Letters

DNA Binding Ligands with Improved in Vitro and in Vivo Potency against Drug-Resistant *Staphylococcus aureus*

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Abstract: Potent in vivo activity against methicillin-resistant *Staphylococcus aureus* (MRSA) has been difficult to achieve with previously reported DNA binding antibacterials. Herein, we describe an efficient access to a focused library of new analogues yielding compounds with improved activity in a mouse peritonitis model. The most potent molecules (**14** and **19**) exhibit efficacy against MRSA at ED_{50} values of ∼1 and ∼5 mg/kg, respectively, and display excellent in vitro activity against vancomycin-resistant *S. aureus*.

Infections caused by drug-resistant bacteria represent an increasing threat to society and pose a real challenge to physicians for selecting the correct antibiotic from the arsenal of existing therapeutics.[1] In addition to methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae* (PRSP), two strains of *S. aureus* with acquired resistance to vancomycin have recently been isolated.[2] The increasing emergence of resistance to virtually all existing antibiotics poses a threat to health care, and novel therapeutics are desperately needed. However, the development of new antibacterial drugs based on novel targets and consequently the lack of cross-resistance have been a major challenge for drug discovery.[3] This is underscored by the fact that despite major efforts within the past 35 years only two novel classes of antibiotics, the oxazolidinones (linezolid) and lipopeptides (daptomycin), have reached the market.[4,5]

We have been optimizing the antibacterial potency and drug-related properties of analogues of distamycin A, a small DNA minor-groove binding natural product.[6] In comparison to distamycin, the first generation of compounds exhibited a significantly improved potency against Gram-positive bacteria.[7] More recently, we reported an optimization process in which we identified compounds bearing novel C- and N-termini.[8] A prototypic structure, the 3-chlorothiophene **1**, is shown in Figure 1. This compound was found to be well tolerated in rodents at doses that might be required for efficacy. In a first proof-of-concept study, **1** given intravenously rescued mice from a lethal infection of methicillin-susceptible *S. aureus* (MSSA) with an ED_{50} of ∼30 mg/kg. However, at a dose of 50 mg/kg, it was not efficacious against MRSA in the same model. Further optimization of **1** has led to an advanced lead molecule (**2**): the isoquinoline **2** showed an ED_{50} of ∼7 mg/kg against MSSA and 11 mg/kg against MRSA.[9,10] In this optimization process, we have found that structural modifications of the internal *N*-methylpyrrole-2-carboxamide units (Py) are critical for in vivo efficacy against drug-resistant *S. aureus*. Interestingly, the in vitro activity in the presence of human plasma albumin (HPA) has correlated relatively well with in vivo efficacy following intravenous administration and was used as a surrogate for the focused lead optimization presented herein. We now report our efforts to further optimize lead **2** for improved in vivo efficacy against MRSA.

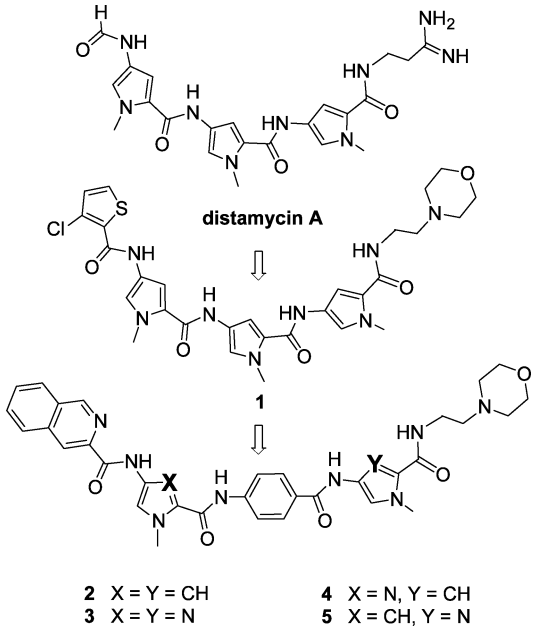

Figure 1. Structures of distamycin A and lead molecules.

The tetrameric isoquinolines **3**−**5** (Figure 1) were prepared from the corresponding isoquinoline, imidazole, and pyrrole building blocks by standard amide bond formations; analogous chemistry has been described in detail.[8] For the preparation of a small library of analogues of the tetrameric isoquinolines **2** and **5** with varying C-terminal amino groups, we developed a one-pot procedure that allows a rapid conversion of the dimethoxyacetals **10** and **11** to the final compounds: reductive amination of the acetal in the presence of solid-phase bound cyanoborohydride and the corresponding amine at ca. 150 °C (microwave) gave the final compounds (Scheme 1). All materials were purified by preparative HPLC and characterized by ^{1}H NMR, mass

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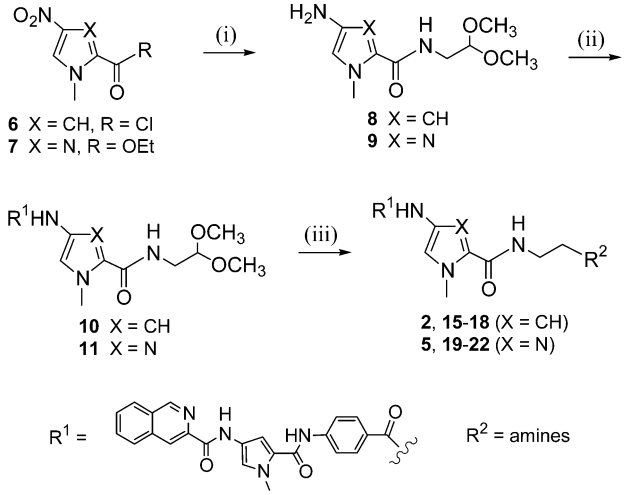
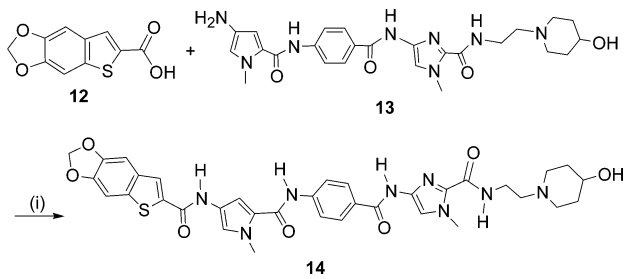
Scheme 1. Synthesis of **2**, **5**, and **15**−**22** (Table 2)[a]



[a] Reagents: (i) for **8**: **6**, THF, DIEA, 2,2-dimethoxyethylamine, 30 min, 25 °C, then H_2, Pd, 25 °C, 2 h, 85%; for **9**: **7**, 2,2-dimethoxyethylamine, 20 min, 110 °C, then H_2, Pd, 25 °C, 2 h, 68%; (ii) R^1OH, BopCl, NMP, DIEA, DMAP, amine, 30 min, 60 °C, **10** (64% from **8**), **11** (75% from **9**); (iii) **10** or **11**, amine, MP-$NEt_3^+BCNH_3^-$, $Cl_2CHCOOH$, THF, 10 min, 150 °C, then preparative HPLC.

Scheme 2. Synthesis of **14**[a]



[a] Reagents: (i) **12**, **13**, HATU, NMP, DIEA, 30 min, 60 °C, 51%.

spectrometry, and analytical HPLC as documented previously.[8] Experimental details and analytical data are available in the Supporting Information. The purity of the compounds was generally greater than 95% (exceptions are specified in Table 2). The reductive amination protocol allowed a very efficient access to a small quantity of compound. At larger scales (>50 mg), compounds were prepared by classical solution-phase chemistry from their corresponding building blocks. Similarly, **14** (Scheme 2) and the intermediates **10** and **11** were prepared from the known nitropyrrole **6**[11] and imidazole **7**,[12] respectively.

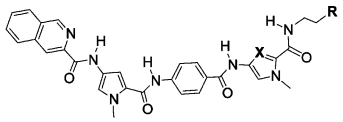
The lead optimization, aimed at improvement of potency of **2**, proceeded stepwise. We started by systematically replacing the internal Py units in **2** by an *N*-methylimidazole-2-carboxamide moiety (Im). We speculated that replacement of Py by an Im might improve the physicochemical properties of the molecules. The Im building block has been studied extensively in polyamides, i.e., larger homologues of distamycin A with improved DNA binding affinity and sequence specificity;[13] it has been shown that in a 2:1 binding motif, an Im opposite a Py specifically recognizes a G·C base pair, whereas in a 1:1 binding mode, it still interacts with A/T rich sequences but lacks the specificity of Py.[14,15] We prepared all three Im analogues of the reference **2** and compared their in vitro potency against drug-

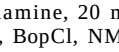
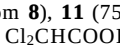
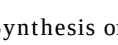
Table 1. Antibacterial Activity of Compounds **2**−**5**[a]

	X	Y	MRSA 27660	MRSA +HPA[b]	MSSA 13709	VRF 51559	PRSP 51422
2	CH	CH	0.06	1	0.06	1	0.06
3	N	N	0.06	1	0.13	0.13	0.25
4	N	CH	16	32	2	0.5	0.5
5	CH	N	0.008	0.13	0.008	0.008	0.03

[a] MIC values in μg/mL against ATCC strains. MRSA, MSSA: methicillin-resistant, -susceptible *S. aureus*. VRF: vancomycin-resistant *E. faecium*. PRSP: penicillin-resistant *S. pneumoniae*. [b] HPA: 2% of human plasma albumin was added to the test medium. MRSA is ATCC strain 27660.

Table 2. Antibacterial Activity of All ^{N}Py-^{p}Bz-Py^C and ^{N}Py-^{p}Bz-Im^C Compounds



	X	R	VRSA HMC3	[a]MRSA 27660	MRSA + HPA[b]	MSSA 13709
2	CH		0.25	0.13-0.06	1	0.03-0.06
15	CH		nd[c]	0.5	16[d]	0.5
16	CH		nd	1	8	1
17	CH		nd	8	32	4
18[e]	CH		nd	16	>32	32
5	N		0.008	0.008	0.13	0.008
19	N		nd	0.03-0.06	0.25-1	<0.03-0.06
20[e]	N		nd	0.03	0.13-0.25	0.03
21	N		nd	0.25-0.5	1-2	0.06-0.13
22	N		nd	0.06	1	0.06
14	*see* Scheme 2		0.008	0.008	0.13-1	0.008

[a] See footnotes to Table 1 for details. [b] 2% of human plasma albumin was added to the test medium. [c] Not determined. [d] MIC in the presence of 4% HPA. [e] 94% purity as determined by HPLC−UV.

resistant and -susceptible *S. aureus*, *E. faecalis*, *E. faecium*, and *S. pneumoniae* (Table 1 and Supporting Information).[16] Interestingly, the bis-imidazole **3** showed similar potency compared to the bis-pyrrole **2**, whereas the activity of the Im-Py hybrids **4** and **5** significantly varied depending on the position of the Im; replacement of the C-terminal Py by Im resulted in excellent potency against all Gram-positive strains (MIC = 0.004−0.03 μg/mL for **5**), while its isomer **4** lost potency, in particular against MRSA (16 μg/mL). More importantly, **5** also exhibited an excellent MIC of 0.13 μg/mL against MRSA in the presence of HPA.

At this point, we studied the influence of the C-terminal amino group on in vitro potency. For this, we prepared a library of analogues (∼100 compounds) consisting of the ^{N}Py-^{p}Bz-Py^C and the ^{N}Py-^{p}Bz-Im^C core scaffolds (^{p}Bz: 4-aminobenzoate). The in vitro susceptibilities of representative compounds are summarized in Table 2. Clearly, the ^{N}Py-^{p}Bz-Im^C motif tolerated more structural diversity at the C-terminus than the ^{N}Py-^{p}Bz-Py^C system. For instance, the morpholine **2** is

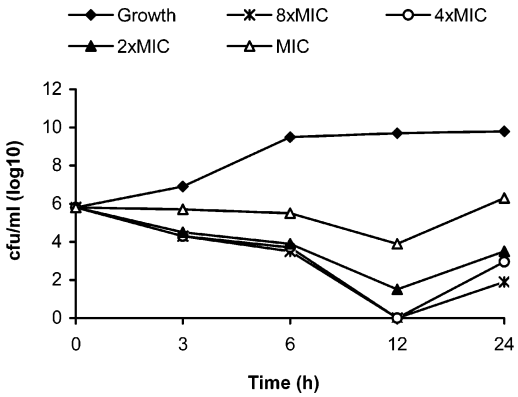


Figure 2. VRSA killing kinetics of **14** at various concentrations.

Table 3. Animal Efficacy (Mouse MRSA Peritonitis)[a]

	survival 25 mg/kg	survival 10 mg/kg	survival 3 mg/kg	survival 1 mg/kg
2	5/5	3/5	0/5	nd[b]
5	nd[b]	1/5	nd[b]	nd[b]
14	nd[b]	nd[b]	4/5	3/5
19	5/5	5/5	2/5	0/5
20	2/5	nd[b]	nd[b]	nd[b]
21	0/5	nd[b]	nd[b]	nd[b]
22	5/5	nd[b]	nd[b]	nd[b]

[a] The mouse peritionitis model was performed as described previously.[8] Mice were infected intraperitoneally with a 5−10 × LD_{50} inoculum with MRSA (ATCC 27660), treated intravenously with vehicle, positive control (vancomycin), or test articles with a single dose 1 h after infection, and monitored for 5 subsequent days with a primary endpoint of survival. [b] Not determined.

potent against all Gram-positve strains, while all derivatives (**15**−**18**) lost activity to various degrees. In contrast, all compounds of the ^{N}Py-^{p}Bz-Im^{C} series showed excellent in vitro potency, irrespective of the nature of their C-terminal amino group (**19**−**22**).

We then tested a few previously identified N-termini in the ^{N}Py-^{p}Bz-Im^{C} scaffold, using the 4-hydroxypiperidine C-terminus. The MIC profile of the benzothiophene **14** (Scheme 2) turned out to be among the most promising and is shown as a representative example. Consistent with the isoquinoline **19**, **14** exhibited remarkable potency against all Gram-positive bacteria and relatively good activity against *E. coli*.

The most potent compounds, **5** and **14**, were tested against one of the existing two clinical isolates of vancomycin-resistant *S. aureus* (VRSA),[17] which was also resistant to methicillin. Both compounds demonstrated excellent potency with identical MIC values of 0.008 μg/mL and were 30-fold more active than the parent compound **2** (Table 2). Time kill kinetics of both compounds were determined against VRSA. Whereas **5** was bacteristatic at the concentrations examined, **14** was bactericidal at 2-, 4-, and 8-fold MICs after 12 h (Figure 2). Similar killing kinetics were observed for both compounds against MRSA (ATCC 27660), however, without the occurrence of regrowth for **14** between 12 and 24 h (data not shown).

Selected compounds were tested in a mouse peritoneal sepsis model against MRSA as documented previously.[8,9] The hydroxy compounds **19** and **22** successfully rescued all animals, similar to the parent compound **2** (Table 3). In contrast, the use of methoxy ether **20**, the direct analogue of **19**, only partially protected animals (40% survival). Clearly, in vitro activity is not the only parameter influencing in vivo efficacy, as evidenced by the identical MIC values of **19** and **20** against *S. aureus*, and pharmacokinetics and ADME properties will impact efficacy as well. The bis(2-methoxyethyl)amine **21** did not show efficacy at 25 mg/kg.

Compound **22** was not pursued further because the animals showed marked lethargic behavior in an acute tolerability test at 50 mg/kg (iv). Instead, the 4-hydroxypiperidine **19** was selected for a dose−response study. It showed complete protection at 10 mg/kg, and two out of five animals survived when administered at 3 mg/kg. The compound was inactive at a dose of 1 mg/kg. From this study, we concluded that **19** has an approximate ED_{50} of 5 mg/kg against MRSA, which is significantly better than the parent compound **2**. The morpholine analogue **5** was directly tested at 10 mg/kg, since the excellent in vitro profile in the presence and absence of HPA suggested good efficacy; however, at 10 mg/kg, only one out of five animals survived.

Last, we investigated the in vivo efficacy of the benzothiophene **14**. Because this compound demonstrated a great in vitro profile, we tested only low doses in vivo. Indeed, it resulted in 80% survival at 3 mg/kg and still 60% protection at 1 mg/kg. Such performance in this stringent model of MRSA infection is rare, and the only reference therapeutic we have observed with comparable efficacy to **14** in this model is vancomycin ($ED_{50} \approx 1$ mg/kg).

Earlier lead compounds such as the thiophene **1** protected mice in a peritonitis model against a lethal infection of MSSA. However, this compound was not very efficacious against the drug-resistant MRSA strain. Lead optimization and internal replacement of selected units of the Py_3 element led to **2**, which protected mice in the same model against MRSA with an approximate ED_{50} of ∼11 mg/kg. A focused optimization of the isoquinoline **3** resulted in molecules with remarkable in vitro potency. In particular the MIC value in the presence of human plasma albumin was greatly improved compared to previously reported DNA binding antibacterials. Some of these new compounds, especially the 4-hydroxypiperidines **14** and **19**, exhibited in vivo efficacy at low doses (ED_{50} of approximately 1 and 5 mg/kg, respectively) comparable to the best of reference drugs such as vancomycin. In vitro, these compounds showed excellent potency against various drug-resistant and -susceptible Gram-positive bacteria (MIC ≥ 0.004 μg/mL). This study clearly demonstrates the potential of DNA binding molecules as antibacterials, not only in vitro but also in a mouse model of MRSA infection.

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Supporting Information Available: Experimental procedures, ^{1}H NMR spectra, mass spectra, and purity analysis data (HPLC−UV). This material is available free of charge via the Internet at http://pubs.acs.org.

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