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2-Piperidin-4-yl-benzimidazoles with Broad Spectrum Antibacterial Activities

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Abstract—A series of 2-piperidin-4-yl-benzimidazoles were synthesized and evaluated for antibacterial activities. Certain compounds inhibit bacterial growth with low micromolar minimal inhibitory concentration (MIC). These benzimidazoles are effective against both Gram-positive and Gram-negative bacteria of clinical importance, particularly entercococci, and represent a new class of potential antibacterial agents.

Almost all the major classes of antibiotics have encountered resistance in clinical applications.[1–5] The emergence of bacterial resistance to β-lactam antibiotics, macrolides, quinolones, and vancomycin is becoming a major worldwide health problem.[4–8] In particular, antibiotic resistance among Gram-positive bacteria (staphylococci, enterococci, and streptococci) is becoming increasingly serious.[9–13] Entercococci, which are frequently resistant to most antibiotics including penicillin, cephalosporin and aminoglycosides, are often treated with either a combination of two antibiotics or vancomycin. However, with the recent increased use of vancomycin in methicillin-resistance *Staphylococcus aureus* (MRSA) infections and colitis due to *Clostridium difficile*, multiple resistant *Entercoccus faecium* has been spreading.[14] As such, the last resort for anti-infective diseases, the Vancomycin family of antibiotics, has now been gravely challenged in recent years due to the emergence of Vancomycin resistance in clinical practice.[11,15]

In order to overcome these emerging resistance problems, there is an urgent need to discover novel antibacterial agents in structural classes distinct from existing antibiotics. In an effort to identify such compounds, we continuously screen our in-house libraries for active leads. Herein, we report on the initial structure–activity relationship (SAR) studies of a series of benzimidazoles that have been discovered to possess broad-spectrum antibacterial activities.

The synthesis of this class of benzimidazoles is shown in Scheme 1. Treatment of commercially available 4,5-dichloro-1,2-phenylenediamine (**1**) and *N*-Boc-isonipecotic acid (**2**) with EDC in the presence of catalytic amount of DMAP led to the formation of the corresponding amide. The crude mixture was then refluxed in aqueous sodium hydroxide solution to give cyclized intermediate **3**, which was reacted with various alkyl, benzyl and aryl halides to give **4b–4i**. Treatment of compound **4g** with various amines or nitrogen-containing heterocylces provided **6a–r**. Deprotection of the Boc group with anhydrous hydrogen chloride (HCl, 4.0 M) in dioxane at room temperature for 30 min formed benzimidazoles **7a–r**. In a similar manner, **3**, **4b–4i** were treated with hydrogen chloride to give benzimidazoles **5a–i**.

In the initial assays, all the benzimidazoles were tested for activity against *S. aureus* and *Eschericia coli*.[16] Their minimal inhibitory concentrations (MIC) are listed in Table 1. The preliminary data showed that all the amine analogues (**7a–r**) exhibited strong ability to inhibit *S. aureus* with most of the MICs in the low micromolar range. Various nitrogen substitutions are accepted including straight alkyl chain amines and diamines (**7a–g**), polyamine (**7h**), cyclic diamines (**7i**, **7j**, **7n–p**), heterocyclics (**7k–m**, **7q**, **7r**). These benzimidazoles are also effective

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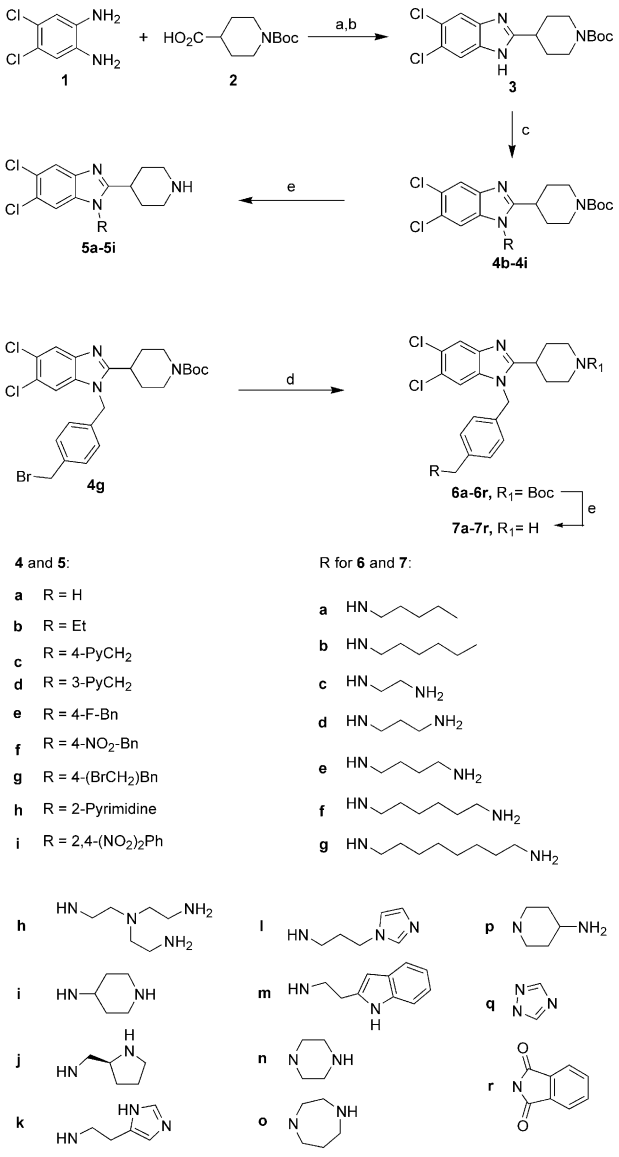

Scheme 1. Synthesis of benzimidazoles: (a) EDC, DMAP; (b) NaOH, H_2O; (c) RX, NaH or K_2CO_3; (d) for amines (RH), DMF 25 °C, 2 h; for heterocycles (RH), K_2CO_3 DMF, 25 °C, 2 h; (e) 4.0 M HCl/dioxane, 0 °C, 0.5 h.

in inhibiting *E. coli* growth, albeit with somewhat reduced potency. Nevertheless, some compounds possess MICs approaching low micromolar concentrations. In comparison, these benzimidazoles are two or more folds more effective against *S. aureus* than *E. coli*. In contrast, compound **5a**, which has no substitution at N1 position, and compounds **5b–i**, which have various alkyl, benzyl or aryl substitutions at N1 positions did not exhibit any appreciable ability to inhibit bacterial growth. This suggests the crucial role of a basic nitrogen distinct from the benzimidazole core.

All benzimidazoles were also screened for their ability to inhibit bacterial translation and transcription using a coupled assay.[16] Several compounds (**7c**, **7d**, **7h**, **7k**) were found to posses low micromolar IC_{50}s. Since these compounds have similar inhibitory activities against *S. aureus* and *E. coli*, their antibacterial activities could

Table 1. Inhibitory effects of benzimidazoles on *S. aureus* and *E. coli* growth and bacterial transcription/translation[17]

Compd	*S. aureus* MIC (μM)	*E. coli* MIC (μM)	T/T IC_{50} MIC (μM)
5a	>100	>100	>100
5b	>100	>100	>100
5c	50–100	50–100	>100
5d	50–100	50–100	>100
5e	>100	>100	>100
5f	50–100	>100	>100
5g	>100	>100	>100
7a	6–12	12–25	>100
7b	3–6	6–12	>100
7c	6–12	12–25	12
7d	12–25	50–100	20
7e	6–12	25–50	50
7f	6–12	12–25	>100
7g	6–12	6–12	>100
7h	3–6	12–25	10
7i	6–12	12–25	>100
7j	12–25	12–25	>100
7k	6–12	12–25	25
7l	12–25	12–25	>100
7m	12–25	12–25	>100
7n	6–12	12–25	>100
7o	6–12	12–25	>100
7p	6–12	50–100	60
7q	12–25	12–25	>100
7r	6–12	6–12	>100
Ciprofloxacin	0.75–1.56	0.75–1.56	>100
Paromomycin	1–3	3–6	0.56

be partially due to the inhibition of bacterial transcription and/or translation. However, most of the IC_{50} values are much higher than the corresponding MICs for *S. aureus* and *E. coli*, it is unlikely that the antibacterial activities for most of these compounds are due to inhibition of either bacterial transcription or translation.

To test effectiveness of these benzimidazoles against other bacteria, the active compounds from the preliminary screening were screened against additional four strains of Gram-positive and four strains of Gram-negative bacteria, and the results are shown in Table 2. Again, these compounds exhibited higher potencies against Gram-positive bacteria (*S. aureus* 13709, *Enterococcus hirae* 29212, *Streptococcus pyogenes* 49399, and *Streptococcus pneumoniae* 6303) as compared to Gram-negative bacteria (*E. coli* 25922, *Proteus vulgaris* 8427, *Klebsiella pneumoniae* 13383, *Pseudomonas aeruginosa* 25416). Several benzimidazoles, in particular **7b**, **7f** and **7g**, showed interesting activities against *E. hirae*. These compounds were screened against seven additional clinically important *Enterococcus* strains, and the results are shown in Table 3. In addition to their original activities, several compounds (**7a**, **7b** and **7r**) displayed strong inhibitory activities against all eight *Enterococcus* strains. It appears that relatively hydrophobic terminal moieties could enhance the antibacterial activities against *Enterococcus*. Further studies are necessary to fully understand their potent antibacterial activities. To study the selectivity for inhibition of bacterial growth, these compounds were also screened against yeast cell line *Candida albicans* 10231 (Table 2). Certain compounds were significantly less inhibitory to yeast growth

Table 2. Minimal inhibitory concentrations (MIC, μM) of benzimidazoles against bacteria and yeast[a]

Compd	Gram+				Gram−				Yeast
	SA1	EH2	SP4	SP6	EC2	PV8	KP1	PA2	CA1
7a	6–12	1–3	3–6	12–25	12–26	25–50	6–12	25–50	50–100
7b	3–6	1–3	3–6	6–12	6–12	12–25	6–12	25–50	25–50
7c	6–12	3–6	6–12	12–25	12–25	25–50	12–25	12–25	>100
7d	12–25	6–12	6–12	25–50	50–100	NT	25–50	25–50	>100
7e	6–12	3–6	6–12	25–50	25–50	NT	25–50	25–50	>100
7f	6–12	1–3	3–6	12–25	12–25	25–50	12–25	25–50	>100
7g	6–12	1–3	3–6	6–12	6–12	12–25	6–12	25–50	>100
7h	3–6	3–6	3–6	6–12	12–25	25–50	12–25	12–25	>100
7i	6–12	3–6	6–12	12–25	12–25	25–50	12–25	12–25	50–100
7k	6–12	3–6	6–12	12–25	12–25	25–50	12–25	25–50	>100
7n	6–12	3–6	6–12	12–25	12–25	12–25	6–12	12–25	50–100
7o	6–12	3–6	6–12	12–25	12–25	25–50	12–25	25–50	>100
7p	6–12	3–6	6–12	25–50	50–100	25–50	25–50	12–25	50–100
7r	6–12	3–6	12–25	6–12	6–12	25–50	25–50	12–25	50–100

NT, not tested.
[a]SA1, *S. aureus* 13709; EH2, *E. hirae* 29212; SP4, *S. pyogenes* 49399; SP6, *S. pneumoniae* 6303; EC2, *E. coli* 25922; PV8, *P. vulgaris* 8427; KP1, *K. pneumoniae* 13383; PA2, *P. aeruginosa* 25416; CA1, *C. albicans* 10231.

Table 3. Minimal inhibitory concentrations (MIC, μM) of benzimidazoles against *Enterococcus*

Compd	*E. faecalis* ATCC 11823	*E. faecalis* ATCC 23241	*E. faecalis* ATCC 4200	*E. faecalis* ATCC 49757	*E. faecalis* ATCC 828	*E. faecium* ATCC 6569	*E. faecium* ATCC 882	*E. faecium* ATCC 29212
7a	3–6	6–12	6–12	6–12	6–12	3–6	6–12	1–3
7b	3–6	3–6	3–6	3–6	3–6	3–6	3–6	1–3
7c	6–12	12–25	12–25	12–25	12–25	6–12	12–25	3–6
7d	12–25	25–50	25–50	12–25	12–25	12–25	25–50	6–12
7e	12–25	12–25	12–25	12–25	12–25	12–25	12–25	3–6
7g	6–12	NT	25–50	50–100	50–100	12–25	NT	1–3
7l	12–25	12–25	12–25	12–25	12–25	12–25	12–25	3–6
7l	6–12	12–25	12–25	12–25	6–12	6–12	12–25	3–6
7n	12–25	6–12	12–25	12–25	6–12	6–12	6–12	3–6
7o	12–25	12–25	12–25	6–12	12–25	6–12	12–25	3–6
7p	12–25	12–25	12–25	12–25	12–25	6–12	12–25	3–6
7r	3–6	6–12	6–12	6–12	6–12	3–6	6–12	3–6

NT, not tested.

as compared to bacterial growth, suggesting a selectivity index for inhibition of bacterial growth.

In summary, we have discovered a novel series of benzimidazoles that exhibit a broad spectrum of antibacterial activities. These benzimidazoles are particularly effective against Gram-positive bacteria, including clinically relevant strains of *Enterococcus*. The simplicity of their structures and their high potencies against different types of bacteria render these benzimidazoles interesting leads for further investigation. The preliminary SAR has pointed to a high degree of tolerance for structure modifications at the N1 of the benzimidazole core. The effect of extensive modification around this core will be the subject of further optimization studies, which shall be reported in due course.

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17. The assays were carried out in 150 mL volume in duplicate in 96-well clear flat-bottom plates. The growth bacterial or yeast suspension from an overnight culture in appropriate medium was added to a solution of test compound in 2.5% DMSO in water. Final bacterial or yeast inoculum is approximately 102–103 CFU/well. The percentage growth of the bacteria or yeast in test wells relative to that observed for a control well containing no compound was determined by measuring absorbance at 595 nm (A595) after 20–24 h at 37 °C (bacteria) or 40–48 h (yeast) at 25 °C. The MIC was determined as a range of concentration where complete inhibition of growth is observed at the higher concentration and bacterial/yeast cells are viable at the lower concentration.