Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 1259–1263

DNA binding ligands targeting drug-resistant Gram-positive bacteria. Part 2: C-terminal benzimidazoles and derivatives

Roland W. Bürli, Peter Jones, Dustin McMinn, Quan Le, Jian-Xin Duan, Jacob A. Kaizerman, Stacey Difuntorum and Heinz E. Moser*

Genesoft Pharmaceuticals, Inc., 7300 Shoreline Court, South San Francisco, CA 94080, USA

Received 30 October 2003; revised 3 December 2003; accepted 9 December 2003

Abstract—The synthesis and in vitro potency of DNA minor-groove binding antibacterials lacking the C-terminal amide bond are described. The crescent shaped molecules bear the positively charged amino group at an internal pyrrole unit instead of the C-terminus. Three structural parameters were investigated: the N-terminal unit, the internal amino group, and the C-terminal ring system. Several compounds demonstrated good in vitro potency against various Gram-positive bacteria and some molecules were moderately active against *Escherichia coli*, a representative Gram-negative strain.

In the past decade, there has been a dramatic increase in the occurrence of bacterial strains that are resistant to multiple classes of antibiotics;[1] for instance, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecalis* (VRE) are known to cause problems particularly in hospital settings, while infections by multidrug-resistant pneumococci occur also in the community. To combat such drug-resistant bacterial strains, there is a demand for antibiotics acting by a novel mechanism and consequently lacking cross-resistance to existing agents.[2] As reported previously,[3–5] we started to optimize analogues of the natural product distamycin A for antibacterial potency and improved drug-like properties; in this context, novel pharmacophores have been identified that show excellent in vitro activity against a wide range of Gram-positive bacterial strains. These molecules exercise their antibacterial activity by recognizing and binding the minor-groove of A/T rich DNA sequences within bacterial genomes. As a result, they are likely to inhibit DNA dependent processes such as DNA replication and RNA transcription. Our optimization process is schematically illustrated in Figure 1. In an initial screening, we identified analogues of distamycin A that consist of three internal *N*-methyl pyrrole carboxamides (Py), an N-terminal 4-chloroisothiazole (It), and a charged amino group at both ends of the molecule.[3] In a small library approach, we have prepared analogues with variable substituents at both termini and have found that the N-terminal charge is not critical for good antimicrobial activity. Thus, we rigorously screened for novel N-terminal units and discovered several promising It-alternatives such as 3-chlorothiophene-2-carboxamide, isoquinoline-3-carboxamide, or 2,4-dihalobenzamides, respectively.[4] A compound from this series demonstrated in vivo efficacy in a mouse peritonitis model against a lethal *S. aureus* infection (MSSA).[4] In a next step, we started to modify the internal Py_3-element and replaced one Py-carboxamide unit by the isosteric benzimidazole group.[5] This formally eliminated one internal amide bond and it was anticipated that consequently the drug-like properties of the corresponding molecules should be improved. Indeed, benzimidazole replacement of the Py unit next to the C-terminus yielded compounds with comparable antibacterial activity and—as demonstrated in one case—improved in vivo efficacy.[5] We were interested in the question whether the drug-like properties of these molecules could be further increased by removal of an additional amide bond through internalization of the C-terminal amino group. Notably, an aliphatic amino group is at least in part positively charged under physiological conditions and cannot be omitted without seriously compromising the solubility of the polyaromatic molecules. Herein, we focus on the synthesis and in vitro antibacterial activity of a small library of compounds in

Keywords: Antibiotics; DNA binder; Minor groove; Lead optimization.
* Corresponding author. Tel.: +1-650-837-1820; fax: +1-650-827-0476; e-mail: hmoser@genesoft.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.12.043

which the amino group is attached at the ring nitrogen of an internal Py moiety.

The synthesis of the antibacterial compounds **17**–**35** (Table 1) is described in Scheme 1. Alkylation of the known pyrrole **1**[6] and subsequent hydrogenation yielded the amino ester **3**.[7] Acylation of amine **3** with either an acyl chloride or an activated carboxylic acid, followed by saponification of the resulting dimeric esters gave the N-terminal dimers; analogous chemistry has been described in detail.[4,5] The C-terminal pyrrole–benzimidazole building block **10** and its aza-analogues **11**–**13** were prepared in two steps from the nitro pyrrole **4**.[8] Coupling of **4** under standard conditions (HBTU activation)[4] to the corresponding 1,2-diamines provided the amino amides **6**–**9**. Heating these amides under acidic conditions led to the corresponding fused ring systems **10**–**13**. Notably, the trichloromethyl ketone **5**[9] can be used instead of the acid **4**; details for the synthesis of benzimidazole **10** starting from ketone **5** are described.[10] Hydrogenolytic reduction of the nitro dimers **10**–**13** followed by in situ coupling to the corresponding N-terminal dimers gave the final compounds. All products were purified by preparative HPLC and analyzed by ^{1}H NMR and mass spectroscopy (general procedure below).[11]

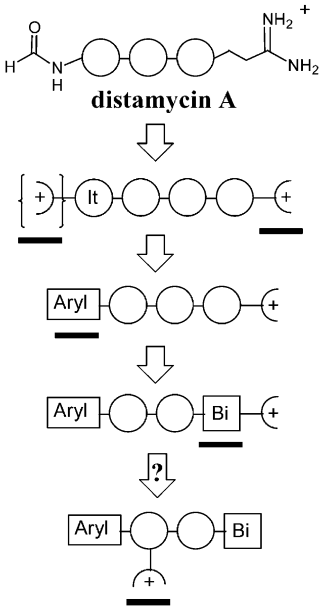
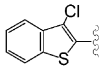
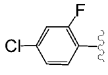
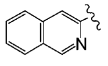


Figure 1. Schematic representation of optimization of distamycin A analogues. Optimized element is underlined (—). Empty circle: *N*-methyl-pyrrole-2-carboxamide. It: 4-chloroisothiazole-2-carboxamide. Aryl: uncharged aromatic/heteroaromatic N-terminus. BI: benzimidazole. (+: alkyl amine.

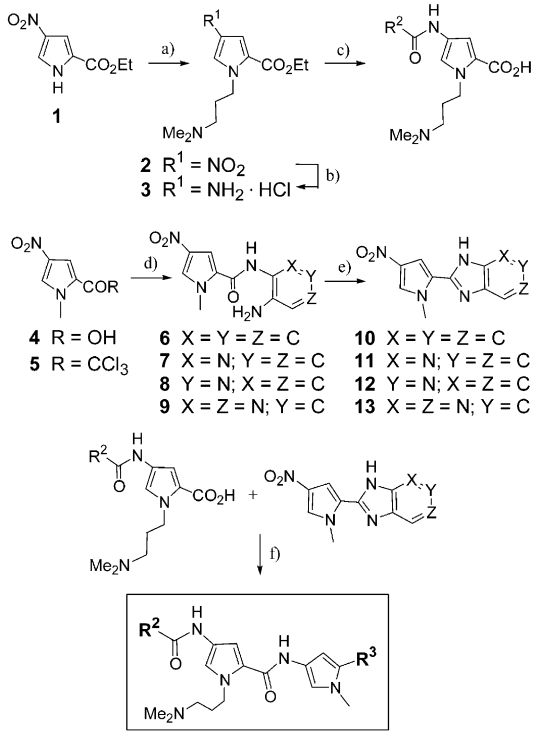
The compounds **36**–**42** (Table 2) were synthesized from the common tetrameric intermediate **16** (Scheme 2). Coupling of activated 4-chloro-2-fluorobenzoic acid (HBTU) to the amino ester **14**,[12] followed by saponification of the dimeric intermediate, provided the hydroxy alcohol **15** in good yields. Reaction of **15** and the in situ generated C-terminal benzamide (obtained from **10**, by hydrogenation) gave the tetramer **16**. Mesylation of the alcohol **16** and treatment with excess of the desired secondary amines led to the final compounds that were purified by HPLC; a general procedure is documented.[13]

In a 'matrix-type' fashion, we first investigated the influence of the nature of the terminal heterocycles on antibacterial activity in compounds that carry a basic diaminopropyl group linked to the ring nitrogen of an internal Py module. It has previously been shown that the DNA binding properties of similar minor-groove binding ligands are not diminished by similar substituents at this position.[12,14] In the case of short linkers, an electrostatic interaction between the pending

Table 1. Antibacterial activity of compounds **17**–**35** (Scheme 1)

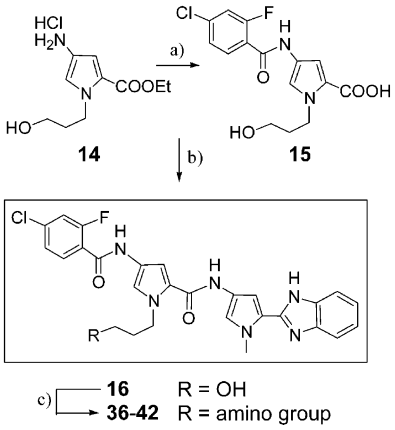
R_3 → / ↓R^2		MRSA[a]	PISP	VSEF		MRSA	PISP	VSEF		MRSA	PISP	VSEF		MRSA	PISP	VSEF
	**17**	2	0.5	1	**22**	4	1	4			—		**31**	16	2	16
	**18**	8	8	8	**23**	2	0.5	2	**27**	1	0.25	2	**32**	0.03	0.03	2
	**19**	1	0.25	1	**24**	2	1	1	**28**	4	1	4	**33**	16	4	8
	**20**	0.5	0.13	0.25	**25**	0.5	0.25	0.25	**29**	0.5	0.5	0.5	**34**	2	0.13	4
	**21**	1	0.5	2	**26**	2	1	2	**30**	0.5	0.25	0.5	**35**	1	0.25	1

[a] MIC values in (μg/mL).[15] MRSA, methicillin-resistant *S. aureus* (ATCC 27660). PISP, penicillin-intermediate *S. pneumoniae* (ATCC 49619). VSEF, vancomycin-susceptible *E. faecalis* (ATCC 29212).



Scheme 1. Synthesis of final compounds (Table 1): (a) 3-(dimethylamino)propyl chloride (1.3 equiv), K_2CO_3 (2.2 equiv), NaI (1.0 equiv), DMF, 75 °C, 16 h, 53%; (b) Pd–C, H_2 (1 atm), AcOEt/MeOH (1:1), 25 °C, 12 h, 91%; (c) RCOOH (1.2 equiv), HBTU (1.14 equiv), **3** (1.0 equiv), DMF/ ^{i}Pr$_2$EtN (10:1), 25 °C, 12 h; (d) **4** (1.0 equiv), HBTU (1.0 equiv), diamine (1.1 equiv), DMF/^{i}Pr$_2$EtN (10:1), 60 °C, 15 h, 76% for **6**, 84% for **7**, 51% for **8**, 35% for **9**; (e) AcOH, 60 °C, 2 h, 52% for **10**; AcOH, 60 °C, 16 h, 61% for **11**, 51% for **13**; aqueous HCl (conc.), reflux, 16 h, 59% for **12**; (f) **10**–**13**, Pd–C, H_2 (1 atm), DMF, 25 °C, 4 h, crude; then, acid (1.2 equiv), HBTU (1.15 equiv), DMF/ ^{i}Pr$_2$EtN (10:1), 25 °C, 12 h, prep. HPLC.

positively charged amine and the phosphodiester backbone of DNA might stabilize the DNA–ligand complex. For the N-terminus, we selected five end caps that previously have shown good activity in the context of the internal Py_3 scaffold,[4] whereas the C-terminus consisted of either benzimidazole or an aza-analogue with one or two additional ring nitrogens at various positions. The antibacterial activities against MRSA, PISP, and VSEF, are summarized in Table 1.[15] In the benzimidazole series, the benzothiophene **18** was only moderately potent, whereas the thiophene **17**, the benzamides **19** and **20**, and the isoquinoline **21** showed MIC (minimum inhibitory concentration) values close to 1 μg/mL or lower. The aza-analogues of the thiophene **17**—namely the imidazo[4,5-*b*]pyridine **22** and the purine **31**—were significantly less potent. Interestingly, the opposite structure–activity relationship was observed for the benzothiophene series; addition of ring nitrogens at the C-terminus resulted in improved in vitro potency (→ **23**, **27**, **32**). In fact, the purine **32** showed excellent MIC values of 0.03 μg/mL against *S. aureus* and *S. pneumoniae*, and good activity against *E. faecalis* (2 μg/mL). The 2,4-difluorobenzamides (**19**, **24**, **28**, **33**) were consistently less potent than their corresponding 4-chloro-analogues (**20**, **25**, **29**, **34**). The 4-chloro-2-fluorobenzamides **20**, **25**, and **29** were active at MIC values well below 1 μg/mL against all strains,

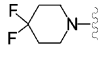
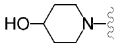
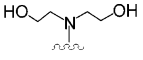


Scheme 2. Synthesis of final compounds (Table 2): (a) 4-chloro-2-fluorobenzoic acid (1 equiv), HBTU (1 equiv), **14** (1 equiv), DMF, ^{i}Pr$_2$EtN (3 equiv), 60 °C, 12 h, 74%; then, NaOH (3 equiv), EtOH/H_2O (1:2), 60 °C, 16 h, 88%; (h) **15** (1.1 equiv), HBTU (1.05 equiv), **10** (1 equiv), DMF/ ^{i}Pr$_2$EtN (10:1), 60 °C, 12 h, crude; (c) $MeSO_2Cl$ (4.8 equiv), ^{i}Pr$_2$EtN (3 equiv), DMF, 40 °C, 1 h; then, amine (10 equiv), 60 °C, 12 h; then, prep. HPLC.

while the purine derivative **34** was somewhat less potent. In the isoquinoline group, no dramatic change in activity was observed upon introduction of ring nitrogens in the C-terminal unit; the imidazo[4,5-c]pyridine **30** was slightly more potent compared to the other derivatives and showed excellent activity against all strains (MIC values: 0.25–0.5 μg/mL).

We next studied the influence of the amino group on antibacterial potency (Table 2). It has previously been shown that the nature of the positively charged group can substantially impact the in vitro potency as well as

Table 2. Antibacterial activity of compounds **36**–**42** (Scheme 2)

	R	MRSA[a] 27660	PISP 49619	VSEF 29212	*E. coli* 25922
36		0.13	0.06	0.5	>32
37		0.13	0.25	0.5	>32
38		0.13	0.25	1	16
39		0.5	1	0.5	>32
40		2	1	4	32
41		2	0.5	2	>32
42		4	4	2	>32

[a] MIC values in (μg/mL) against ATCC strains.[15] MRSA, methicillin-resistant *S. aureus*. VSEF, vancomycin-susceptible *E. faecalis*. PISP, penicillin-intermediate *S. pneumoniae*.

other pharmacologically important parameters. For this, we concentrated on analogues of the 4-chloro-2-fluorobenzamide **20**, as this compound showed the most promising in vitro profile against all Gram-positive strains. The morpholine **36**, the thiomorpholine **37**, and the unsubstituted piperidine **38** exhibited similar potency (MIC values of 0.06–1 μg/mL against all Gram-positive strains), while the 4,4-difluoropiperidine **39**, the 4-hydroxypiperidine **40** and the piperazine **41** were slightly less active. The bis(hydroxyethyl)amine **42** was significantly less potent. Possibly, the hydroxy groups might be highly solvated in aqueous medium and thus reduce cellular penetration. Interestingly, out of this set of compounds, three molecules showed moderate activity against *E. coli*, a representative Gram-negative bacterial strain (16 μg/mL for **20**, 16 μg/mL for **38**, 32 μg/mL for **40**). As outlined in the preceding publication,[5] some benzimidazole-containing antibacterials exhibited activity against *E. coli*, whereas the corresponding Py_3 derivatives did not.[4] Removal of the C-terminal amide bond, however, did not improve *E. coli* potency.

The goal of this study was to investigate whether the C-terminal amide bond could be eliminated by internalization of the charged function without loss of antibacterial potency. Three structural parameters were varied: the N-terminal unit, the internal amino group, and the C-terminal ring system, respectively. The N-termini and the internal amines were selected based on previous results, whereas the C-terminus consisted of a benzimidazole-type structure. We also studied the effect of one or two additional ring nitrogens in the benzimidazole unit and found that, depending on the nature of the N-terminus, extra ring nitrogens will enhance or reduce antibacterial potency of the molecules. In summary, some compounds bearing the charged group at an internal unit demonstrated good in vitro potency against various Gram-positive bacteria and are structurally quite different from the original natural product distamycin A and earlier analogues thereof. Internalizing the amino group clearly opens a new avenue for structural optimization DNA minor-groove binding ligands, since the nature of the C-terminal aryl group has largely remained unexplored. The in vivo behavior of this new subclass of DNA minor-groove binding antibacterial agents remains to be assessed.

Acknowledgements

This work was supported in part by the Defense Advanced Research Projects Agency (DARPA Grant Number N65236-99-1-5427). The authors would like to thank Vernon Jiang, Zhijun Ye, and Hsiu Chen for analytical support.

References and notes

1. McDevitt, D.; Rosenberg, M. *TRENDS in Microbiology* **2001**, *9*, 611 and references cited therein.
2. Projan, S. J.; Youngman, P. J. *Curr. Opin. Microbiology* **2002**, *5*, 463.
3. Bürli, R. W.; Ge, Y.; White, S.; Baird, E. E.; Touami, S. M.; Taylor, M.; Kaizerman, J. A.; Moser, H. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2591.
4. Kaizerman, J. A.; Gross, M. I.; Ge, Y.; White, S.; Hu, W.; Duan, J. X.; Baird, E. E.; Johnson, K. W.; Tanaka, R. D.; Moser, H. E.; Bürli, R. W. *J. Med. Chem.* **2003**, *46*, 3914.
5. Bürli, R.; McMinn, D.; Kaizerman, J.; Hu, W.; Pack, Q.; Le, Q.; Ge, Y.; Gross, M.; Difuntorum, S.; Moser, H. *Bioorg. Med. Chem. Lett*., submitted.
6. Bremer, R. E.; Szewczyk, J. W.; Baird, E. E.; Dervan, P. B. *Bioorg. Med. Chem.* **2000**, *8*, 1947.
7. Synthesis of **3**. A mixture of pyrrole **1** (10.0 g, 54.3 mmol), 3-(dimethylamino)propyl chloride (HCl salt, 10.74 g, 67.9 mmol), NaI (8.14 g, 54.3 mmol), and K_2CO_3 (16.51 g, 119.4 mmol) in DMF (150 mL) was stirred at 75 °C for 16 h, cooled to 25 °C, poured into 1 M aqueous HCl (500 mL), and washed with AcOEt (2×300 mL). The mixture was neutralized by addition of Na_2CO_3 and extracted with AcOEt (3×300 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to give nitro pyrrole **2** (7.77 g, 53%). A suspension of **2** (7.65 g, 28.4 mmol) and PdC (10%, 0.38 g) in AcOEt/MeOH (1:1, 150 mL) was stirred under H_2 (1 atm) at 25 °C for 12 h, and filtered through Celite. The filtrate was evaporated and the residue dissolved in AcOEt (200 mL), and treated with HCl gas. Evaporation of solvents gave amino pyrrole **3** (8.05 g, 91%).
8. Dudouit, F.; Goossens, J.-F.; Houssin, R.; Henichart, J.-P.; Colson, P.; Houssier, C.; Gelus, N.; Bailly, C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 553.
9. Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6141.
10. Synthesis of **10**. A mixture of ketone **5** (25.1 g, 92.5 mmol) and 1,2-phenylenediamine (10.0 g, 92.47 mmol) in THF (300 mL) and $^{i}Pr_2EtN$ (30 mL) was stirred at 60 °C for 15 h and added dropwise into H_2O (ca. 1 L). The resulting precipitate was collected by filtration and dried to give amide **6** (16.05 g, 67%). A solution of **6** (16.05 g, 61.67 mmol) in glacial AcOH (200 mL) was heated at 60 °C for 2 h and concentrated in vacuo. Addition of Et_2O resulted in precipitation of **10**, which was collected by filtration and dried (→7.82 g, 52%).
11. General procedure for the synthesis of **17**–**35**. A solution of nitro compound **10**–**13** (150 μmol) and PdC (10%, 30 mg) in DMF (0.75 mL) was stirred under H_2 (1 atm) at 25 °C for 4 h, and filtered through Celite. The filtrate was treated with a mixture of preactivated acid (1.2 equiv acid, 1.15 equiv HBTU in DMF/$^{i}Pr_2EtN$ = 10:1, 25 °C, 30 min), stirred at 37 °C for 12 h, diluted with 50% aqueous AcOH to a volume of 15 mL, and purified by preparative HPLC (Hamilton PRP-1 column, 250×21.5 mm, A: 0.5% AcOH in H_2O, B: CH_3CN, 0–60% B in 60 min, 20 mL/min, UV detection at 310 nm). Analytical data for **17**: ^{1}H NMR (DMSO-d_6) δ 12.51 (br. s, 1H), 10.22 (s, 1H), 10.06 (s, 1H), 7.87 (d, J = 5.2 Hz, 1H), 7.62–7.55 (m, 1H), 7.48–7.41 (m, 1H), 7.34 (d, J = 1.3 Hz, 1H), 7.32 (d, J = 1.5 Hz, 1H), 7.17 (d, J = 5.3 Hz, 1H), 7.16–7.13 (m, 2H), 7.06 (d, J = 1.5 Hz, 1H), 7.03 (d, J = 1.5 Hz, 1H), 4.34 (t, J = 7.0 Hz, 2H), 4.08 (s, 3H), 2.17 (t, J = 6.8 Hz, 2H), 2.13 (s, 6H), 1.86–1.80 (m, 2H). ESI MS 552.1 (40%), 550.2 (100%, $[M+H]^+$). Purity (analyt. HPLC,[4] 310 nm) 95%. All compounds were characterized by ^{1}H NMR and mass spectrometry and showed purity of at least 90%. The isolated yields after HPLC purification ranged from ca. 10–30%.
12. Bremer, R. E.; Wurtz, N. R.; Szewczyk, J. W.; Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2093.
13. General procedure for the synthesis of **36**–**42**. A solution of alcohol **16** (25 mg, 47 μmol) in DMF (1 mL) and $^{i}Pr_2EtN$ (0.1 mL) was treated with $MeSO_2Cl$ (18 μL, 227

μmol), stirred at 40 °C for 1 h, treated with amine (470 μmol), and stirred at 60 °C for 12 h. The mixture was diluted with 50% aqueous AcOH to a volume of 15 mL and purified by preparative HPLC (as above). Data for **36**: ^{1}H NMR (DMSO-d_6) δ 12.51 (s, 1H), 10.44 (s, 1H), 10.06 (s, 1H), 7.68 (t, J = 8.1, 1H), 7.61 (br. d, J = 8.0 Hz, 1H), 7.59 (br. t, J = 8.0 Hz, 1H), 7.42 (br. d, J = 7.4 Hz, 2H), 7.37 (br. s, 1H), 7.32 (br. s, 1H), 7.15–7.13 (m, 2H), 7.02 (br. s, 2H), 4.36 (t, J = 6.6 Hz, 2H), 4.07 (s, 3H), 3.65 (t, J = 4.8 Hz, 2H), 3.58–3.56 (m, 4H), 3.07 (t, J = 4.8 Hz, 2H), 2.21 (t, J = 7.0 Hz, 2H), 1.88–1.86 (m, 2H). ESI MS 606.2 (40%), 604.3 (100%, $[M+H]^+$). Purity (analyt. HPLC, 310 nm) 95%.

14. Bruice, T. C.; Mei, H.-Y.; He, G.-X.; Lopez, V. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1700.
15. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 5th ed; Approved Standard (M7A5); National Committee for Clinical Laboratory Standards, Wayne, PA, 2000.