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Synthesis and biological evaluations of novel benzimidazoles as potential antibacterial agents

Yun He,* Jun Yang, Baogen Wu, Lisa Risen and Eric E. Swayze

Ibis Therapeutics, A Division of Isis Pharmaceuticals, Inc., 2292 Faraday Av., Carlsbad, CA 92008, USA

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Abstract—A series of novel benzimidazole derivatives were synthesized via parallel solution-phase chemistry. Many of these compounds were found to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. Several analogues exhibited low micromolar minimal inhibitory concentrations (MIC) against both Gram-positive and Gram-negative bacteria of clinical relevance and could serve as leads for further optimizations for antibacterial research.

The emergence of resistance to the major classes of antibacterial agents is recognized as a serious health concern.[1–8] Particularly, the emergence of multidrug-resistant strains of Gram-positive bacterial pathogens is a problem of ever increasing significance. Organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE), vancomycin-resistant enterococci (VRE), and penicillin- and cephalosporin-resistant streptococci are continually challenging scientist, physicians and patients.[5,9–14] The search for antibacterial agents with new mode of actions will always remain an important and challenging task. We have initiated a research program to discover novel antibiotics by targeting bacterial *r*RNA utilizing our unique MS-based screening technologies.[15–19] Previously, we reported the discovery of a series of novel benzimidazoles with general structure **1** that exhibit potent broad-spectrum antibacterial activities, particularly against Gram-positive bacteria (Fig. 1).[20] In this work, we report on the design and synthesis of a library of novel benzimidazoles related to **1** and the evaluation of their antibacterial activities.

To explore the SAR in the xylenyl region of these benzimidazoles and search for potentially better antibacterial agents, additional heterocycles were attached to the benzimizadole core with various linkers. The first

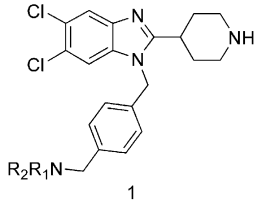

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Figure 1.

series of benzimidazole analogues contained various alkane spacers (**10–14**, Scheme 1). Since our earlier studies suggested that a nitrogen atom at the terminal site of the xylenyl moiety in **1** is important for their antibacterial activities,[20] all these new analogues bear nitrogen-containing heterocycles and their syntheses are shown in Scheme 1. 4,5-Dichloro-1,2-dianiline (**2**) reacted smoothly with *N*-Boc-isonipecotic acid (**3**) to give the corresponding amide, which cyclized upon treatment with sodium hydroxide to give benzimidazole **4**. Reaction of **4** with different diiodides furnished **5–9** in good yields. A variety of nitrogen-containing heterocycles were introduced in good yields by simple alkylation in the presence of sodium hydride or potassium carbonate. Deprotection of the Boc group furnished the target molecules **10–14** in almost quantitative yields. These benzimidazoles were first screened against *S. aureus* and *Escherichia coli*, and their minimum inhibitory concentrations (MICs) are shown in Table 1. While the simple alkyl analogues from **5–9** after removal of the Boc group exhibited no antibacterial activities, several of the heterocyclic analogues (**11i**, **13a**,**b**,**d**,**g**,**i**, **14i**) were

* Corresponding author at present address: Department of Medicinal Chemistry, Genomics Institute of the Novartis Research Foundation (GNF), 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA. Tel.: +1-858-332-4706; fax: +1-858-332-4351; e-mail: yhe@gnf.org

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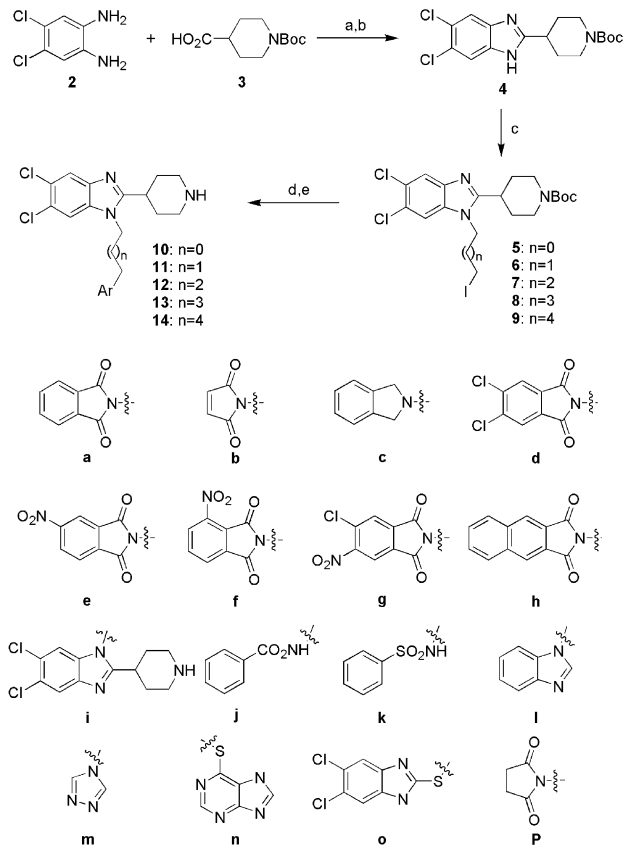
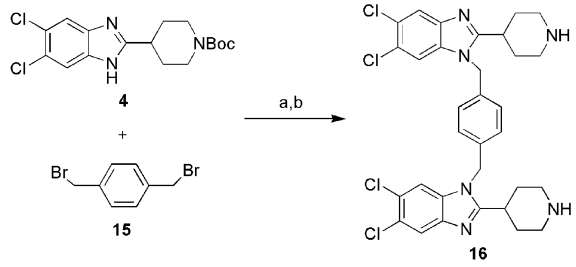


Scheme 1. Synthesis of benzimidazoles **10**–**14**. Reagents and conditions: (a) EDC (1.2 equiv), DMAP (cat.), CH_2Cl_2, 25 °C, 12 h; (b) NaOH, H_2O, 100 °C, 10 h, 65% over 2 steps; (c) $ICH_2(CH_2)_nCH_2I$ (n = 0–4, 3.0 equiv), NaH or K_2CO_3, DMF, 25 °C, 1–8 h; 69–88%; (d) ArH, NaH or K_2CO_3, 65–82%; (d) 4.0 M HCl/dioxane, CH_2Cl_2, 25 °C, 0.5 h, 90–95%.

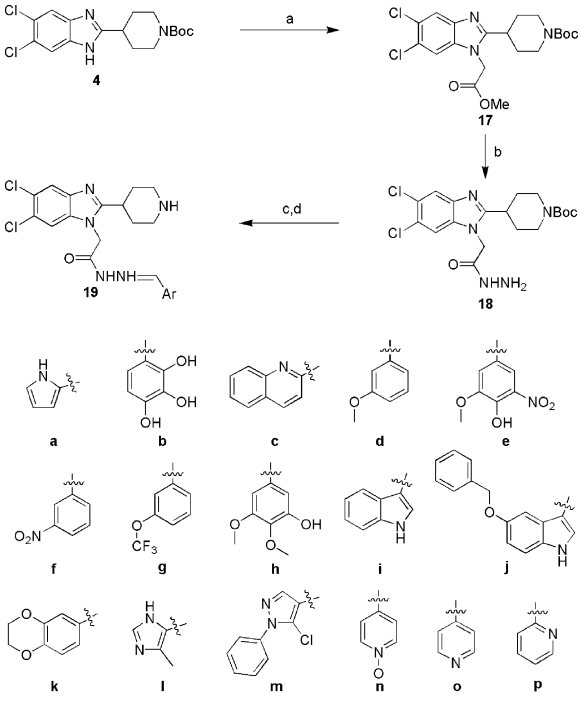
indeed found to possess good activities. Interestingly, all three dimers **11i**, **13i** and **14i** are similarly potent against both *S. aureus* and *E. coli*. Encouraged by these results, we then prepared the corresponding dimer **16**, which has the xylenyl group as the spacer. The synthesis of **16** was accomplished by first reacting **4** with 0.5 equivalents of α,α-dibromo-*p*-xylene (**15**), followed by deprotection of the Boc group using hydrogen chloride (Scheme 2). As expected, **16** exhibited low μM MICs against both *S. aureus* and *E. coli* (Table 1).

Encouraged by the antibacterial activities of these heterocyclic and dimeric benzimidazoles, we decided to further explore this heterocyclic region of these benzimidazole derivatives by synthesizing a larger library of analogues for quick screening. We thus focused on the chemistry that would be compatible with combinatorial synthesis, so that analogues could be quickly and cleanly generated for biological evaluations. A series of acylhydrazone containing various aryl or heterocyclic moieties were designed and synthesized (Scheme 3). Acylhydrazide **18** was synthesized as a key intermediate for the combinatorial generation of benzimidazoles. Since the acyl hydrazide could serve as both a hydrogen donor and acceptor to potentially add additional contacts with the target, analogues based on **18** could be potentially more potent than the parent benzimidazoles.

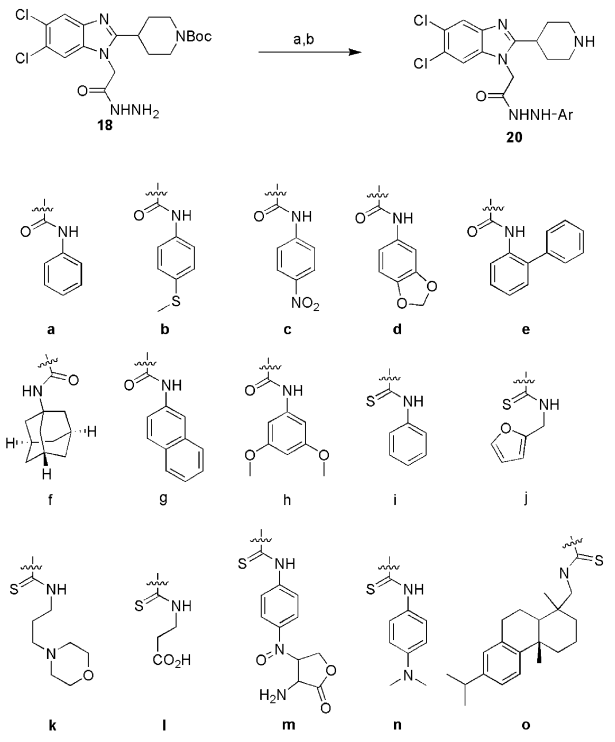
Acyl hydrazide **18** was easily prepared in gram quantity in excellent overall yield from **4** by alkylation with methyl α-bromoacetate followed by a nucleophilic displacement of the methoxy group with hydrazine. Many derivatives could then be easily synthesized in high yields from **18** without the need of vigorous purification. The first series of analogues with the general structure **19** were prepared by simply reacting **18** with different aldehydes, followed by the removal of the Boc protecting group with hydrogen chloride. All the benzimidazole analogues obtained this way have more than 95% purity based on LC/MS analysis and were thus used directly for antibacterial assays. Gratifyingly, most of these analogues (**19a**–**m**) inhibited *S. aureus* growth with MICs in the low μM range (Table 1). In particular, **19j** and **19m** showed 3–6 and 6–12 μM MICs against *S. aureus* respectively. In contrast to most of the active analogues found in the first library (**11i**, **13a**,**i**, **14i**, **16**) that were effective against both *S. aureus* and *E. coli*,



Scheme 2. Synthesis of benzimidazole dimer **16**. Reagents and conditions: (a) α,α-dibromo-*p*-xylene (0.5 equiv), NaH, DMF, 0 °C, 2 h, 56%; (b) 4.0 M HCl/dioxane, 25 °C, 2 h, 98%.



Scheme 3. Synthesis of benzimidazoles **18a**–**p**. Reagents and conditions: (a) NaH (3.0 equiv), $BrCH_2CO_2Me$ (1.2 equiv), DMF, 25 °C, 0.5 h, 92%; (b) H_2NNH_2 (5.0 equiv), DMF, 25 °C, 2.0 h, 98%; (c) ArCHO (1.02 equiv), CH_2Cl_2, *p*TsOH (cat.) 25 °C, 0.5 h, >95%; (d) 4.0 M HCl/dioxane, CH_2Cl_2, 25 °C, 0.5 h, >95%.



Scheme 4. Synthesis of benzimidazoles **20a**–**o**. Reagents and conditions: (a) RNCO or RNCS (1.05 equiv), CH_2CH_2, 25 °C, 0.5 h, >95%; (b) 4.0 M HCl/dioxane, CH_2Cl_2, 25 °C, 0.5 h, >95%.

Table 1. Inhibitory effects of benzimidazoles on *S. aureus* and *E. coli* growth[20,21]

Compd	*S. aureus* MIC (μM)	*E. coli* MIC (μM)	Compd	*S. aureus* MIC (μM)	*E. coli* MIC (μM)
10a	>100	>100	**19e**	25–50	>100
11a	>100	>100	**19f**	25–50	>100
11i	25–50	25–50	**19g**	25–50	>100
12a	>100	>100	**19h**	25–50	>100
12n	>100	>100	**19i**	25–50	>100
12o	>100	>100	**19j**	3–6	>100
13a	12–50	25–50	**19k**	50–100	>100
13b	6–12	>100	**19l**	50–100	>100
13c	50–100	25–50	**19m**	6–12	>100
13d	12–25	50–100	**19n**	>100	>100
13e	>100	50–100	**19o**	>100	>100
13f	>100	>100	**19p**	>100	>100
13g	25–50	50–100	**20a**	50–100	>100
13h	>100	>100	**20b**	12–25	25–50
13i	12–25	12–25	**20c**	25–50	>100
13j	>100	>100	**20d**	25–50	50–100
13k	>100	>100	**20e**	12–25	25–50
13l	50–100	50–100	**20f**	6–12	12–25
13m	>100	>100	**20g**	6–12	12–25
13p	>100	>100	**20h**	6–12	25–50
14a	>100	>100	**20i**	25–50	50–100
14i	6–12	12–25	**20j**	50–100	>100
16	3–6	6–12	**20k**	>100	>100
19a	12–25	>100	**20l**	>100	>100
19b	12–25	>100	**20m**	>100	>100
19c	25–50	>100	**20n**	50–100	>100
19d	25–50	>100	**20o**	25–50	25–50
19e	25–50	>100	Paromomycin	1–3	3–6

none of analogues in this series had any activity for *E. coli*. These data suggested that the spacer attached to the benzimidazole nitrogen plays an important role in determing the antibacterial activities of these analogues. All three pyridine analogues (**19n**–**p**) had no effect against these bacteria, which were possibly due to the presence of the basic pyridine nitrogen.

Next, a variety of isocyanides and isothiocyanides were then allowed to react with acyl hydrazide **18**, and the corresponding ureas and thioureas were obtained in excellent yields and purity (Scheme 4). The resulted *N*-Boc protected intermediates were directly treated with hydrogen chloride to give the corresponding products of general structure **20** in almost quantitative yields and more than 95% purity. In the urea series, a variety of functional moieties with different sizes were tolerated and all these analogues (**20b**–**h**) except **20a** showed good antibacterial activities. Interestingly, similar to the analogues with alkyl spacers, many analogues in this series again exhibited good activities against both *S. aureus* and *E. coli*. However, among the thioureas analogues, only **20i** and **20o** showed moderate activities (Table 1).

Similar to the xylenylamine analogues (**1**), most of these compounds did not show appreciable inhibitory activities in the transcription/translation assay (selected data shown in Table 2), suggesting that the antibacterial activities of these compounds are most likely not due to the inhibition of the transcription/translation machinery.

Table 2. Minimal Inhibitory Concentrations (MIC) of selected benzimidazoles against bacteria and their inhibitory concentrations (IC_{50}) in the Transcriptions/Translation (T/T) assay[a,21]

Compd	MIC (μM, Gram+)				MIC (μM, Gram−)				IC_{50} (μM)
	SA1	EH2	SP4	SP6	EC2	PV8	KP1	PA2	T/T
13a	6–12	25–50	25–50	12–25	25–50	25–50	25–50	50–100	25
13b	6–12	25–50	25–50	6–12	>100	>100	50–100	>100	>100
13i	3–7	0.75–1.5	25–50	6–12	12–26	25–50	25–50	6–12	35
14i	6–12	1–3	3–6	6–12	12–25	NT	6–12	12–25	>100
16	3–6	1–3	3–6	6–12	6–12	NT	6–12	12–25	>100
19j	3–6	3–6	6–12	12–25	>100	50–100	6–12	50–100	>100
19m	6–12	1–3	6–12	12–25	>100	25–50	25–50	50–100	>100
20f	6–12	3–6	6–12	12–25	12–25	12–25	12–25	25–50	>100
20g	6–12	3–6	6–12	12–25	12–25	25–50	6–12	25–50	>100
20h	6–12	1–3	6–12	12–25	25–50	>100	12–25	>100	>100

[a] SA1: *S. aureus* 13709; EF2: *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; NT: Not tested.

To further evaluate the potential of these benzimidazole derivatives, the active compounds were screened against a panel of clinically relevant bacteria, and most of these compounds were found to be active against these bacteria (Table 2). In particular, **13i**, **14i**, **16**, **20f**,**g** exhibited low μM broad-spectrum activities. The promising activities and easy access of these benzimidazole derivatives render them as very attractive antibacterial leads. Further optimizations and detailed SAR studies are the subject of future studies and shall be reported in due course.

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21. The bacterial strains were from ATCC (American Type Culture Collection). The numbers in the note of Table 2 are ATCC numbers.