Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2591–2594

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

DNA Binding Ligands with Excellent Antibiotic Potency Against Drug-Resistant Gram-Positive Bacteria

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Received 18 April 2002; accepted 18 June 2002

Abstract—An efficient synthesis of DNA binding molecules consisting of four heterocyclic carboxamide units and various substituents at both termini is described. The minor-groove binding ligands showed excellent activity against a broad range of Gram-positive bacteria; no cross-resistance to known antibacterial drugs was observed.

There has been an alarming increase in resistance of numerous Gram-positive bacterial strains to different classes of antibiotics such as β-lactams, macrolides, and quinolones; more recently, resistance has also been observed to the glycopeptide vancomycin and the oxazolidinone linezolid.[1,2] Thus, there is an increasing need for novel classes of antibiotics, particularly for agents with a novel mechanism of action and no cross-resistance to known antibacterial drugs. Bacterial DNA could potentially serve as an attractive and novel target for the development of new antibiotics. Specifically, bacterial promoter regions contain highly conserved RNA polymerase binding sites, including the σ-subunit regulatory elements for the initiated transcription of essential genes.[3–6] The DNA replication origin is another potential target conserved among bacterial species.[7] DNA binding ligands targeting important sequences within these regions could potentially interfere with bacterial RNA transcription and/or DNA replication and kill the bacterial organism.[4–12]

The natural product distamycin, a DNA minor-groove binding ligand, has shown weak antibacterial activity.[13] However, its DNA binding affinity is low (micromolar range) and the chemical stability is limited due to the terminal formamido group. Nevertheless, distamycin has served as a prototype for the development and refinement of minor-groove binding ligands with subnanomolar binding affinity and remarkable sequence-specificity.[14,15] Analogous minor-groove binding ligands have recently been reported to show antimicrobial activity.[16]

We herein report an efficient synthesis of DNA minor-groove binding molecules with enhanced binding affinities to a functionally relevant target sequence and excellent activities against various drug sensitive and resistant bacterial strains.

The tetrameric acid **5**, consisting of an isothiazole and three *N*-methyl pyrrole carboxamide units, is the common intermediate for the preparation of the antibacterial compounds shown in Table 1. It has been synthesized from the known starting materials **1** and **4** (Scheme 1):[17,18] Saponification of the trimer **1** and Boc-deprotection of the resulting carboxylate **2** led to the amino acid **3** in excellent yields.

Subsequent coupling of this amino acid to the isothiazole **4** resulted in the desired intermediate **5**. This tetramer was converted in a one-pot synthesis (3 steps) to the final products: in situ activation of **5**, coupling of the C-terminal amine, and nucleophilic aromatic substitution at C(5) of the isothiazole using excess of the N-terminal amine. The regioselectivity of the SN_{Ar} reaction has been reported and was confirmed by an X-ray analysis of a model compound (data not shown).[18] The final compounds were purified by HPLC and characterized by ^1H NMR and mass spectrometry. A representative protocol is given below.[19]

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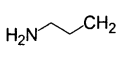
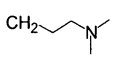
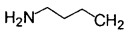
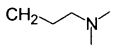
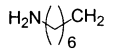
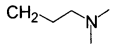
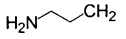
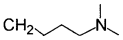
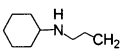
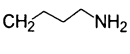
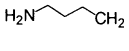
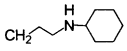
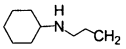
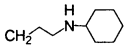
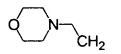
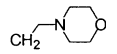
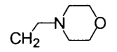
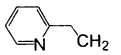
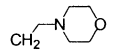
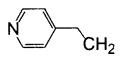
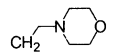
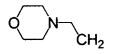
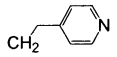
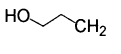
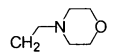
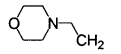
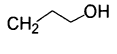
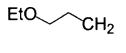
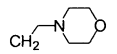
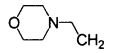
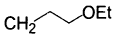
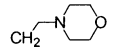
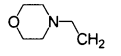
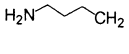
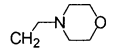
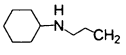
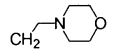
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00515-2

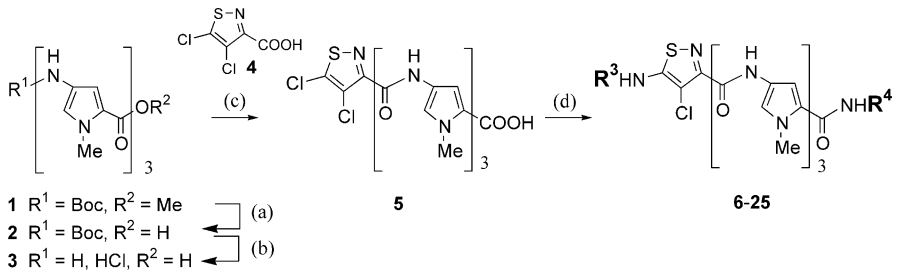
Table 1. Antimicrobial activity and DNA binding affinity of compounds **6**–**25**

	R^3	R^4	M_w	[a]*MRSA* 27660	[a]*MSSA 29213*	[a]*VREF* 51559	[a]*VSEF 29212*	[a]*PRSP 51422*	[a]*PSSP* 49619	[a]*C. albicans* 38247	[b]K_d (app.) (nM) 5′-ACAATTAA-3′
6			686	1	2	2–4	1	4	0.5	8	1.74 (±0.55)[d]
7			700	2	2	1	1	1	0.5	8	1.04 (±0.19)[d]
8			742	32	32	1	8	16	32	> 32	1.30
9			700	0.25	0.5	0.25	0.125	0.125	0.064	0.25	0.53
10			754	2	2	16	2	16	1	4	2.00
11			754	1	0.5	2	0.5-2	4	1	32	0.43
12			822	1	1	2	2	0.5-2	2	16	1.19
13			770	4	1-2	2	0.25	0.25	0.062	> 32	9.09
14			725	1-2	1	1-2	1	0.062	0.125	> 32	4
15			762	2	1[c]	4	0.5	0.5	0.125	> 32	0.83
16			762	1	1[c]	2	0.25	0.25	0.062	> 32	0.29
17			762	0.5	0.125 [c]	0.25	0.062	0.5	0.062	> 32	4.35
18			715	2	1	0.5	0.125	0.031	0.5	> 32	1.03 (±0.97)[d]
19			715	> 32	> 32	> 32	32	1	32	> 32	32.1 (±11.7)[d]
20			743	2	1	2	0.25	0.125	0.25	> 32	1.34 (±0.59)[d]
21			743	16	16	4	16	1	0.5	> 32	83.3 (±16.2)[d]
22			711	1	0.5	1	0.25	0.125	0.125	> 32	1.01
23			711	8	16	1	0.5	0.5	1	> 32	208
24			728	4	4	16	4	0.5	0.5	8	0.37
25			796	8	2	16	2	0.25	0.062	16	0.59

[a]MIC values in [μg/mL]. MRSA, methicillin-resistant *S. aureus*. MSSA, methicillin-susceptible *S. aureus*. VREF, Vancomycin-resistant *E. faecalis*. VSEF, Vancomycin-susceptible *E. faecalis*. PRSP, penicillin-resistant *S. pneumoniae*. PSSP, penicillin-susceptible *S. pneumoniae*.
[b]Equilibrium dissociation constants determined by quantitative DNase I footprint titrations (22 °C, pH 7.0, 10 mM Tris–HCl, 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$).
[c]Tested against MSSA 13709.
[d]Mean values from at least two experiments.

Screening our library of DNA minor-groove binding molecules for antimicrobial activity identified the two diamines **6** and **7** as active against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus pneumoniae* (Table 1).[20,21]

Both compounds showed similar activity against those strains regardless of their susceptibility to other classes of antibiotics. They also had moderate activity against yeast (*Candida albicans*), but no activity against a Gram-negative species (*Escherichia coli*, data not



Scheme 1. Synthesis of compounds **6**–**25**: (a) 3 M NaOH, MeOH, H_2O, 50 °C, 30 h, 87%; (b) AcOEt (sat. HCl), 0 °C, 45 min, 97%; (c) **4** (1.2 equiv), HBTU (1.14 equiv), DMF/$^{i}Pr_2EtN$ (9:2), rt, 11 h, 91%; (d) BOPCl (1.2 equiv), R^4NH_2 (2 equiv), NMP/$^{i}Pr_2EtN$ (10:1), 3 h, 37 °C; then, R^3NH_2 (20 equiv), 60 °C, 24 h.

shown). A DNase I footprint analysis revealed that both compounds bind at low nanomolar concentrations at the sequence 5′-ACAATTAA-3′, a site proximal to the σ70 RNA polymerase subunit binding site within the *E. coli* Trc promoter.[22,23]

We set out to investigate the influence of the nature (i.e., basicity, steric demand) of the terminal amino groups on the antimicrobial potency, the spectrum of activity, and the DNA binding affinity, while keeping the heterocyclic core intact. Given that all marketed antibacterial agents that are systemically administrated have good selectivity without significant antifungal activity, one of our objectives for lead optimization was to synthesize compounds with improved antibacterial and reduced antifungal potency.

Extension of the N-terminal amino propyl/butyl group of the first leads **6** and **7** to an aminoheptyl moiety (compound **8**) significantly decreased the antibacterial activity despite its high DNA binding affinity. Modifying the C-terminal dimethylaminopropyl group of the diamine **6** to a dimethylaminobutyl group (C_4-homologue **9**) increased the potency against all strains, including yeast. A set of compounds bearing the sterically more demanding cyclohexylaminopropyl group at one or both termini showed good antimicrobial activity, but still had moderate antifungal potency (compounds **10**–**12**). DNA binding studies for these molecules indicated that the bulkier six-membered ring did not greatly affect DNA interaction as determined for the target site.

All molecules discussed so far are mostly protonated under biological conditions; the pK_a value of propyl ammonium groups ranges between 9 and 10.[24] Replacing these amino groups by less basic functions such as the ethylmorpholine or ethylpyridine groups (pK_a values ca. 6 and 5, respectively),[25] led to antimicrobial agents with a modified spectrum of activity; compounds **13**–**23** bearing the ethylmorpholine group as the most basic function showed no activity against *C. albicans*. Some of these molecules exhibited excellent potency against Gram-positive bacteria. Among these less basic compounds, we have studied four isomeric pairs in which the position of the terminal R^3 and R^4 substituents is interchanged (**16**/**17**, **18**/**19**, **20**/**21**, and **22**/**23**). The dibasic pyridino-morpholines **16** and **17** bind with high affinity to their target sequence ($K_d = 0.29$ and 4.35 nM, respectively) and show similar anti Gram-positive activity. In contrast, the DNA binding affinity and antibacterial activity of the monobasic compounds **18**–**23** appeared to strongly depend on the position of the basic function: the molecules bearing the basic group at the C-terminus (**18**, **20**, and **22**) consistently showed higher DNA binding affinity and better antibacterial potency than their corresponding isomers (**19**, **21**, and **23**). This correlation of DNA binding affinity and antibacterial activity for isomeric pairs may indicate that DNA binding plays an important role in the antibacterial mechanism of action. For non-isomeric structures, such a correlation is of limited value as uptake/efflux characteristics and metabolic stability could influence the potency. Interestingly, the dibasic compounds **24** and **25** bearing a C-terminal morpholine unit and a more basic 4-aminobutyl or 3(cyclohexylamino)propyl substituent at the isothiazole showed antifungal activity, suggesting that the spectrum of activity strongly depends on the basicity of the molecule and not only on the presence of a morpholine unit.

A small library of minor-groove binding ligands consisting of a four-ring core element and diverse substituents at both termini was studied for antimicrobial activity and DNA binding affinity. The nature and position of the basic groups in the molecules appeared to strongly influence both parameters. For a series of isomeric pairs bearing the basic group at either of the termini, we observed better in vitro activity and higher DNA binding affinity for the isomer with the basic function at the C-terminus. The antifungal activity appeared to strongly depend on the basicity of the compounds; less basic molecules showed significantly reduced antifungal activity.

Molecules with reduced antifungal potency might also be more selective and tolerated by other eukaryotic systems. Studies regarding the tolerability as well as pharmacokinetics and in vivo efficacy are under investigation and will be reported in due course.

Acknowledgements

This work was supported in part by the Defense Advanced Research Projects Agency (DARPA Grant Number N65236–99–1-5427).

References and Notes

1. For example: Chu, D. T. W.; Plattner, J. J.; Katz, L. *J. Med. Chem.* **1996**, *39*, 3833.
2. Jones, R. N.; Della-Latta, P.; Lee, L. V.; Biedenbach, D. *J. Diagn. Microbiol. Infect. Dis.* **2002**, *42*, 137.
3. Harley, C. B.; Reynolds, R. P. *Nucleic Acids Res.* **1987**, *15*, 2343.
4. Collado-Vides, J.; Magasanik, B.; Gralla, J. D. *Microbiol. Rev.* **1991**, *55*, 371.
5. Lisser, S.; Margalit, H. *Nucleic Acids Res.* **1993**, *21*, 1507.
6. Gross, C. A.; Lonetto, M.; Losick, R. In *Transcriptional Regulation*; McKnight, S. L.; Yamamoto, K. R., Ed.; Cold Spring Harbor Laboratory: Cold Spring Harbor, 1992; p 129.
7. Kornberg, A.; Baker, T. A. *DNA Replication*, 2nd ed.; W. H. Freeman & Co: New York, 1992.
8. Hawley, D. K.; McClure, W. R. *Nucleic Acids Res.* **1983**, *11*, 2237.
9. Siebenlist, U.; Simpson, R. B.; Gilbert, W. *Cell* **1980**, *20*, 269.
10. Naryshkin, N.; Revyakin, A.; Kim, Y.; Mekler, V.; Ebright, R. H. *Cell* **2000**, *101*, 601.
11. Jaros-Kaminska, B.; Malagocka, E.; Ciesielska, E.; Gniazdowski, M. *Studia Biophysica* **1981**, *86*, 211.
12. Straney, D. C.; Crothers, D. M. *Biochemistry* **1987**, *26*, 1987.
13. For distamycin, see: Arcamone, F.; Penco, S.; Orezzi, P.; Nicolella, V.; Pirelli, A. *Nature* **1964**, *203*, 1064. In our assays, distamycin showed MIC values of 32–128 μg/mL against various Gram-positive strains.
14. Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215.
15. Sharma, S. K.; Reddy, B. S. N.; Lown, J. W. *Drugs Future* **2001**, *26*, 39.
16. Dyatkina, N. B.; Roberts, C. D.; Keicher, J. D.; Dai, Y.; Nadherny, J. P.; Zhang, W.; Schmitz, U.; Kongpachith, A.; Fung, K.; Novikov, A. A.; Lou, L.; Velligan, M.; Khorlin, A. A.; Chen, M. S. *J. Med. Chem.* **2002**, *45*, 805.
17. Boger, L. D.; Fink, B. E.; Hedrick, M. P. *J. Am. Chem. Soc.* **2000**, *122*, 6382.
18. Kaberdin, R. V.; Potkin, V. I.; Ol'dekop, Y. A. *Zh. Org. Khim.* **1990**, *26*, 1560.
19. Synthesis of **11**. A mixture of **5** (80 mg, 0.14 mmol), BOPCl (bis(2-oxo-3-oxazolidinyl)-phosphinic chloride, 44 mg, 0.17 mmol), in NMP (1 mL) and $^{i}Pr_2EtN$ (0.1 mL) was stirred at 37 °C for 30 min, treated with *N*-cyclohexyl-1,3-propanediamine (46 μL, 0.28 mmol) and stirred for 3 h. The mixture was treated with putrescine (250 mg, 2.8 mmol) and stirred for 24 h at 60 °C. 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, CH_3CN/0.5% aq AcOH, 0% to 60% in 60 min) gave **11** (22 mg, 21%). ^1H NMR (DMSO-d_6) δ 10.52 (s, 1H), 9.97 (s, 1H), 9.89 (s, 1H), 8.09 (br. t, $J = 5$ Hz, 1H), 7.27 (br. s, 1H), 7.24 (br. s, 1H), 7.16 (br. s, 1H), 7.11 (br. s, 1H), 7.04 (br. s, 1H), 6.85 (br. s, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.79 (s, 3H), 3.10–3.25, 2.32–2.68, 1.45–1.86, 0.99–1.26 (several m, 25H). ESI MS 756.5 (40%), 754.6 (100%, $[M+H]^+$). 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 10–30%.
20. *National Committee for Clinical Laboratory Standards. Methods for Determining Bactericidal Activity of Antimicrobial Agents*. Proposed Guideline (M26-P); Approved standard (M11-A3); National Committee for Clinical Laboratory Standards: Wayne, PA, 1987.
21. *National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 4th ed; Approved Standard (M7-A4); National Committee for Clinical Laboratory Standards: Wayne, PA, 1997.
22. DNase I footprinting protocol as described in Trauger, J. W.; Dervan, P. B. *Methods Enzymol.* **2001**, *340*, 450. The plasmid pTrc99a (from Amersham Pharmacia Biotech, Inc) was used as a DNA-binding probe for all DNase I footprint titration experiments. The 3′-^{32}P end-labeled 352-bp DNA fragment was prepared by digesting pTrc99a with EcoRI and PvuII with simultaneous fill-in using Sequenase v. 2.0, [α-^{32}P]-ATP, and [α-^{32}P]-TTP.
23. Brosius, D. J.; Erfle, M.; Storella, J. *J. Biol. Chem.* **1985**, *260*, 3539.
24. Anker, L.; Testa, B.; van de Waterbeemd, H.; Bornand-Crausaz, A.; Theodorou, A.; Jenner, P.; Marsden, C. D. *Helv. Chim. Acta* **1983**, *66*, 542.
25. Yung, D. K.; Lo, E. K. F.; Vohra, M. M. *J. Pharm. Sci.* **1972**, *61*, 1953.