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DNA binding ligands targeting drug-resistant Gram-positive bacteria. Part 1: Internal benzimidazole derivatives

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Abstract—Novel DNA minor-groove binding ligands with a promising antibacterial profile are described. Apart from excellent in vitro potency against multiple Gram-positive bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), and penicillin-intermediate *Streptococcus pneumoniae* (PISP), a small subset of compounds was active against Gram-negative bacteria such as *Escherichia coli* (*E. coli*).
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The steadily increasing frequency of bacterial resistance to antibiotics has become a severe health care problem and has revitalized the search for bactericidal molecules acting by a novel mechanism.[1] Functional genomics has identified a number of essential bacterial targets;[2] however, subsequent lead discovery and optimization has often failed to meet the stringent requirements such as whole-cell antibacterial potency, broad spectrum of activity, and tolerability.[3] Alternatively, there are many natural products that show antibacterial potency. In an evolutionary process, these compounds have been selected for specific purposes, often as part of a defense mechanism between microorganisms. In order to serve a therapeutic application, such natural products likely will need to be optimized for several parameters such as in vitro potency and ultimately in vivo efficacy at a well-tolerated dosage. Promising advances in the area of natural product optimization have been reported recently (e.g., TAN-1057 A/B,[4] negamycin,[5] globomycin,[6] saphenamycin[7]), and likely, bacterial genomics and functional studies will be pivotal in determining the precise mode of action of such agents.

We have focused on the optimization of distamycin A[8] analogues for therapeutic application in the treatment of severe infections caused by drug-resistant, Gram-positive bacteria. Originally, the discovery of this DNA-binding natural product initiated the development of more complex minor-groove binding ligands with subnanomolar affinity for predetermined target sequences.[9–11] We reported a first generation of distamycin A analogues with significantly improved in vitro potency against drug-resistant Gram-positive bacteria.[12] These molecules have been shown to bind A/T rich target sequences that are commonly found in bacterial promoters and replication origins. As a result, they inhibit DNA replication and RNA transcription and consequently kill bacteria.[13] More recently, we described an optimization process in which the influence of the N- and C-terminal units of tetra-heterocyclic compounds on in vitro potency and acute tolerability was investigated.[14] In this study, several new end-terminal substituents were identified; a prototypic structure (**1**), which has shown in vivo efficacy against a lethal *S. aureus* infection in the mouse peritonitis model, is shown in Figure 1. Compound **1** consists of three consecutive *N*-methyl-pyrrole-2-carboxamido units (Py), an N-terminal 3-chlorothiophene-2-carboxamido group, and a C-terminal 4-ethylmorpholine function. In order to improve the aqueous solubility and ultimately oral bioavailability,[15] we asked the question whether the molecular weight (M_w) and/or the number of amide bonds in molecules like **1** could be reduced without loss of potency. Replacement of a Py unit by a benzimidazole moiety would formally eliminate one internal

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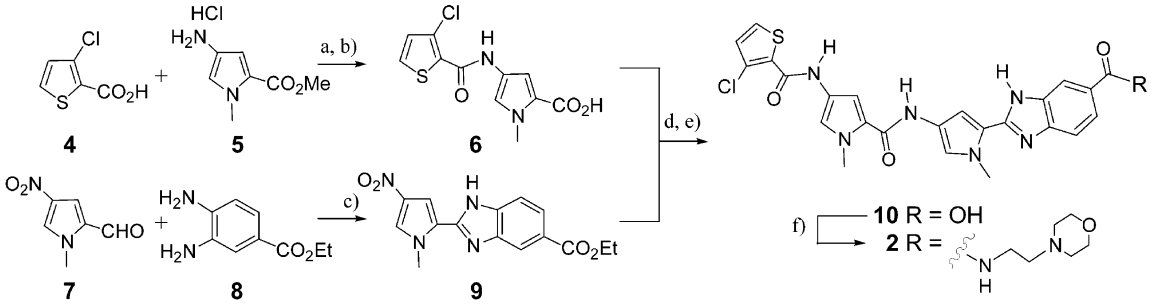


Scheme 1. Synthesis of compound **2**: (a) **4** (1.2 equiv), HBTU (1.14 equiv), DMF/^{i}Pr$_2$EtN 10:1, 25 °C, 30 min; then, **5** (1.0 equiv), 25 °C, 12 h; (b) KOH (3.9 equiv), EtOH/H_2O 1:1, 25 °C, 12 h, 90% (2 steps); (c) **7** (1.0 equiv), **8** (1.06 equiv), DMF, 80 °C, 1 h; then, 1,4-benzoquinone (1.5 equiv), 120 °C, 2 h, 61%; (d) **9** (1.0 equiv), PdC, H_2 (1 atm), DMF, 25 °C, 20 h; then, **6** (1.2 equiv), HBTU (1.14 equiv), DMF/ ^{i}Pr$_2$EtN 10:1, 25 °C, 17 h; (e) KOH (30 equiv), EtOH/H_2O 4:5, 60 °C, 4 h, 74% (2 steps); (f) **10** (1.0 equiv), HBTU (1.0 equiv), 4(2-aminoethyl)morpholine (5 equiv), DMF, ^{i}Pr$_2$EtN, 37 °C, 2 h, prep. HPLC, 32%.

amide bond while retaining all hydrogen bond donor functions that are critical for DNA recognition in the minor-groove. In fact, the benzimidazole element per se is an important structural motif of various types of DNA minor-groove binding ligands.[16–20] Additionally, the inherent fluorescent property of benzimidazole containing compounds renders them attractive tools for non-therapeutic applications such as cellular localization studies.[21,22]

The synthesis of compound **2** is given in Scheme 1 as a representative example. By analogy to the preparation of building block **6**,[23] all N-terminal aryl-pyrrole dimers were synthesized in two steps by coupling the corresponding acids or acid chlorides to the known amino pyrrole **5**,[24] followed by saponification of the dimeric intermediate. Reacting the aldehyde **7**[25] and the diamine **8** under oxidative conditions yielded the benzimidazole ethyl ester **9** in 61% yield: a similar procedure has recently been described for the corresponding methyl ester.[19] The nitro ester **9** was reduced under hydrogenolytic conditions and the resulting amine was coupled in situ to either a monomer or a dimer. Saponification of the resulting trimeric and tetrameric esters provided the corresponding acids. Activation (HBTU) followed by coupling of the C-terminal amine (DMF/^{i}Pr$_2$EtN, 37 C, 212 h) yielded the final compounds, which were purified by preparative HPLC and characterized by ^{1}H NMR and mass spectroscopy as described previously.[12,14] Details for the synthesis of the thiophene tetramer **10** and the final compound **2** are described below.[26,27] The isomeric benzimidazole **3** was prepared in analogy by standard amide bond formations from the corresponding building blocks.

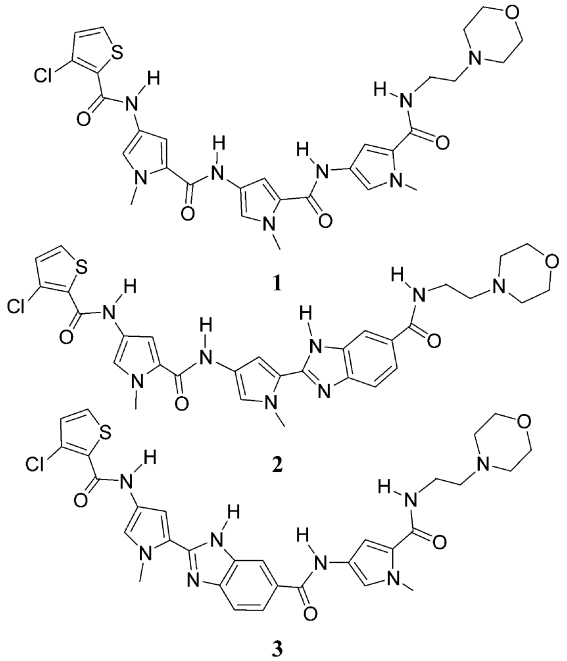


Figure 1. Structure of compounds **1**–**3**.

We first synthesized compound **2**, in which the C-terminal Py is replaced by a benzimidazole unit. In an initial antimicrobial screen (Table 1),[28] this compound exhibited excellent potency against MSSA, VSEF, and PISP. In contrast to the previously reported DNA binding antibacterials,[12,14] **2** was also moderately active against *E. coli*, a representative strain of Gram-negative bacteria. Its isomer **3**, carrying the benzimidazole group between two Py units, was similarly potent against the Gram-positive strains, but lacked activity against *E. coli*. The third isomer, in which the Py unit adjacent to the thiophene is substituted by a benzimidazole, was significantly less potent (data not shown).

We next studied whether a selection of the N- and C-terminal modules that showed good antibacterial potency in the context of the internal Py_3 structure[14] would give rise to active compounds in the benzimidazole series. All compounds were tested against methicillin-resistant and -sensitive *S. aureus*, vancomycin-resistant and -sensitive *E. faecalis*, penicillin-resistant and -intermediate *S. pneumoniae*, and *E. coli* (Table 2).[28] Replacement of the C-terminal morpholine in **2** by six-membered ring analogues such as a thiomorpholine (**11**)

Table 1. Antibacterial activity of compounds **1**–**3** (Fig. 1)

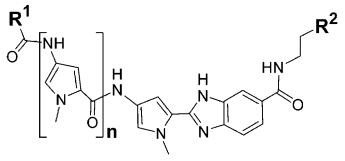
	MSSA[a] 13709	VSEF 29212	PISP 49619	*E. coli* 25922
1	1–2	1	0.13	>32
2	0.25	0.25	0.25	4
3	0.13	0.25	0.25	>32

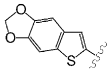
[a] MIC values in (μg/mL) against ATCC strains. MSSA, methicillin-susceptible *S. aureus*. VSEF, vancomycin-susceptible *E. faecalis*. PISP, penicillin-intermediate *S. pneumoniae*.

or a piperidine (**12**) led to molecules with good potency against all Gram-positive strains but no activity against *E. coli*. The N-terminal 3-chlorobenzothiophene (**13**) and the benzamides **14** and **15** revealed comparable potencies to thiophene **2** against Gram-positive bacteria. Both **14** and **15** showed moderate potency against *E. coli*, while benzothiophene **13** was not active. These results indicate that this class of antibiotic agents can be optimized in a modular fashion for in vitro potency; that is, the terminal subunits that were previously identified in the Py_3 series also showed activity as benzimidazole analogues.

To reduce the molecular weight (M_w), we synthesized compounds that lack an internal Py unit. The 3-chlorothiophene **16** ($M_w = 513$)—the smaller homologue of compound **2** (M_w 635)—significantly lost potency, whereas the benzothiophene **17** ($M_w = 563$) and the 4-chloro-2-fluorobenzamide **18** ($M_w = 524$) showed excellent activity against all Gram-positive strains. As it appeared that the larger N-terminal units were beneficial for potency, we incorporated the bicyclic isoquinoline (**19**) and the tricyclic benzothiophene (**20**) in the smaller series. Both end caps have been shown to exhibit excellent in vitro potency in the Py_3 series;[14] the isoquinoline **19** ($M_w = 523$) demonstrated good activity against the Gram-positive strains, but no *E. coli* activity. Interestingly, the benzothiophene **20** ($M_w = 572$) was extremely potent and showed MIC values of 0.03–0.06 μg/mL against *S. aureus*, *E. faecalis*, and *S. pneumoniae*, irrespective of their resistance to other drugs. Additionally, compound **20** exhibited excellent activity against various Gram-negative strains such as *E. coli* (MIC 0.5 μg/mL), *Haemophilus influenzae* (ATCC 78025, MIC 0.06 μg/mL), and *Moraxella catharrhalis* (ATCC 25238, MIC 0.031 μg/mL), but was inactive against *Pseudomonas aeruginosa* (ATCC 27853) up to 32 μg/mL.

Table 2. Antibacterial activity against drug-resistant and -sensitive ATCC strains



	n	R^1	R^2	M_w	MRSA[a] 27660	MSSA 13709	VREF 51559	VSEF 29212	PRSP 51422	PISP 49619	*E. coli* 25922
2	1			635	0.25	0.25	0.13	0.25	0.03	0.25	4
11	1			651	0.5	1	0.13	0.13	0.06	0.13	>32
12	1			633	0.5	2	0.5	0.5	0.13	0.25	>32
13	1			685	0.5	0.13	0.25	0.25	0.13	0.13	>32
14	1			630	0.13	0.25	0.25	0.06	0.06	0.06	4
15	1			647	0.13	0.25	0.13	0.03	0.13	0.03	8
16	0			513	8	8	N/A[b]	4	N/A	4	>32
17	0			563	0.13	0.25	0.25	0.5	0.25	0.13	>32
18	0			524	0.25	0.13	0.06	0.03	0.03	0.03	>32
19	0			523	0.5	0.5	1	0.5	0.5	0.25	>32
20	0			572	0.03	0.03	0.03	0.03	0.06	0.06	0.5

[a] MIC values in (μg/mL). MRSA, methicillin-resistant *S. aureus*. VREF, vancomycin-resistant *E. faecalis*. PRSP, penicillin-resistant *S. pneumoniae*.
[b] N/A, not analyzed.

To date, the structure–activity relationship regarding *E. coli* activity is not fully understood. Clearly, subtle structural modifications have an incisive effect on in vitro potency and likely, both penetration and efflux behavior through the cell wall of Gram-negative bacteria are important parameters that influence activity.

For compounds **2**, **16**, and **19**, DNA binding was studied by quantitative DNase I footprint titration at the sequence 5′-ACAATTAA-3′, a site proximal to the σ70 RNA polymerase subunit binding site within the *E. coli* Trc promoter.[12,14] Compound **2** showed high affinity for this target site (K_d <0.1 nM), whereas the smaller homologue **16** bound this sequence only at higher concentrations (K_d≈500 nM). This is in accordance with the loss of potency of **16** as compared to **2**. The low-molecular-weight compound **19** bound the same DNA sequence with a K_d of ca. 10 nM, indicating that the smaller compounds still act by DNA binding.

In an exploratory in vivo profiling study, compound **2** was tested for efficacy against a lethal infection of methicillin-resistant *S. aureus* in a mouse peritoneal sepsis model: the compound showed full protection of mice at IV dosages of 50 and 20 mg/kg.[29]

In conclusion, we showed that the number of amide bonds and the molecular weight of analogues of the thiophene **1** can be reduced without loss of in vitro potency against a wide range of Gram-positive bacteria. In fact, the benzimidazole **2** showed excellent potency against various Gram-positive pathogens and moderate activity against Gram-negative bacteria such as *E. coli*. The same compound was further shown to bind with high affinity to a functionally relevant A/T rich DNA sequence and has demonstrated improved efficacy in a mouse model against a lethal MRSA infection as compared to **1**. In a next step, C- and N-terminal units that were previously identified to lead to active molecules in the Py_3 series were investigated in the context of the benzimidazole-pyrrole backbone. This limited study suggests that units can be optimized in a modular fashion. Removal of an internal Py group further reduced the molecular weight and the number of amide bonds within the molecules and ultimately led to compound **20**, which showed excellent MIC values against all Gram-positive strains (0.03–0.06 μg/mL) and good in vitro potency against various Gram-negative pathogens such as *E. coli*, *H. influenzae*, and *M. catharrhalis*. The antimicrobial profile of compound **20** clearly demonstrates the potential of this class of antibiotics for a broad-spectrum therapeutic application.

Acknowledgements

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23. Synthesis of **6**. A mixture of acid **4** (0.90 g, 5.53 mmol) and HBTU (1.99 g, 5.25 mmol) in DMF (5 mL) and $^{i}Pr_2EtN$ (0.5 mL) was stirred at 25 °C for 30 min, treated with amino ester **5** (0.88 g, 4.60 mmol), stirred at 25 °C for 12 h, and poured into ice water (100 mL). The resulting precipitate was collected by filtration, washed (H_2O), and dried in vacuo. The crude solids were dissolved in EtOH (15 mL), treated with an aqueous solution of KOH (1.0 g in 15 mL), and stirred at 25 °C for 12 h. The mixture was diluted with H_2O (100 mL), cooled to 0 °C, and acidified

to pH = 2 (2 M aqueous HCl). The precipitated solids were collected by filtration, washed (H_2O), and dried to give dimer **6** (1.18 g, 90%).

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26. Synthesis of **10**. A mixture of nitro ester **9** (1.00 g, 2.97 mmol) and PdC (10%, 0.30 g) in DMF (20 mL) was stirred under H_2 (1 atm) at 25 °C for 20 h and filtered through Celite. The filtrate was added to a preactivated solution of acid **6** and HBTU (1.01 g, 3.56 mmol of **6**; 1.29 g, 3.39 mmol of HBTU in 20 mL DMF and 2 mL $^{i}Pr_2EtN$; stirred for 30 min at 37 °C). The combined reaction mixture was stirred at 25 °C for 17 h and poured into ice water (ca. 400 mL, containing 30 mL satd aqueous K_2CO_3). The resulting precipitate was collected by filtration and dried. The solids were dissolved in EtOH (40 mL) and H_2O (50 mL), treated with KOH (5.0 g, 0.09 mol), and stirred for 4 h at 60 °C. The mixture was diluted with H_2O (ca. 300 mL), cooled to 4 °C, acidified to pH≈5 (1 M aqueous HCl), and extracted with AcOEt (2×150 mL). The combined organic layers were dried ($MgSO_4$), and evaporated to give tetramer **10** as a solid (1.15 g, 74%).
27. Synthesis of **2**. A mixture of **10** (0.17 g, 0.32 mmol) and HBTU (0.12 g, 0.32 mmol) in DMF (1 mL) and $^{i}Pr_2EtN$ (0.1 mL) was stirred at 25 °C for 30 min, treated with 4(2-aminoethyl)morpholine (0.21 mL, 1.6 mmol), and stirred at 37 °C for 2 h. The solution was diluted with 50% aqueous AcOH (15 mL) and washed with Et_2O (2×, each 10 mL). HPLC purification (Hamilton PRP-1 column, 250×21.5 mm, A: 0.5% AcOH in H_2O, B: CH_3CN, 0% to 60% B in 60 min, 20 mL/min, UV detection at 310 nm) gave **2** (67 mg, 32%). ^1H NMR (DMSO-d_6) δ 12.73 (s, 1H), 10.22 (s, 1H), 10.08 (s, 1H), 8.38–8.32 (m, 1H), 8.15 (br. s, 0.5H), 7.91 (br. s, 0.5H), 7.87 (d, J = 5.3, 1H), 7.72–7.59 (m, 2H), 7.45 (br. d, J 8.2 Hz, 0.5H), 7.35 (br. s, 1H), 7.30 (s, 1H), 7.19 (d, J = 5.3, 1H), 7.12 (br. s, 0.5H), 7.08 (br. s, 2H), 4.08 (s, 3H), 3.89 (s, 3H), 3.61–3.56, 3.44–3.38, 2.45–2.39 (3 m, 12H). ESI MS 637.2 (40%), 635.2 (100%, $[M+H]^+$). Purity (analyt. HPLC,[14] 310 nm) 94%. All compounds were characterized by ^1H NMR and mass spectrometry and showed purity of at least 90%. The isolated yields after HPLC purification ranged from ca. 10–30%.
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29. Mice were infected intraperitoneally with a lethal dose of MRSA (ATCC 27660, inoculum of 1.8×10^7 cfu in 0.5 mL) and treated IV 1 and 3 h post infection with positive control, test compound, or vehicle alone. Survival of animals, the primary endpoint of this study, was monitored for five days. For further details, see ref 14.