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Amidino Benzimidazole Inhibitors of Bacterial Two-Component Systems

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Abstract—Amidino benzimidazoles have been identified as inhibitors of the bacterial KinA/Spo0F two-component system (TCS). Many of these inhibitors exhibit good in vitro antibacterial activity against a variety of susceptible and resistant Gram-positive organisms. The moiety at the 2-position of the benzimidazole was extensively modified. In addition, the regioisomeric benzoxazoles, heterocyclic replacements for the benzimidazole, have been synthesized and their activity against the TCS evaluated.

The number of cases of multidrug resistant bacterial infections is increasing at an alarming rate. Clinicians have become reliant on vancomycin as the antibiotic for serious infections resistant to traditional agents. Due to this, the incidence of vancomycin-resistant *Enterococcus faecium* (VRE) infections in intensive-care units of US hospitals has increased from 0.5% in 1989 to 22% in 1997.[1] New classes of antibacterial agents with novel mechanisms of action are urgently needed.

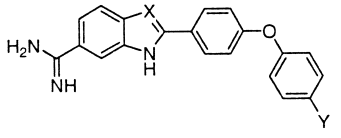
Bacterial two-component systems (TCSs), which include a histidine protein kinase (HPK) and a response regulator (RR), are signal transduction pathways that sense and respond to environmental changes thus allowing the bacteria to adapt and survive within the host.[2] Inhibitors of TCS should block these signaling pathways and may lead to agents which would either slow bacterial cell growth or result in bacterial cell death.

HPKs respond to environmental signals by undergoing autophosphorylation of a conserved histidine residue. The histidine *N*-phosphate is then transferred to an aspartic acid residue in the active site of the RR that regulates gene transcription.[3]

Screening of our chemical library in the model KinA/Spo0F TCS[4,5] from *Bacillus subtilis* resulted in the identification of the bisamidine indole **1** as a lead structure (Table 1, $IC_{50} = 31\,\mu M$). Indole **1** is a known antibacterial/antifungal agent, structurally related to the bisbenzamidine class of DNA minor-groove binders.[6,7] The monoamidine analogue **2** was synthesized to reduce the potential for DNA binding. This compound showed a 4-fold increase in potency ($IC_{50} = 7.7\,\mu M$), though the antibacterial activity was diminished (Table 6).

Since the synthesis of this class of indoles is tedious and low yielding, the indole nucleus was replaced with a benzimidazole. This modification has the advantage that a large variety of compounds with diverse substitution patterns can be easily synthesized and, therefore, the SAR could be rapidly delineated.

Table 1. KinA inhibitory activity of initial leads



Compd	X	Y	IC_{50} (μM)
1	CH	$C(=NH)NH_2$	31
2	CH	H	7.7
3	N	H	42

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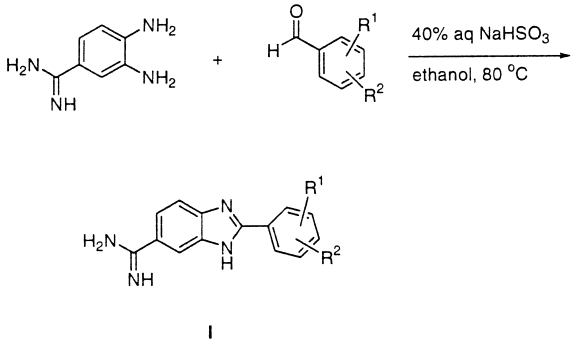
Benzimidazole **3** was about 5 times less potent than the analogous indole **2** in the biochemical assay, and exhibited moderate potency against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and VRE. Encouraged by this data, the synthesis of benzimidazole analogues was undertaken. The HPK inhibitory potency and antibacterial activity of these compounds are reported in this paper.

The target amidines **I** were synthesized in one step by condensing the bisulfite adduct of the appropriately substituted benzaldehyde with 4-amidinophenylenediamine in warm ethanol.[8] The compounds were either screened as the free base or the hydrochloride salts (formed by recrystallization from 1 N HCl). In this manner, over 200 compounds were prepared in a parallel synthetic effort (Scheme 1).[9]

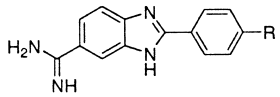


I

Scheme 1.

The 4-phenoxyphenyl analogue **3** was slightly more potent than the 3-phenoxyphenyl analogue with IC_{50}'s of 42 and 53 μM, respectively. In addition, substitutions on the phenoxyphenyl portion did not significantly affect HPK inhibitory potency (data not shown). Replacement of the phenoxy moiety was briefly explored (Table 2). Biphenyl analogue **4** was less potent than phenoxy derivative **3**, as was the isopropyl analogue **5**. Removal of the phenoxy moiety completely abolished all HPK inhibitory activity (**6** vs **3**). Introduction of a diphenylamino group afforded compound **7** which was about 2.5 times more potent than parent benzimidazole **3**.

Table 2. Modification of phenyl substituents

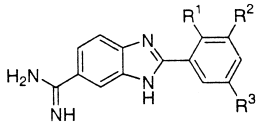


Compd	R	IC_{50} (μM)
3	OPh	42
4	Ph	88
5	$CH(CH_3)_2$	220
6	H	>500
7	NPh_2	17

By continuing to employ diverse aldehydes in the benzimidazole forming reaction, it was discovered that the 2-hydroxy-3,5-di-*t*-butyl analogue **8** was 4-fold more potent than phenoxyphenyl analogue **3**, but, more importantly, it exhibited superior antibacterial activity (Tables 3 and 6).

Compounds **9**–**14** were synthesized to probe the SAR for this subseries of amidino benzimidazoles. As is evident from the data in Table 3, removal of the hydroxyl group resulted in a decrease in activity (**8** vs **10** and **11** vs **14**). Methylation of the hydroxyl was also detrimental to activity (**8** vs **9**). However, the hydroxyl group itself was not sufficient for HPK inhibitory activity. Compound **13**, which lacks both *t*-butyl groups, was essentially inactive. To explore the significance of the individual *t*-butyl groups, the regioisomeric mono-*t*-butyl compounds **11** and **12** were synthesized. The results suggested that KinA contains a hydrophobic pocket near the 3-position of the phenyl ring, since the 3-*t*-butyl moiety is necessary for good potency (**8** vs **11** and **11** vs **12**).

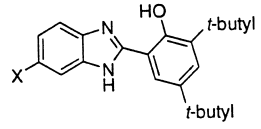
Table 3. KinA inhibitory activity of 2-hydroxyphenyl series



Compd	R^1	R^2	R^3	IC_{50} (μM)
8	OH	*t*-Butyl	*t*-Butyl	12
9	OMe	*t*-Butyl	*t*-Butyl	76
10	H	*t*-Butyl	*t*-Butyl	25
11	OH	*t*-Butyl	H	6
12	OH	H	*t*-Butyl	64
13	OH	H	H	470
14	H	*t*-Butyl	H	163

Modification of the amidine moiety was also examined (Table 4). Cyclic amidine **15** was equipotent to compound **8** in the biochemical assay. Replacement of the basic amidine with a carboxylic acid resulted in approximately a 7-fold decrease in potency (**8** vs **16**). The analogous methyl ester **17** was essentially inactive. However, the aminoamide **18** retained good HPK

Table 4. Modification of the charged substituent

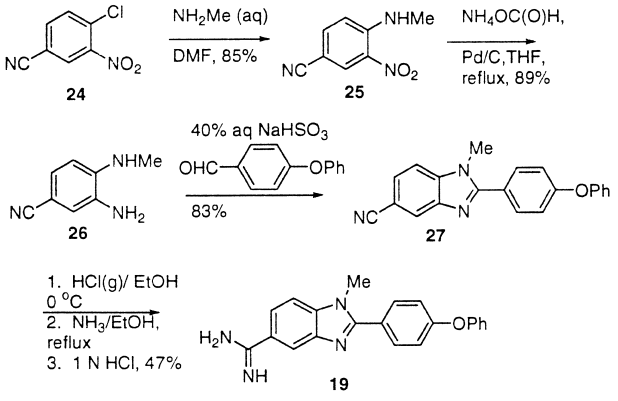


Compd	X	IC_{50} (μM)
8	$C(=NH)NH_2$	12
15		10
16	CO_2H	86
17	CO_2CH_3	380
18	$C(O)NHCH_2C(CH_3)_2NH_2$	10

inhibitory activity. These data indicate that a basic functional group is the preferred functionality on the benzimidazole portion of the substrate.

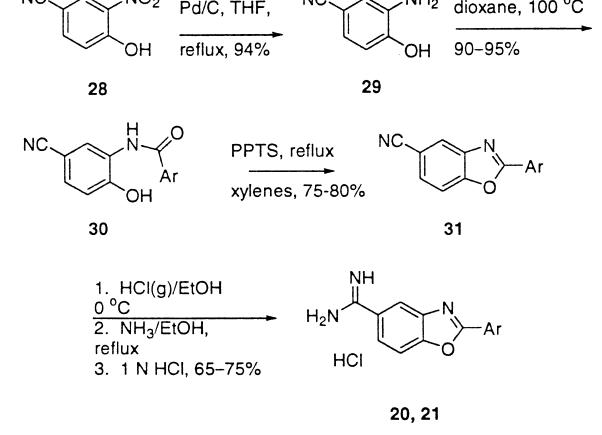
Variation of the heterocyclic nucleus was examined in the 4-phenoxyphenyl and 2-hydroxy-3,5-di-*t*-butylphenyl subseries in order to determine the importance of the benzimidazole NH on TCS and antibacterial activity. To this end, the methylated benzimidazole **19** and benzoxazole derivatives **20**–**23** were prepared and screened for biological activity.

The *N*-methylbenzimidazole **19** was synthesized according to Scheme 2. Nitro derivative **25**, prepared by reaction of aqueous methylamine with 4-chloro-3-nitrobenzonitrile (**24**), was reduced to aniline **26**. This was reacted with the bisulfite adduct of 4-phenoxybenzaldehyde to afford the benzimidazole nucleus **27**. Pinner reaction of nitrile **27** afforded amidine **19**.



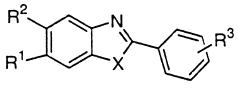
Scheme 2.

The synthesis of the regioisomeric benzoxazole analogues for each subseries was undertaken. The 5-amidinobenzoxazoles **20** and **21** were synthesized as depicted in Scheme 3. The appropriately substituted aryl acid chloride was coupled[5] with 3-amino-4-hydroxybenzonitrile (**29**), prepared by hydrogenation of the corresponding nitro compound **28**, to afford amide **30**. This amide was then cyclized to benzoxazole **31** with PPTS in refluxing xylenes. Elaboration of nitrile **31** into targeted amidines **20** and **21** was readily accomplished in two steps via the imidate. The desired amidines were converted to the hydrochloride salt by recrystallization from hydrochloric acid (1 N). The 6-amidinobenzoxazoles **22** and **23** were synthesized by an analogous route utilizing 4-nitro-3-hydroxybenzonitrile.



Scheme 3.

Table 5. Modification of the heterocycle



Compd	R^1	R^2	R^3	X	KinA/Spo0F (IC_{50}, μM)
3	Am[a]	H	4-OPh	NH	42
19	H	Am	4-OPh	NMe	44
20	H	Am	4-OPh	O	138
22	Am	H	4-OPh	O	49
8	Am	H	2-OH; 3,5-*t*-Bu	NH	9.8
21	H	Am	2-OH; 3,5-*t*-Bu	O	14
23	Am	H	2-OH; 3,5-*t*-Bu	O	15.4

[a]Am is an amidine moiety.

N-Methyl derivative **19** was equipotent to the unalkylated benzimidazole **3** (Table 5) thus indicating that the benzimidazole hydrogen was not absolutely necessary for activity in the biochemical assay. Within the phenoxyphenyl series, the 6-amidino benzoxazole **22** was approximately 3-fold more potent than the 5-amidino isomer **20**. However, in the di-*t*-butyl series, both isomers **21** and **23** are equipotent to the analogous benzimidazole **8**. These data confirm that neither the hydrogen bond donating ability nor the basicity of the benzimidazole is essential for TCS inhibitory activity. In addition, since oxygen can only accept a hydrogen bond, it is possible that a hydrogen bond acceptor at that position of the heterocycle is sufficient for activity.

Table 6. MIC (μg/mL) for select compounds[a]

Compd	*S. aureus* ATCC 29213	MRSA OC 2089	*E. faecalis* OC 3041	*E. faecium* (VRE) OC 3312
1	1	0.5	1	0.5
2	4	4	4	4
3	8	16	16	16
7	4	16	4	4
8	1	2	2	1
9	4	4	8	4
10	2	4	4	4
11	1	1	2	2
12	8	8	16	16
15	1	1	1	1
19	16	32	>64	32
21	1	1	1	1
23	2	2	2	1
Oxacillin	0.25	>64	16	>64
Vancomycin	1	2	4	>128

[a]The variance in the determination of MIC values is 2-fold such that an MIC difference of more than two dilutions is significant.[10]

Several of the more potent analogues were tested for in vitro antibacterial activity against important Gram-positive pathogens (Table 6). The TCS inhibitory activity of the benzimidazoles generally correlated with antibacterial activity within each subseries. In particular, there was a uniform 8-fold difference in the MIC values for the organisms studied between the regioisomeric mono-*t*-butyl compounds **11** and **12**. This is consistent with the IC_{50} values observed for these two compounds. Benzimidazole **8** and benzoxazoles **21** and **23** displayed comparable antibacterial activity thus indicating that the NH is not essential for activity. However, for *N*-methyl analogue **19**, a free NH may contribute to antibacterial activity, since there is a 2- to >4-fold decrease in the MIC values.

Generally, the compounds exhibited consistent antibacterial activity against both susceptible and resistant Gram-positive organisms compared to vancomycin and oxacillin. In spite of the apparent correlation between HPK inhibitory potency and antibacterial activity, recent studies have shown that bacterial killing may be due to multiple mechanisms of action for compound **8**.[11]

In conclusion, a series of amidino benzimidazoles has been identified as potent inhibitors of the HPK KinA. The optimum group at the 2-position of the benzimidazole is 2-hydroxy-3-*t*-butylphenyl. The benzimidazole NH is not essential for activity in the biochemical assay nor is it essential for antibacterial activity. Many of these compounds exhibited good in vitro antibacterial activity against susceptible as well as resistant strains of several Gram-positive organisms. They exhibited good activity against VRE, a significant nosocomial pathogen especially among immunocompromised patients. The SAR results from this study will be applied to the design of future compounds.

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