculated on ACD/Labs website.[13]

Gram-positive and Gram-negative strains at the level of nmol mL^{-1}. However, the overall results indicated that the tested compounds possessed a slightly stronger inhibitory activity against *E. coli*, than that against *S. aureus*.

In-depth analysis of the structure activity relationship of these structures revealed that the MIC was found to decrease after the introduction of substituents on the hydroxyl group of compound **4**. This could be explained by their ClogP values, as it is known that compounds with modest ClogP can easily cross the cell membrane. Firstly, a study of the data shown in Table 1 demonstrates that most of the test compounds exhibited antibacterial activity against *S. aureus* (MIC 8-128 nmol mL^{-1}). Among these benzyl-substituted derivatives, those containing a Cl atom, i.e., **5b** (*o*-Cl) and **5d** (*p*-Cl) were found to be the most active with an MIC value of 64 nmol mL^{-1} each, against *S. aureus*. On the other hand, derivatives **5e** (*p*-F) and **5g** (*p*-Br) were found to be less active with a MIC value of 128 nmol mL^{-1} each, against *S. aureus*. Hence, these results showed that the presence of a halogen atom in the *para*-position of the benzyl ring yielded a better inhibitory activity, with the derivative **5b** (*o*-Cl) being an exception. Furthermore, the derivatives **5a**, **5c**, **5f**, and **5h**, each having halogen substituents on the *ortho*- or *meta*-position of the benzyl group, exhibited no inhibition at the dose of 256 nmol mL^{-1}. As compared to compound **5a**, the derivatives with an electron-donating group (**5i**, *p*-CH$_3$) did not improve the inhibitory potency. A preliminary study suggests that electron-donating groups may contribute more to the inhibitory activity against *S. aureus* than electron-withdrawing groups.

Most of the tested derivatives exhibited antibacterial activity against *E. coli* in the dose range of 4-256 nmol mL^{-1}. Compound **4**, having no substitution on the phenyl ring, had a weaker activity against *E. coli* than compounds with different substitutions on the phenyl ring, i.e., **5a-5q**. As discussed earlier, the introduction of a benzyl or alkyl group

on compound **4** improves the antibacterial activity against *E. coli*. Amongst the derivatives **5a**-**5i**, the compound **5d**, with a *p*-Cl benzyl group possesses the most potent inhibitory activity, with an MIC value of 16 nmol mL^{-1}. Compounds **5b**, **5c**, and **5d**, were found to be active against *E. coli* at concentrations of 32, 64, and 16 nmol mL^{-1}, respectively. This indicated that the position of the halogen atom on the phenyl ring greatly influenced the antibacterial activity, with the *para*-substituted compounds being more active than the *ortho*- and *meta*-substituted compounds. We also found that the Cl atom contributed more to the inhibitory activity against *E. coli* than the Br and F atoms. Derivative **5i**, containing an electron-donating group (*p*-CH$_3$), had an MIC value of > 256 nmol mL^{-1} against *E. coli*. Thus, this further illustrated that electron-donating groups may contribute more to the antibacterial activity than electron-withdrawing groups.

Analysis of the inhibitory activity of compounds **5j**-**5q** against *S. aureus* and *E. coli* showed that the length of the alkyl chain appeared to have a direct impact on the antibacterial activity of the 2-ethyl-1-(4-substituted) phenyl-1*H*-imidazole derivatives. As the alkyl chain length increased starting from **5j** onwards, the MIC gradually decreased, with the compound **5m** (with an *n*-hexyl substituent), being the most active against *S. aureus*, and the compound **5l** (with an *n*-amyl substituent) being the most active against *E. coli*. However, this trend was found to be reversed when the alkyl chain consisted of more than six carbon atoms. Compound **5l** was found to be the most potent inhibitor with an MIC value of 8 nmol mL^{-1} against *S. aureus*, and 4 nmol mL^{-1} against *E. coli*, and showed comparable activity with the reference agents, ciprofloxacin and amoxicillin.

Compound **5l** was further screened against a range of Gram-negative bacteria (*P. aeruginosa*, *K. pneumoniae*, *P. vulgaris*, *S. typhimurium*, and *P. multocida*) and Gram-positive bacteria (*M. luteus*, *S. pneumoniae*, and *S. mutans*). These bacteria easily cause severe infection illness or food poisoning, and at the same time, they are widely applied to the antimicrobial activity screening experiments.[14-17] Analysis of the data in Table 2 showed that the compound **5l** exhibited inhibitory activity against *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris*, and *S. typhimurium* in the entire dose range of 8-256 nmol mL^{-1}, but was found to be inactive against *P. multocida* at even the highest concentration of 256 nmol mL^{-1}. The MIC of **5l** against *P. aeruginosa* was found to be 8 nmol mL^{-1}, which was similar to that of the control ciprofloxacin, and lower than that of amoxicillin. The compound **5l** was found to be as potent as amoxicillin against *K. pneumoniae* and much weaker than ciprofloxacin. Notably, the compound **5l** showed

a fourfold increase in potency against *P. vulgaris* as compared to the controls, ciprofloxacin, and amoxicillin. Meanwhile, **5l** exhibited inhibitory activity against *S. typhimurium* at 128 nmol mL^{-1}, whereas the two controls were found to be inactive at 256 nmol mL^{-1}. The compound **5l** was found to be active at doses of 64, 8, and 64 nmol mL^{-1}, against the Gram-positive bacteria, *M. luteus*, *S. pneumoniae*, and *S. mutans*, respectively. The potency of this activity was comparable with that of ciprofloxacin against *S. pneumoniae*.

Table 2. Inhibitory activity[a] of compound **5l** and controls against several bacterial strains

Gram	Bacterial strain	MIC[b] / (nmol mL^{-1})		
		5l	Amoxicillin	Ciprofloxacin
N[c]	*P. aeruginosa*	8	32	8
N	*P. multocida*	> 256	32	256
N	*K. pneumoniae*	256	256	8
N	*P. vulgaris*	64	> 256	256
N	*S. typhimurium*	128	> 256	> 256
P[d]	*S. mutans*	64	64	32
P	*M. luteus*	64	32	128
P	*S. pneumoniae*	8	64	8

[a]Anti-bacterial testing was carried out in triplicate; [b]MIC (minimum inhibitory concentration) values represent the average of three readings; [c]N: Gram-negative; [d]P: Gram-positive.

The above pharmacological data revealed that our previous design strategy is feasible. The open-chain analogues of 7-alkoxyl-4,5-dihydroimidazo[1,2-*a*]quinolines (**I**) retained the original activity against Gram-negative bacteria and gave a more potent inhibition of Gram-positive bacteria. Given the present biological data, such processes can be impacted by the hydrophobicity of the prepared derivatives. While in the present study the inclusion of ClogP value in the design strategy was used as a predictor of crossing of the biological barrier, it can also be used to determine if hydrophobicity contributes to the obtained pharmacological results. A bidirectional analysis between MIC and ClogP value yielded a good correlation. This may provide instructions for further structural optimization of imidazole analogues with higher affinity and better clinical therapeutic properties. Further investigations on enzyme inhibition, cytotoxicity, and pharmacokinetic properties are underway in order to confirm the exact mechanism of antibacterial activity.

Conclusions

In this study, a series of novel 2-ethyl-1-(4-substituted) phenyl-1*H*-imidazole derivatives was synthesized and evaluated for its antibacterial activity against Gram-positive

and Gram-negative bacterial strains. Most of the prepared compounds possess antibacterial activity against both these bacterial strains. Especially, the compound **5l** with an MIC value of 4 nmol mL^{-1} against *E. coli*, and 8 nmol mL^{-1} against *S. aureus*, was found to be the most potent inhibitor, and showed an even better activity as compared to the reference agents, ciprofloxacin and amoxicillin. This suggests that hybrid compounds containing substituted imidazole moieties may possess potent antibacterial properties. The results of this evaluation suggest that further development of such compounds may be of therapeutic interest.

Supplementary Information

Supplementary data (^{1}H and ^{13}C NMR spectra) are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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