# Antimicrobial Lexitropsins Containing Amide, Amidine, and Alkene Linking Groups

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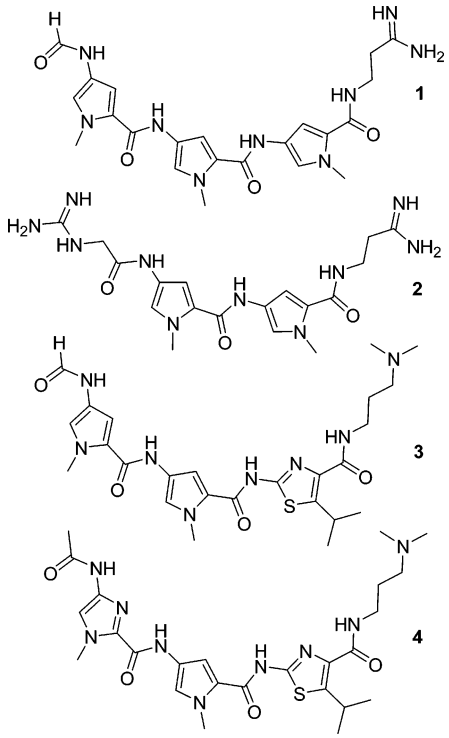
The synthesis and properties of 80 short minor groove binders related to distamycin and the thiazotropsins are described. The design of the compounds was principally predicated upon increased affinity arising from hydrophobic interactions between minor groove binders and DNA. The introduction of hydrophobic aromatic head groups, including quinolyl and benzoyl derivatives, and of alkenes as linkers led to several strongly active antibacterial compounds with MIC for *Staphylococcus aureus*, both methicillin-sensitive and -resistant strains, in the range of 0.1−5 $\mu$g mL$^{-1}$, which is comparable to many established antibacterial agents. Antifungal activity was also found in the range of 20−50 $\mu$g mL$^{-1}$ MIC against *Aspergillus niger* and *Candida albicans*, again comparable with established antifungal drugs. A quinoline derivative was found to protect mice against *S. aureus* infection for a period of up to six days after a single intraperitoneal dose of 40 mg kg$^{-1}$.

## Introduction

The lexitropsins are analogues of the natural products distamycin **1** and netropsin **2**, both of which bind selectively and with high affinity to adenine/thymine sequences of DNA, in the minor groove. A substantial effort has been invested in finding ways of controlling the selectivity of binding for other DNA sequences based on the preference of distamycin for binding two molecules side-by-side, which allows for the pairing of different heterocycles and the development of the "pairing rules".[1] Thus, pyrrole/pyrrole pairs confer A/T selectivity, as with distamycin itself, while analogues with pyrrole/imidazole, imidazole/pyrrole, or pyrrole/hydroxypyrrole pairs can be designed to read C/G or G/C and distinguish A/T from T/A.[2] From the conventional viewpoint of drug design, most of the compounds that were used to explore the pairing rules, such as the hairpin polyamides,[3] are too large and have too many hydrogen-bonding groups. However, there is good evidence that this principle can be extended to much shorter molecules, such as thiazotropsins A **3** and B **4**, where 2:1 binding in the minor groove occurs with limited overlap between the minor groove binders.[4−6]

When the ligand is reduced in length, the use of hydrophilic subunits such as imidazole and hydroxypyrrole results in reduced affinity; there is evidence that a significant proportion of the binding energy may result from hydrophobic interactions in some classes of minor groove binder.[7] The challenge is thus to produce drug-like molecules with high affinity for DNA while maintaining sufficient sequence selectivity to have useful therapeutic effects. While there are many areas of therapy that might benefit from DNA-directed intervention, there is currently an urgent need for new antimicrobials with novel modes of action. The Center for Disease Control and Prevention (U.S.A.) considers antibiotic resistance to be the most important public health issue today, and medical experts anticipate record increases of antibacterial resistance in the next decade.[8]




*Staphylococcus aureus* is one of the major causes of hospital-acquired infections and is becoming more widespread in the community. In hospitals, it is likely to cause wound and blood-

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[a] Abbreviations: DMAP, dimethylaminopropyl; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; MGB, minor groove binder; MIC, minimum inhibitory concentration; MRSA, methicillin resistant *Staphylococcus aureus.*

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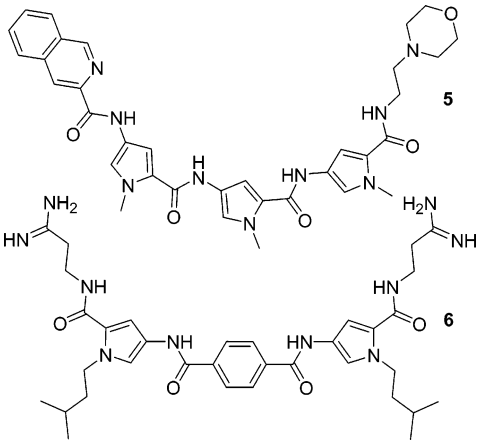
stream infections with significant attendant morbidity and mortality. The prevalence of methicillin-resistant *S. aureus* (MRSA[a]) has increased markedly in the past decade, primarily associated with hospital-acquired infection, and is a continuing cause for concern. Resistance to methicillin itself is widespread, and most methicillin-resistant strains also exhibit multiple resistance to other antibiotics.[9]

One of the best strategies for the discovery of new anti-infective drugs is to target biological mechanisms against which resistance has not yet emerged. The minor groove binders (MGBs) exemplify this approach as they target DNA in place of proteins. While there have been many studies of new MGBs, relatively few have been specifically directed toward the search for new antimicrobial agents.[10] Two significant research programs have been described by Genesoft[11−15] and Genelabs.[16] Both are based on polyamide lexitropsins, the former with extended analogues of distamycin having novel head-groups, for example, **5**, and the latter with "head-to-head" molecules in which two distamycin-like units are joined through amide bonds to a central diacid, as, for example, in **6**.




As an extension of our earlier work,[17] our first stratagem to increase the hydrophobicity of the MGB was to introduce an *N*-alkyl or *C*-alkyl group larger than methyl. Such a group, which sits on the outside of the helix, has the additional property of preventing side-by-side binding if placed in the middle of the molecule. If placed toward the tail, side-by-side binding occurs but with an offset, increasing the length of DNA that can be covered. Such extended overlap has the effect of extending the reading frame of the minor groove binder from four to six base pairs. The first, well-characterized example of this effect was thiazotropsin A **3**; the mode of binding, established by NMR,[18] is shown in Figure 1.

In this paper, we describe the extension of this concept by the incorporation of different hydrophobic head groups in oligoamides and also to two further series of compounds in which one of the amide links has been replaced. In the first of these, the hydrophobic head group is linked to the rest of the molecule by an amidine, bringing the structure closer to that of netropsin, which tends to bind as single molecules in the minor groove yet shows good affinity. In the second, one of the hydrophilic amide links is replaced by a hydrophobic alkene. One alkene-linked MGB had been described previously, but this compound had no hydrophobic head group and was shown to bind only weakly to DNA.[19]

## Chemistry

The new MGBs were obtained by applications and developments of established methods following the strategy shown in Scheme 1. For simplicity, the reactions shown in Scheme 1 are illustrated entirely by *N*-methylpyrrole oligomers; the full range of components used in this study is given in Tables 1−3. Polyamides were synthesized by using HBTU, dicyclohexylcarbodiimide, or acid chloride coupling methods.[11−17] Amidines were prepared via methylation of thioamides and the subsequent displacement of methane thiol by an aminopyrrole oligomer.[20−22] MGBs with stilbenoids head groups were prepared using two different methods. The reaction of arylmethylphosphonates with aromatic aldehydes in the presence of sodium hydride, under Horner−Wadsworth−Emmons conditions,[23] gave ester intermediates, which were hydrolyzed using aqueous sodium hydroxide to give the desired carboxylic acids. For pyridyl derivatives with active methyl substituents, a condensation reaction with aromatic aldehydes in the presence of zinc chloride in acetic anhydride was used. However, sometimes, the corresponding carboxylic acid as well as the ester was obtained, as has been reported previously.[24]

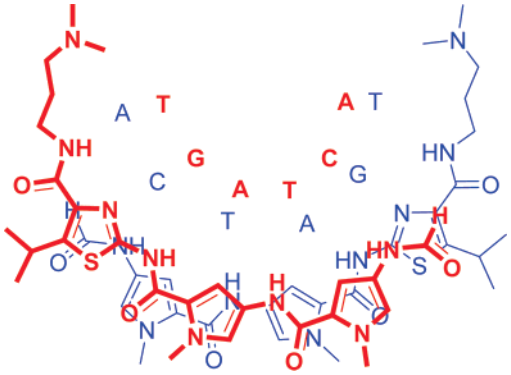
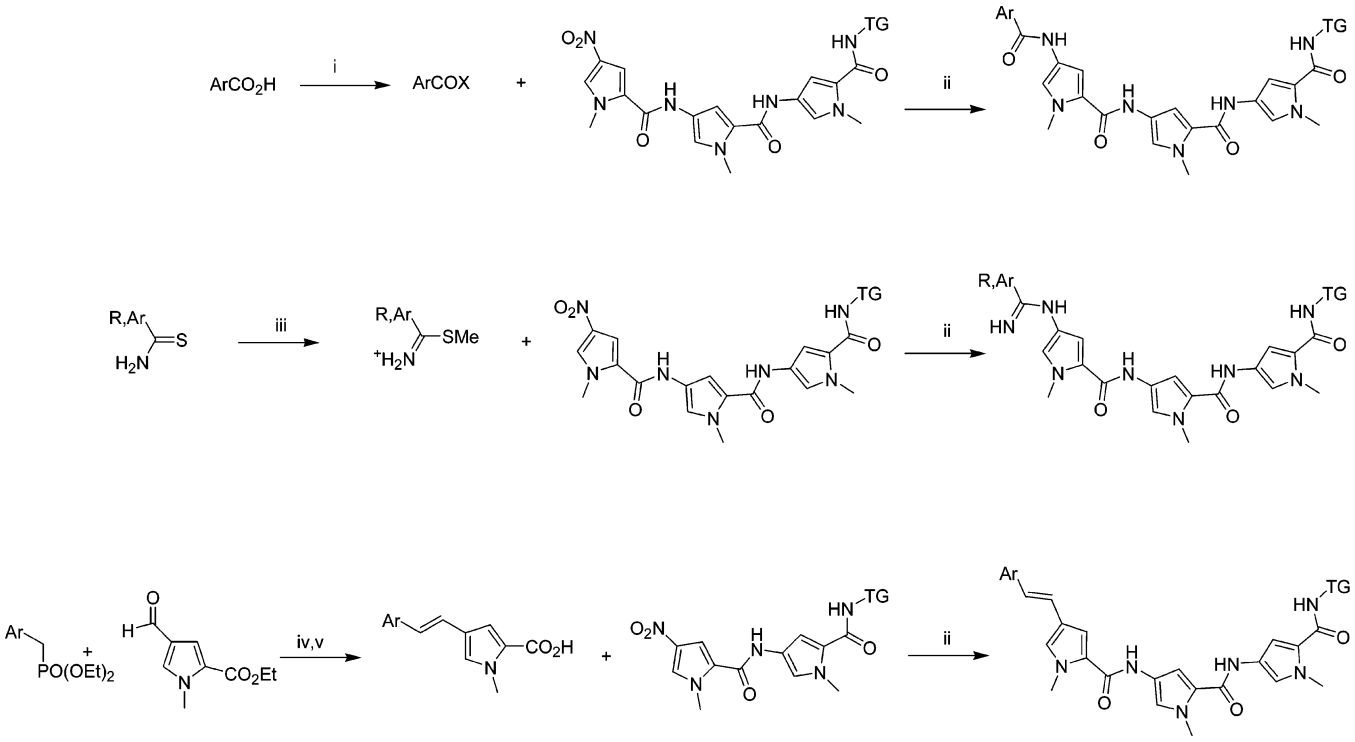


**Figure 1.** The overlap of two molecules of thiazotropsin A when bound to DNA, showing the extended base-pair coverage induced by the bulky isopropyl substituents and sulfur atoms in preventing the complete overlap of all three rings. The thiazole nitrogen in the red-coded ligand hydrogen bonds to the guanine in the red-coded DNA strand and similarly for the blue-coded ligand and DNA base.

In all three series, the final step involved coupling a head group (either a single residue or a dimer) to an amino pyrrole derivative (either a dimer or a trimer) prepared by reduction of the appropriate nitropyrrole intermediate using 10% palladium on carbon in an atmosphere of hydrogen gas in methanol. This methanolic amine solution was used without further purification in the next step. Suitably activated head groups were added to aliquots of the amine solution in parallel syntheses, which were all performed at room temperature. When the formyl head group was required, ethyl formate was added to the amine solution, and the reaction mixture was heated under reflux until the reaction was completed. HPLC purification of the final compounds was carried out using a Vydac protein and peptide C18 column on a gradient eluting system with water/acetonitrile containing 0.1% trifluoroacetic acid. Single peaks corresponding to the required products were collected and freeze-dried; the quality of the product before biological assay was assured by clean 400 MHz $^1$H proton NMR spectra and high-resolution mass spectra, consistent with the expected composition of the MGB. The full range of structural components used is shown in Figures 2−4.

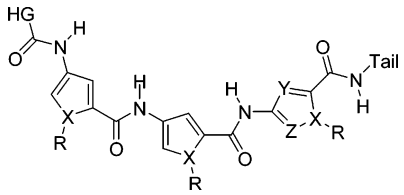
As will be described below, the most significant antibacterial activity was found with MGBs containing alkene links. For such MGBs to bind to DNA in the same manner as a fully amide linked analogue, it is essential that the alkene be in the *E*-configuration. The synthetic methods used prepared building blocks that were obtained by standard methods known to afford

**Scheme 1.** Synopsis of Synthesis of MGBs[a]



[a] Ar and R represent head groups (Figure 2), and TG stands for tail group (Figure 4). *N*-methylpyrroles have been shown throughout as the linked components, but the full range is shown in Figure 3. Reagents. *i* coupling agent: HBTU, dicyclohexylcarbodiimide, or thionyl chloride as described in the experimental section; *ii*: $H_2$/Pd−C then immediate reaction with the coupling partner after filtration of the catalyst; *iii*: MeI; *iv*: NaH; *v*: NaOH(aq).

**Table 1.** Activities of Polyamides against Representative Bacterial and Fungal Species[a]

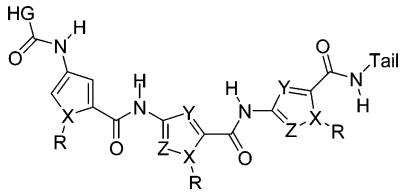


| compound | structure | *Staphylococcus aureus* | *Streptepcoccus faecalis* | *Mycobacter fortuitum* | *Aspergillus niger* | *Candida albicans* |
|---|---|---|---|---|---|---|
| **3** | $HG_1$-AmPy-AmPy-*i*PrThi-DMAP | 5 | 10 | 19 | 76 | 76 |
| **7** | $HG_1$-AmPy-AmPy-*i*PrThi-Met | 13 | 25 | >50 | >50 | >50 |
| **8** | $HG_8$-AmPy-AmPy-AmPy-DMAP | >50 | >50 | >50 | 50 | 25 |
| **9** | $HG_8$-AmPy-*i*PrPy-AmPy-DMAP | 6 | 50 | >50 | 50 | >50 |
| **10** | $HG_7$-AmPy-AmPy-*i*PrThi-DMAP | 50 | 50 | 50 | >50 | 50 |
| **11** | $HG_{12}$-AmPy-AmPy-*i*PrThi-Met | >50 | >50 | >50 | >50 | >50 |
| **12** | $HG_5$-AmPy-AmPy-AmPy-PyrEt | >50 | >50 | >50 | >50 | >50 |
| **13** | $HG_5$-AmPy-PePy-AmPy-DMAP | 13 | 6 | >50 | >50 | >50 |
| **14** | HG-AmPy-*i*PePy-AmPy-DMAP | 50 | >50 | >50 | >50 | >50 |
| **15** | $HG_3$-AmPy-AmPy-*i*PrThi-DMAP | 25 | 25 | >50 | >50 | >50 |
| **16** | $HG_5$-AmPy-AmPy-*i*PrThi-DMAP | 13 | 6 | >50 | >50 | >50 |
| **17** | $HG_6$-AmPy-AmPy-*i*PrThi-DMAP | 13 | 25 | >50 | >50 | >50 |
| **18** | $HG_1$-*i*PrThi-AmPy-AmPy-DMAP | 50 | 25 | >50 | >50 | >50 |
| **19** | $HG_3$-*i*PrThi-AmPy-AmPy-Amd | 13 | 13 | >50 | >50 | >50 |
| **20** | $HG_7$-AmPy-AmPy-Im-DMAPr | >50 | >50 | >50 | >50 | >50 |
| **21** | $HG_3$-AmPy-AmPy-Im-DMAPr | >50 | >50 | >50 | >50 | >50 |
| **22** | $HG_4$-AmPy-AmPy-Im-DMAPr | >50 | >50 | >50 | >50 | >50 |
| **23** | $HG_1$-AmPy-AmPy-Im-DMAPr | >50 | >50 | >50 | >50 | >50 |
| **24** | $HG_7$-*i*PeThi-AmPy-DMAP | >50 | >50 | >50 | >50 | >50 |
| **25** | $HG_7$-AmPy-*i*PeThi-DMAP | 50 | 50 | >50 | >50 | >50 |
| **26** | $HG_3$-AmBenz-AmPy-AmPy -MEt | >50 | >50 | >50 | >50 | >50 |
| **27** | $HG_{26}$-AmBenz-AmPy-AmPy -MEt | >50 | >50 | >50 | >50 | >50 |

[a] All data are minimum inhibitory concentrations, given as $\mu$g $mL^{-1}$. Organisms used: *Staphylococcus aureus* NCTC 6571, *Streptococcus faecalis* NCTC 775, *Mycobacterium fortuitum* NCTC 10394, *Candida albicans* NCPF 3179, *Aspergillus niger* IMI 149007. For compound structure codes, see Figures 2−4.

the *E*-configuration, many of which had been made previously. In all cases, the configuration was confirmed by the coupling constants of the alkene protons, which were typically in the range of 16−16.5 Hz. Data in the same range for the purified MGBs that underwent assay assured that there had been no change in configuration during the final synthetic operations.

**Table 2.** Activities of Amidines against Representative Bacterial and Fungal Species[a]




| compound | structure | *Staphylococcus aureus* | *Streptococcus faecalis* | *Mycobacter fortuitum* | *Aspergillus niger* | *Candida albicans* |
|---|---|---|---|---|---|---|
| **28** | $HG_{10}$-AmPy-AmPy-*i*PrThi-DMAP | 13 | 25 | >50 | >50 | >50 |
| **29** | $HG_{10}$-AmPy-*i*PrPy-AmPy-DMAP | 50 | >50 | >50 | >50 | >50 |
| **30** | $HG_{11}$-AmPy-*i*PrPy-AmPy-DMAP | 50 | >50 | >50 | 25 | >50 |
| **31** | $HG_{11}$-AmPy-AmPy-*i*PrThi-DMAP | 13 | 50 | >50 | 50 | >50 |
| **32** | $HG_{10}$-AmPy-AmPy-AmPy-DMAP | 50 | >50 | >50 | 50 | >50 |
| **33** | $HG_{11}$-AmPy-AmPy-AmPy-DMAP | 25 | >50 | >50 | 25 | >50 |
| **34** | $HG_9$-AmPy-AmPy-AmPy-DMAP | 13 | 50 | >50 | 50 | >50 |
| **35** | $HG_9$-AmPy-*i*PrPy-AmPy-DMAP | 13 | 50 | >50 | 50 | >50 |
| **36** | $HG_9$-AmPy-AmPy-*i*PrThi-DMAP | 50 | 50 | >50 | 50 | >50 |
| **37** | $HG_9$-AmPy-AmPy-*i*PeThi-DMAP | >50 | >50 | >50 | >50 | 50 |
| **38** | $HG_{11}$-AmPy-AmPy-*i*PeThi-DMAP | 3 | 13 | >50 | >50 | >50 |
| **39** | $HG_{11}$-AmPy-AmPy-AmPy-MEt | >50 | >50 | >50 | >50 | >50 |
| **40** | $HG_2$-AmPy-AmPy-AmPy-MEt | 50 | >50 | >50 | 50 | >50 |
| **41** | $HG_{11}$-AmPy-AmPy-*i*PrThi-MEt | >50 | >50 | >50 | >50 | >50 |
| **42** | $HG_9$-AmPy-AmPy-*i*PrThi-MEt | >50 | >50 | >50 | >50 | >50 |
| **43** | $HG_{11}$-AmPy-AmPy-AmPy-MPr | >50 | >50 | >50 | >50 | >50 |
| **44** | $HG_9$-AmPy-AmPy-AmPy-MPr | >50 | >50 | >50 | >50 | >50 |
| **45** | $HG_{11}$-AmPy-AmPy-AmPy-PyrEt | 50 | >50 | >50 | >50 | >50 |
| **46** | $HG_{12}$-AmPy-AmPy-AmPy-DMAP | >50 | 50 | >50 | 50 | 50 |
| **47** | $HG_{12}$-AmPy-AmPy-*i*PeThi-DMAP | 50 | 50 | >50 | >50 | 50 |
| **48** | $HG_{11}$-AmPy-AmPy-Im-DMAP | >50 | >50 | >50 | >50 | >50 |

[a] All data are minimum inhibitory concentrations, given as $\mu$g mL$^{-1}$. For structure codes, see Figures 2−4.

**Measurement of Antimicrobial Activity.** All of the compounds were initially tested in a broad screen, including four bacterial and two fungal species, using 96 well microtiter plates with resazurin as the growth indicator.[25] Most of the compounds were then evaluated against further clinical isolates of *Staphylococcus aureus* including MRSA, used according to NCCLS guidelines.

## Structure−Activity Relationships

**Antibacterial Activity. Polyamides−Head Group Modification.** The lead compound for this study was thiazotropsin A **3**, which had MIC values against three bacterial species in the range of 5−20 $\mu$g mL$^{-1}$ (Table 1; for structures, see Table 1 and Figures 2−4). The disclosure of the highly beneficial effect of a morpholinoethyl rather than dimethylaminopropyl (DMAP) tail group on the potency of a series of similar compounds[12,15] prompted the synthesis of **7**, but in our series, the effect was deleterious (Table 1). A possible explanation lies in the observed close packing of the head and tail groups in thiazotropsin A, which is not compatible with the increased steric requirement of morpholinoethyl compared to DMAP.

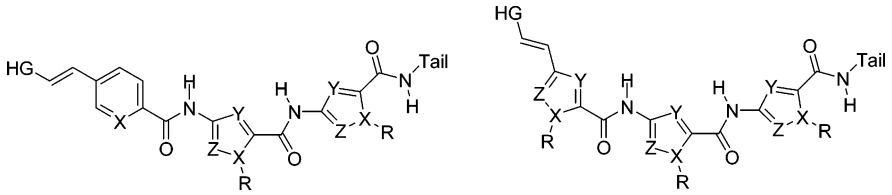
All but 6 of the 20 further compounds **8**−**27** synthesized and tested in this group therefore retained the DMAP tail but failed to improve on the potency of **3**. It may be noted that an imidazole next to the tail as in **19**, **20**, **21**, and **22** was very deleterious to antimicrobial potency, consistent with a reduction in lipophilicity. An imidazole would normally tolerate any DNA base, and these compounds would be expected, simply from the viewpoint of hydrogen bonding to DNA, to have some activity. However, as discussed below, binding to DNA is presumably necessary for antimicrobial activity but not sufficient as physicochemical properties appear to play an important role.

The high potency of the isoquinoline-terminated lexitropsin **5**, reported by the Genesoft group,[11] prompted the incorporation of an isoquinoline head in the thiazotropsin A analogues **10** and **11**, but this failed to increase the potency. These analogues were expected to read a longer A/T sequence than **5** and may have exceeded the length of the critical binding site, by analogy with the spatial relationships in Figure 1. However, the analogues with a pyrrole deletion, **24** and **25**, had little or no activity.

**Amidines.** Having failed to improve on the potency of thiazotropsin A by modification of the head, chain, or tail, we transferred our attention from polyamides with a single basic tail to a series that has a closer resemblance to the structure of netropsin, with two basic groups, while retaining the hydrophobic head group consistent with our design philosophy. The major change was to introduce an amidine at the head end (see structures of head groups in Figure 3). It was anticipated that such amidines would bind as single molecules in the minor groove, a possible advantage in their pharmacodynamics, and that the extra positive charge would enhance affinity for DNA. Of the 21 compounds synthesized and tested, only **38** had potency comparable with that of thiazotropsin A **3** (Table 2), and it was evident that the structure−activity relationships were quite different from those in the simple polyamide series. In particular, the use of morpholinoethyl as the tail unit in place of DMAP was disadvantageous; for example, compare **33** and **39**. In addition, an isoquinoline head group, as that in **46** and **47**, did not improve potency. Overall, the amidine series was characterized by modest potency, with many compounds that were only weakly active in the test system.

**Alkene Linkers.** An *E*-alkene is isosteric with the amide linker normally used in the design and synthesis of lexitropsins. While amide linkers are convenient for synthesis, they are hydrophilic and potentially could be replaced by alkenes to improve DNA binding. One alkene linker had been reported but apparently with a negative effect on binding.[19] Despite this,

**Table 3.** Activities of Stilbenoids against Representative Bacterial and Fungal Species[a]




| compound | structure | *Staphylococcus aureus* | *Streptococcus faecalis* | *Mycobacter fortuitum* | *Aspergillus niger* | *Candida albicans* |
|---|---|---|---|---|---|---|
| **49** | $HG_{13}$-*p*Benz-AmPy-AmPy-MEt | 6 | 50 | >50 | >50 | >50 |
| **50** | $HG_{14}$-*p*Benz-AmPy-AmPy-MEt | 3 | 25 | 50 | 25 | 50 |
| **51** | $HG_{15}$-*p*Benz-AmPy-AmPy-MEt | 3 | 13 | >50 | >50 | >50 |
| **52** | $HG_{13}$-*p*Benz-AmPy-AmPy-DMAP | 6 | >50 | >50 | 50 | >50 |
| **53** | $HG_{14}$-*p*Benz-AmPy-AmPy-DMAP | 32 | 13 | >50 | >50 | >50 |
| **54** | $HG_{13}$-*m*Benz-AmPy-AmPy-DMAP | 25 | 25 | >50 | >50 | >50 |
| **55** | $HG_{16}$-*p*Benz-AmPy-AmPy-DMAP | 50 | 50 | >50 | >50 | >50 |
| **56** | $HG_{17}$-*p*Benz-AmPy-AmPy-DMAP | 50 | 25 | >50 | >50 | >50 |
| **57** | $HG_{13}$-AmPy-AmPy-AmPy-DMAP | >50 | 25 | >50 | >50 | 50 |
| **58** | $HG_{14}$-*p*Benz-AmPy-*i*PeThi-DMAP | 50 | >50 | >50 | >50 | >50 |
| **59** | $HG_{13}$-*p*Benz-AmPy-*i*PeThi-DMAP | >50 | >50 | >50 | >50 | >50 |
| **60** | $HG_{13}$-*m*Benz-AmPy-*i*PeThi-DMAP | >50 | >50 | >50 | >50 | >50 |
| **61** | $HG_{16}$-AmPy-AmPy-*i*PeThi-DMAP | >50 | >50 | >50 | >50 | >50 |
| **62** | $HG_{19}$-*p*Benz-AmPy-AmPy-MEt | 25 | >50 | >50 | >50 | >50 |
| **63** | $HG_{20}$-*p*Benz-AmPy-AmPyep-MEt | 50 | >50 | >50 | >50 | >50 |
| **64** | $HG_{20}$-Py-AmPy-AmPy-DMAP | >50 | >50 | >50 | 50 | 25 |
| **65** | $HG_{14}$-*p*Benz-AmPy-*i*PeThi-MEt | >50 | >50 | >50 | >50 | 50 |
| **66** | $HG_{21}$-*p*Benz-AmPy-*i*PeThi-MEt | >50 | >50 | >50 | >50 | >50 |
| **67** | $HG_{20}$-*p*Benz-AmPy-*i*PeThi-MEt | >50 | >50 | >50 | >50 | >50 |
| **68** | $HG_{19}$-*p*Benz-AmPy-*i*PeThi-Met | >50 | >50 | >50 | >50 | >50 |
| **69** | $HG_{20}$-AmPy-AmPy-AmPy-Met | 13 | 13 | >50 | >50 | >50 |
| **70** | $HG_{14}$-AmPy-AmPy-AmPy-Met | >50 | >50 | >50 | >50 | >50 |
| **71** | $HG_{20}$-Thi-AmPy-AmPy-Met | 3 | 25 | >50 | >50 | >50 |
| **72** | $HG_{25}$-*p*Benz-AmPy-*i*PeThi-MEt | 3 | 3 | >50 | >50 | 50 |
| **73** | $HG_{22}$-3Pyr-AmPy-AmPy-Met | 6 | 6 | 50 | 50 | 25 |
| **74** | $HG_{22}$-Quin-AmPy-AmPy-Met | >50 | 25 | >50 | 50 | >50 |
| **75** | $HG_{23}$-Quin-AmPy-AmPy-Met | >50 | >50 | >50 | >50 | >50 |
| **76** | $HG_{23}$-3Pyr-AmPy-AmPy-Met | 6 | >50 | >50 | >50 | >50 |
| **77** | $HG_{24}$-*p*Benz-AmPy-AmPy-Met | 25 | 25 | >50 | >50 | >50 |
| **78** | $HG_{18}$-*p*Benz-AmPy-AmPy-Met | 35 | 35 | >50 | >50 | >50 |
| **79** | $HG_{25}$-*p*Benz-AmPy-AmPy-Met | 13 | 25 | >50 | >50 | >50 |
| **80** | $HG_{17}$-*p*Benz-AmPy-AmPy-Met | 2 | 25 | >50 | >50 | 50 |

[a] All data are minimum inhibitory concentrations, given as $\mu$g mL$^{-1}$. For structure codes, see Figures 2−4.

our molecular modeling suggested that an alkene-linked lexitropsin would fit the DNA minor groove better than the polyamides. In particular, a polyamide docked into the minor groove of DNA underwent significantly more distortion upon binding than the equivalent isosteric polyalkene (data not shown). The natural curvature of the polyalkene lexitropsin is more compatible with the DNA helical minor groove, which translated into a higher energy penalty arising from the conformational distortion of the polyamide upon binding to DNA.

We had noted that **6**, one of the most active antibacterials described by Genelabs,[11−15] had a p-disubstituted benzene ring as the central unit in a head-to-head arrangement. One of our first alkene linkers was therefore placed between two benzene rings in the head group to give **49**; such an analogue was also more synthetically accessible than related compounds with alkene links between pyrrole rings. The p-linked benzene ring replaced one of the pyrroles of the related polyamide. Compound **49** had comparable potency to thiazotropsin A against *S. aureus* (MIC 3−11 $\mu$g mL$^{-1}$), which encouraged the synthesis of further analogues by variation of the head group. While the incorporation of a 2-quinolinyl residue in **63** was not beneficial (MIC 67−134 $\mu$g mL$^{-1}$), the similar molecule **50** with 3-quinolinyl was extremely potent, with MIC values in the range of 0.5−13 $\mu$g mL$^{-1}$ against several strains of *S. aureus* in the two test systems. Such potency is comparable with that of many recently introduced antibacterial drugs. A possible explanation of the difference in potency is that the 3-quinolinyl nitrogen can form a hydrogen bond with a guanine amino group at the base of the minor groove, while the 2-quinolinyl nitrogen cannot. This hypothesis was supported by the observation of inactivity of the naphthyl analogue **62**. The 3-pyridyl analogue **78**, lacking the benzene ring of the quinoline head group in **50**, was also a potent antibacterial (MIC 1−8 $\mu$g mL$^{-1}$) against *S. aureus*, consistent with an H-bonding role for the pyridine nitrogen. The 4-pyridyl analogue **80** was slightly more active (MIC 0.5−6 $\mu$g mL$^{-1}$) than **78**, and it is possible that either could H bond to a similarly situated guanine amino group in the minor groove.

All of these alkene-linked analogues had morpholinoethyl tails, in accord with the observations made by the Genesoft group.[11−15] In view of the improved potency among the amidines when a DMAP tail was used, the DMAP analogue of **50** was synthesized and tested, but **53** was less potent (MIC 9−35 $\mu$g mL$^{-1}$). The lower antibacterial activity of compounds with DMAP tails was general (Table 3). This result is similar to that found with the above polyamides and different from the amidines tested in this study.

The apparently optimum structure found with **50** was underlined by the reductions in potency seen when the 3-quinolinyl head group was replaced by *N*-methylpyrrole to give **51** or when the p-disubstituted benzene was replaced by the m-disubstituted analogue, as in **54**. The pyrrole head group is

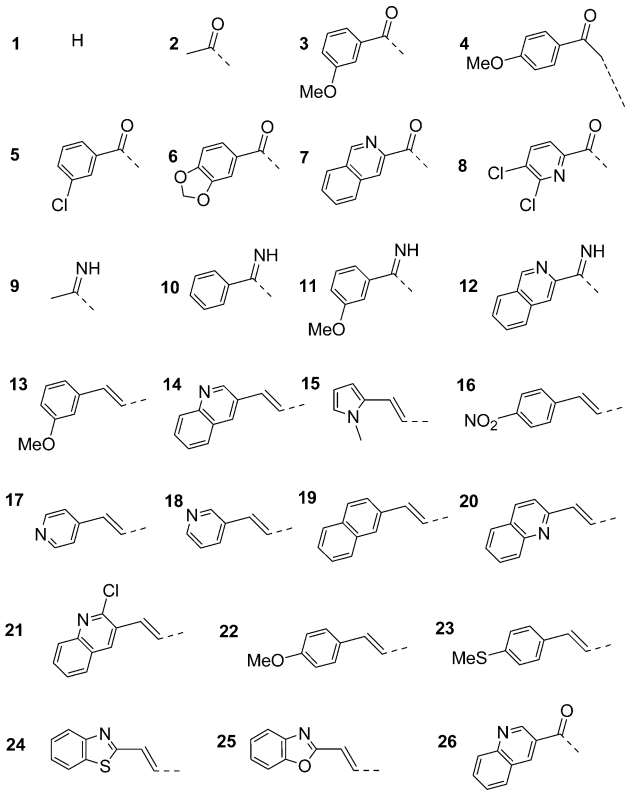



**Figure 2.** Head groups used in the present study. The numbers refer to the head group designations used in the tables and experimental section.

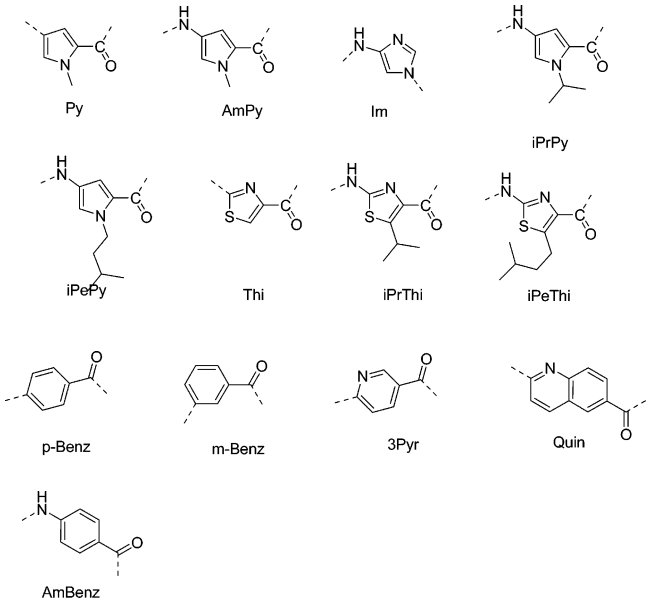



**Figure 3.** Groups used to form the central chain of the minor groove binders. The abbreviations are used in the tables to designate the structures of the MGBs.

incapable of hydrogen bonding with a guanine amino group in the base of the groove, and the m-disubstituted benzene causes a large change in the curvature of the molecule. It is interesting that replacement of p-disubstituted benzene with pyrrole as in **70**, retaining helicity, was deleterious (MIC more than 120 $\mu$g mL$^{-1}$).

Simple inspection indicates that a para-disubstituted benzene ring is not likely to produce an optimum fit to the DNA helix in that part of the molecule. However, the fit to DNA of the most suitably shaped conformer of **50** is shown in Figure 5 and

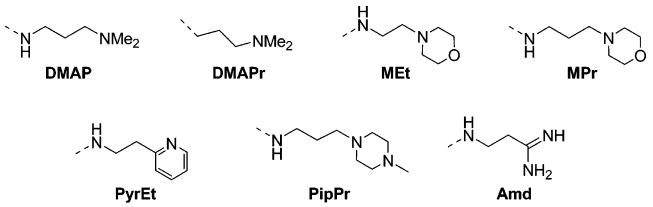



**Figure 4.** Tail groups used in the present study. The abbreviations are used in the tables to designate the structures of the MGBs.

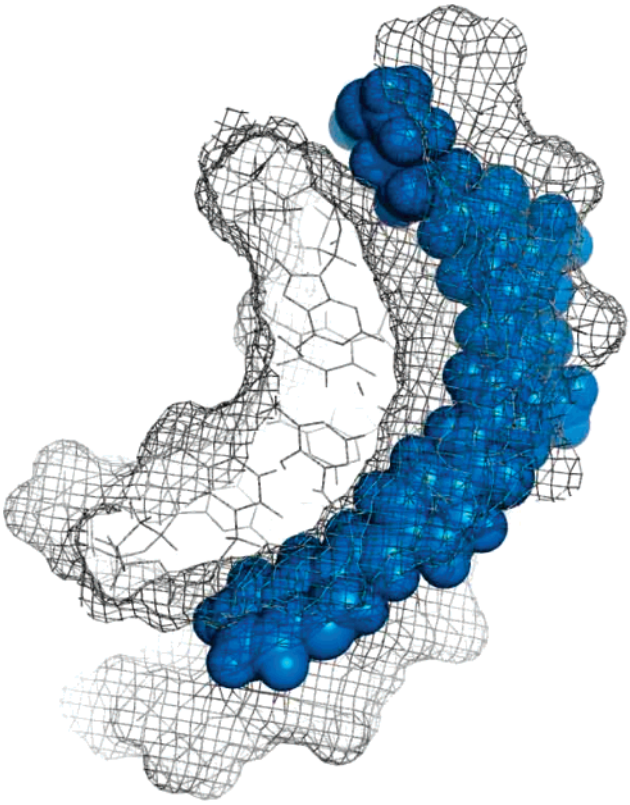


**Figure 5.** Model of **50** bound to DNA in conformation A as shown in Figure 6. A single molecule of ligand is shown for clarity.

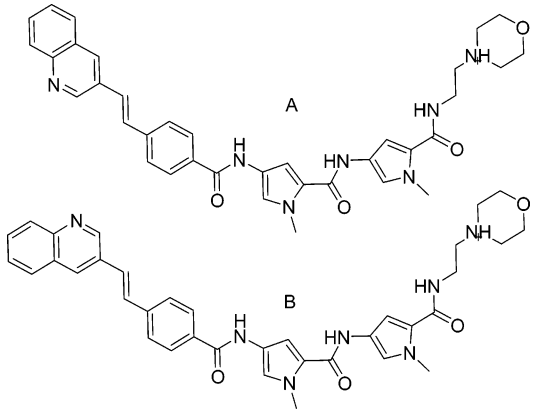



**Figure 6.** The conformation (A) of **50** that produces the best fit to DNA, as modeled in Figure 5, and the conformation (B), which would allow H bonding to a guanine amino group at the base of the minor groove.

illustrates that occupancy of the minor groove is possible. Such a conformer could not bind to a guanine amino group at the base of the minor groove, and it is possible that ring-flipping occurs between conformers A and B, as in Figure 6 (see also under DNA Binding below).

**Table 4.** Activity of Selected Compounds against Clinical Isolates of *S. aureus*[a]

| | MIC ($\mu$g/mL) versus *S. Aureus* strain | | | | |
|---|---|---|---|---|---|
| compound | (1) | (2) | (3) | (4) | (5) |
| **7** | 32 | >64 | 64 | 64 | 64 |
| **11** | >64 | >64 | >64 | >64 | >64 |
| **12** | >64 | >64 | >64 | >64 | >64 |
| **13** | 16 | 16 | 16 | 16 | 16 |
| **14** | 8.0 | 16 | 16 | 16 | 16 |
| **15** | 16 | 32 | 32 | 32 | 32 |
| **16** | 11.0 | 11.0 | 11.0 | 0.50 | 2.0 |
| **17** | 8.0 | 64 | 64 | 64 | 64 |
| **18** | 64 | >64 | >64 | >64 | >64 |
| **19** | 8.0 | 16 | 16 | 8.0 | 8.0 |
| **24** | 32 | 32 | 32 | 32 | 32 |
| **25** | >64 | >64 | >64 | >64 | >64 |
| **41** | 16 | 64 | 32 | 32 | 32 |
| **42** | 64 | >64 | >64 | >64 | >64 |
| **43** | >64 | >64 | >64 | >64 | >64 |
| **44** | >64 | >64 | >64 | >64 | >64 |
| **45** | >64 | >64 | >64 | >64 | >64 |
| **46** | >64 | >64 | >64 | >64 | >64 |
| **47** | 8.0 | 64 | 16 | 64 | 64 |
| **49** | 2.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| **50** | 0.12 | 0.25 | 0.12 | 0.25 | 0.12 |
| **51** | 4.0 | 16.0 | 16.0 | 16.0 | 16.0 |
| **52** | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 |
| **53** | 25 | 25 | 25 | 25 | 25 |
| **55** | >64 | >64 | >64 | >64 | >64 |
| **56** | 16 | 32 | 64 | >64 | >64 |
| **57** | 32 | 32 | 32 | 32 | 32 |
| **58** | >64 | >64 | >64 | >64 | >64 |
| **59** | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| **60** | 32 | 32 | 32 | 32 | 32 |
| **61** | >64 | >64 | >64 | >64 | >64 |
| **69** | 4.0 | 64 | 64 | 32 | 16 |
| **70** | 4.0 | >64 | >64 | >64 | 16 |
| **71** | 8 | 8 | 8 | 8 | 8 |
| **73** | 0.5 | 4 | 2 | 2 | 0.5 |
| **74** | 4.0 | >64 | >64 | >64 | >64 |
| **75** | 4.0 | >64 | >64 | >64 | >64 |
| **76** | 0.50 | 4.0 | 2.0 | 1.0 | 0.50 |
| **79** | >64 | >64 | >64 | >64 | >64 |
| **78** | 2 | 8 | 4 | 8 | 1 |
| **79** | 1 | 16 | 1 | 1 | 1 |
| **80** | 0.5 | 2 | 1 | 1 | 0.5 |

[a] All data are minimum inhibitory concentrations, given as $\mu$g/mL; (1)−(5) refer to collected clinical strains of *S. aureus*.

As with the polyamides described above, replacement of *N*-methylpyrrole with isopentylthiazole to give **65** resulted in a drastic loss of potency. It may be inferred in both the polyamide and alkene series that the bulky substituent forces a frame shift in the overlap so that the two molecules can no longer be accommodated in the critical binding site. Since it was anticipated that the alkene linker was an isosteric replacement for an amide, the polyamide analogues of **49** and **50** were prepared, but **26** and **27** were completely inactive, a result for which we have, at present, no explanation. As expected, the active compounds were just as effective against clinical isolates of MRSA as they were against the methicillin-sensitive strains (Table 4).

**Antifungal Activity.** The major interest in this study was to find antibacterial activity, but all compounds synthesized were routinely tested against two fungal species, *Aspergillus niger* and *Candida albicans*. Both are significant pathogens, particularly in immunocompromised patients. Typically, antifungal agents are less potent than antibacterials, generally at least by an order of magnitude; typical values for the MIC of the standard fluconazole were ~50 $\mu$g/mL. Antifungal minor groove binders of the polyamide type with linkers containing indole substructures have been described.[26] Our primary lead, thiazotropsin A **3**, had MIC values for both fungal species in the range of 70−80 $\mu$g mL$^{-1}$. The observation of this level of antifungal activity may be an indication that thiazotropsin A is a nonselective toxin, as are distamycin and netropsin, consistent with high-affinity binding to a relatively short binding site. Of all of the other simple polyamides tested in this study, only **8** showed greater antifungal activity, at 66 and 33 $\mu$g mL$^{-1}$ against *A. niger* and *C. albicans*, respectively (Table 1).

In the amidine series, **30** and **33** had a MIC of ~30 $\mu$g mL$^{-1}$against *A. niger*, with much less effectiveness against *C. albicans*. The structures of **30** and **33** differ only in the N-substituent on the heterocycle nearest the tail. It would be expected that the amidines, with charges at both ends, would occupy the minor groove as single molecules. The isopropyl group in **30** would not then affect the degree of overlap or the length of the binding site. In the alkene series, only **50** showed activity, at 30 $\mu$g mL$^{-1}$. This compound was the most potent antibacterial in this study, at concentrations 2 orders of magnitude lower than the antifungal MIC, an indication that **50** has a good degree of selectivity. Overall, antifungal activity was promising, especially by comparison with the antifungal standard, but a search for this type of activity requires further research directed specifically at fungi.

The detailed analysis of structure−activity relationships is complicated in this study because of the large number of variables both in the molecular structure and in the biological events that the compounds encounter during assay. It will be necessary to merge both molecular modeling and principal component analysis approaches to obtain clearer and quantitative insight.

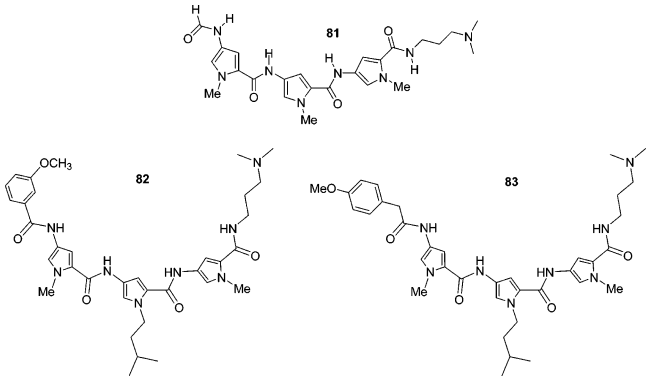
**Molecular Mechanism of Action. DNA Binding.** Preliminary NMR studies have shown that two molecules of **49** bind side-by-side to the self-complementary oligonucleotide CGATATATCG, covering the central eight bases. Some proton resonance broadening was apparent, an indicator of conformational dynamics or exchange between binding modes that could be attributable to flipping of the head group. NMR studies of the binding of the most active quinoline derivative **50** have, so far, proved inconclusive, an unusual occurrence within our experience of studying these classes of compounds. The observed excessive resonance broadening is consistent with exchange between two or more binding modes and is usually attributed to behavior associated with DNA intercalator binding. The possibility that ligands such as **50** could intercalate is not unexpected as an alternative mode of binding since they possess a substantial planar area in the head group region. Although these observations could also have been associated with a greater requirement for DNA sequence-specificity, CE measurements have shown that **50** forms a clean 2:1 complex with the duplex GCGATATATGCG/CGCTATATACGC. Aggregation effects at the higher concentrations used for NMR studies, and the possibility of salting-out effects on the ligand, may also be factors that play a role in these observations, and further NMR investigations are therefore in progress to further understand our observations. The CE results nevertheless make it clear that alkene-containing minor groove binders associate strongly with DNA duplexes. In most respects also, these data imply very similar behavior to that which we have established with the thiazotropsins.[4,6,18]

**Structure and Antibacterial Activity.** There being no obvious impediment to the binding of alkene-containing minor groove binders with hydrophobic head groups to DNA, the other structural features responsible for high antibacterial activity can be sought. It was noted above that a component capable of

**Table 5.** Antibacterial Activity of MGBs as a Function of the p$K_a$ of the Tail Group and Its Implications for Distribution and Partition

| compound | tail | tail p$K_a$ | log *P* | log $D^{7.4}$ | structural feature | biological activity |
|---|---|---|---|---|---|---|
| distamycin **1** | amidine | 12.40 | −2.94 | −7.94 | amidine | toxic |
| dist-DMAP **81** | *t*-amine | 9.99 | −1.98 | −4.57 | no lipophilic groups | weak antibacterial |
| thiazotropsin B **4** | *t*-amine | 9.99 | 0.85 | −1.74 | imidazole and *C*-alkyl thiazole | inactive antibacterial |
| thiazotropsin A **3** | *t*-amine | 9.99 | 0.61 | −1.98 | *C*-alkyl thiazole | antibacterial and antifungal |
| **82** | *t*-amine | 9.99 | 1.25 | −1.34 | H-bond head and *N*-pentyl | selective antibacterial |
| **73** | morpholine | 7.41 | 1.10 | 0.79 | H-bond head and alkene | potent selective antibacterial |
| **50** | morpholine | 7.41 | 2.22 | 1.91 | H-bond head and alkene | potent selective antibacterial |

hydrogen bonding to a guanine residue of DNA appeared to be important. Such hydrogen bonding come from the head group, as discussed, or from another component such as the thiazole in thiazotropsin A. While this requirement cannot be considered absolute because so many factors are interlinked in obtaining a highly active antibacterial drug, the consensus that emerges from the compounds described here is that a hydrophobic head group with a hydrogen-bond acceptor is an important requirement. To be effective in cellular assays, potential drugs penetrate the cell wall or cell membrane. It is therefore important to examine the physicochemical properties of candidate compounds. We have therefore compared the log *P* and log $D^{7.4}$ of selected compounds in our collection,[27] including those described in this paper, in order to investigate these factors. The importance of distribution has been emphasized in the context of minor groove binders before.[17] Table 5 presents calculated log *P* and log *D* data in comparison with the major structural features of selected compounds. In contrast to distamycin, our best compounds have a positive log *D*, an outcome associated with both the hydrophobicity of the head group and the low p$K_a$ of the *N*-alkyl morpholine tail group. It is therefore reasonable that the proportion of minor groove binder that is nonprotonated in solution at physiological pH is an important factor in its ability to cross bacterial cell walls, in this case of Gram positive bacteria. Lower activity in two minor groove binders that differ only in the tail group may be more a consequence of transport into cells than of intrinsic binding to a target sequence of DNA. We do not suggest that these factors will necessarily be dominant in the structure−activity relationships of minor groove binders in other series, even in polyamides, because of the multiplicity of chemical and biological phenomena involved.




A further notable feature of our lead compounds is their lack of cytotoxicity to a number of mammalian cell lines. For example, **50** is not toxic to HS27 or L929 cells, giving it a therapeutic window of over 1000-fold in vitro; **73** also has a strong profile, showing no in vitro cytotoxicity to L929, H9C2, TE671, Jurkat, and A375 cells. This is clearly in strong contrast with minor groove binders that have been developed for anticancer activity.[28,29] These compounds and others from different classes of minor groove binders[30] commonly contain amidines, suggesting an important differentiating role for this type of tail group. The uptake of amidine-containing minor groove binders not only into cells but also into the nuclei of eukaryotic cells is significant[31] and relevant to the selectivity shown by our compounds. In addition to selectivity, the avoidance of resistance is important in a new antibacterial compound. Selectivity for bacterial DNA as explained above will play a role, but we have found that **37** shows synergy as an antibacterial compound in combination with amoxicillin. Thus, the MIC for **37** against a strain of *S. aureus* was found to be 3.1 $\mu$M in the absence of amoxicillin, but in the presence of amoxicillin (0.25 $\mu$M), the MIC was reduced to 0.19 $\mu$M.[32]

**Table 6.** Purities of Compounds Used for Antimicrobial Assay

| % purity by HPLC and 1H NMR | compounds |
|---|---|
| 98 | **3**, **8**, **11**, **15**, **16**, **17**, **20**, **22**, **56**, **55**, **56**, **51**, **23**, **28**, **31**, **33**, **34**, **35**, **39**, **40**, **41**, **44**, **46**, **47**, **48**, **57**, **58**, **59**, **64** |
| 97 | **7**, **9**, **10**, **12**, **18**, **19**, **25**, **26**, **27**, **52**, **60**, **61**, **62**, **68**, **69**, **72** |
| 96 | **13**, **14**, **21**, **24**, **29**, **30**, **32**, **36**, **37**, **50**, **54**, **76**, **79**, **80** |
| 95 | **38**, **42**, **43**, **45**, **49**, **53**, **63**, **65**, **66**, **67**, **70**, **71**, **73**, **74**, **75**, **77**, **78** |

**In Vivo Antibacterial Activity.** The mammalian cell toxicity of several of the most active antibacterial compounds has been tested; details will be reported elsewhere. Within the range of active compounds, mammalian cell viability varied greatly. Compounds that showed an acceptable balance of activity and toxicity (**50** and **73**) were selected for in vivo evaluation in a mouse model described by Bremell et al.[33] Preliminary results indicate efficacy at a single dose of 40 mg/kg for **73** and 20 mg/kg for **50**, given either intravenously or intraperitoneally.

## Conclusions

Our results, together with the results of the Genesoft and Genelabs groups,[11−16] show that lexitropsin antimicrobial agents can be obtained with very high potency, particularly against methicillin-resistant *S. aureus*. High antibacterial activity against Gram positive bacteria appears to require a minor groove binder that contains a hydrophobic head group with a hydrogen-bonding atom or substituent and a low p$K_a$ tail group. The major questions that remain to be satisfactorily answered concern the detailed molecular mechanism of activity and the lethal biological processes with which these minor groove binders interfere. Such studies will point to the structural features that lead to the wider different biological activities, and we are pursuing these questions.

## Experimental Section

MGBs and their precursors were prepared essentially by established methods, as described in detail in the Supporting Information. Samples for assay were purified by HPLC, and the purity of the products was between 95 and 98%, as assessed by HPLC and $^1$H NMR (Table 6).

**Examples of Preparation of Lead Compounds. 1-Methyl-*N*-[1-methyl-5-({[2-(4-morpholinyl)ethyl]amino}carbonyl)-1*H*-pyrrol-3-yl]-4-nitro-1*H*-pyrrole-2-carboxamide.[11]** 1-Methyl-*N*-[2-(4-

morpholinyl)ethyl]-4-nitro-1*H*-pyrrole-2-carboxamide (1.42 g, 3.41 mmol) was dissolved in methanol (10 mL) and cooled to 0 °C; 10% palladium on carbon (0.055 g) was then added in small portions at room temperature, and the solution stirred under hydrogen gas for 3 h. The solution was then filtered and the solvent removed under reduced pressure. The amine so formed was dissolved in dichloromethane (25 mL), to which *N*-methylmorpholine (0.5 mL) was added. A solution of 1-methyl-4-nitro-1*H*-pyrrole-2-carbonyl chloride (0.642 g, 3.42 mmol) in dichloromethane (10 mL) was then added and allowed to stir for 24 h at room temperature. Sodium hydroxide (1 g) was dissolved in water (25 mL) and then added to the reaction mixture. The solid material formed was filtered, washed with water and dichloromethane, and dried to give the required product as a yellow solid. The organic layer was separated from the filtrate and dried ($MgSO_4$), and the solvent was removed under reduced pressure to give the crude product, which was triturated with a small amount of methanol and filtered. The combined pyrrole carboxamide dimer product obtained was 0.923 g, 67%, mp >230 °C. IR (KBr): 3339, 3284, 3135, 3068, 2929, 2867, 1666, 1635, 1537, 1306, 1112 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): 2.42 (4H, m), 3.30 (4H, q, $J = 6.8$ Hz), 3.57 (4H, t, $J = 4.6$ Hz), 3.80 (3H, s), 3.94 (3H, s), 6.82 (1H, d, $J = 1.6$ Hz), 7.20 (1H, d, $J = 1.6$ Hz), 7.57 (1H, d, $J = 1.6$ Hz), 7.93 (1H, t, $J = 5.6$ Hz), 8.16 (1H, d, $J = 1.6$ Hz), 10.2 (1H, s).

**General Procedure for the Reduction of Nitro Dimers or Trimers.** The nitro dimer or trimer (0.332 mmol) was dissolved in methanol (25 mL), to which 10% palladium on carbon (10%, 117 mg) was added at 0 °C under nitrogen with stirring. The reaction mixture was hydrogenated for 5 h at room temperature and atmospheric pressure. The catalyst was removed over Kieselguhr, and the methanolic solution was divided into a number of equal portions at room temperature or used as a whole. Methanol was removed under reduced pressure at 50 °C for the following coupling reactions.

**General Coupling Procedure using HBTU.** The nitro compound (1-methyl-*N*-[1-methyl-5-({[2-(4-morpholinyl)ethyl]amino}-carbonyl)-1*H*-pyrrol-3-yl]-4-nitro-1*H*-pyrrole-2-carboxamide for the two following examples) was reduced, as above, and then, methanol was removed under reduced pressure at 50 °C. The amine so formed was dissolved in DMF (1 mL, dry), to which *N*-methylmorpholine (40 $\mu$L) was added at room temperature with stirring, followed by 3-isoquinolinecarboxylic acid (12 mg, 0.070 mmol) and HBTU (52 mg, 0.140 mmol). The stirring was continued at room temperature overnight. The product was purified by HPLC, and fractions containing the required material were combined and freeze-dried to give the products.

**1-Methyl-4-({[1-methyl-4-({4-[(*E*)-2-(3-quinolinyl)ethenyl]-benzoyl}amino)-1*H*-pyrrol-2-yl]carbonyl}amino)-*N*-[2-(4-morpholinyl)ethyl]-1*H*-pyrrole-2-carboxamide (50).** The reaction was carried out on a 0.12 mmol scale coupling 4-[(*E*)-2-(3-quinolinyl)-ethenyl] benzoic acid with the nitro compound reduced as described above.The product was obtained as a pale-yellow solid (36 mg, 39%) with no distinct melting point. $^1H$ NMR (DMSO-$d_6$): 10.35 (1H, s), 9.98 (1H, s), 9.55 (1H, br), 9.28 (1H, d, $J = 2.0$ Hz), 8.59 (1H, d, $J = 2.0$ Hz), 8.23 (1H, t, $J = 8.0$ Hz), 8.05−7.97 (5H, m), 7.83−7.75 (4H, m), 7.70−7.60 (4H, m), 7.34 (1H, d, $J = 1.7$ Hz), 7.21 (1H, d, $J = 1.7$ Hz), 7.13 (1H, d, $J = 1.7$ Hz), 7.01 (1H, d, $J = 1.7$ Hz), 4.03−3.99 (2H, m), 3.88 (3H, s), 3.83 (3H, s), 3.69−3.63 (2H, m), 3.59−3.54 (4H, m), 3.28 (2H, m), 3.15 (2H, m). IR (KBr): 1681, 1642, 1577, 1464, 1435, 1404, 1266, 1202, 1134 $cm^{-1}$. HRFABMS: found, 632.2982; calculated for $C_{36}H_{38}N_7O_4$, 632.2985.

**6-[(*E*)-2-(4-Methoxyphenyl)ethenyl]-*N*-[1-methyl-5-({[1-methyl-5-({[2-(4-morpholinyl)ethyl]amino}carbonyl)-1*H*-pyrrol-3-yl]amino}carbonyl)-1*H*-pyrrol-3-yl]nicotinamide (73).** The reaction was carried out on the 0.12 mmol scale coupling 6-[(*E*)-2-(4-methoxyphenyl)ethenyl]nicotinic acid with the reduced nitro compound. The product was obtained as an orange solid (29 mg, 32%) with no distinct melting point. IR (KBr): 3427, 1673, 1588, 1402, 1253, 1202, 1174, 832, 720 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): 10.48 (1H, s), 9.98 (2H, s, br), 9.07 (1H, s), 8.28 (1H, d, $J = 2.3$ Hz), 8.26 (1H,d, $J = 2.3$ Hz), 7.77 (1H, d, $J = 16.0$ Hz), 7.67 (3H, d, $J = 8.8$ Hz), 7.35 (1H, d, $J = 1.6$ Hz), 7.27 (1H, d, $J = 16.0$ Hz), 7.22 (1H, d, $J = 1.6$ Hz), 7.12 (1H, d, $J = 1.6$ Hz), 7.01 (3H, m), 4.01 (2H, m), 3.88 (3H, s), 3.83 (3H, s), 3.80 (3H, s), 3.73 (2H, m), 3.56 (4H, m), 3.27 (2H, m), 2.99 (2H, m). HRFABMS: found, 611.2990; calculated for $C_{34}H_{39}O_5N_6$, 611.2982.

**In Vivo Evaluation.**[33] Lead compounds were evaluated in a model *S. aureus* strain LS-1, which, when injected intravenously into mice, consistently caused transient bacteraemia followed by joint localization in 3−4 days. This model is effective in assessing the likely clinical efficacy of the MGBs. Normal control mice showed inflammation of 50−60% of their joints within 3−4 days. Any significant reduction in this degree of involvement was recognized as clinical efficacy. The severity of joint sepsis was measured using calipers to determine the diameter of the effected joints. In this evaluation, seven groups of mice weighing 18−22 g (five mice per group) were included in the experiment. Each mouse was inoculated with 50 million cfu, and the treatment was as follows:

Group 1: *S. aureus* LS-1 only.
Group 2: *S. aureus* LS-1 plus 60 mg/kg of **50** or **73**.
Group 3: *S. aureus* LS-1 plus 40 mg/kg of **50** or **73**.
Group 4: *S. aureus* LS-1 plus 20 mg/kg of **50** or **73**.
Group 5: *S. aureus* LS-1 plus 10 mg/kg of **50** or **73**.

**In Vitro Assessment of Antimicrobial Activity.**[34] Sample dilutions were typically prepared by dissolving the test sample (2 mg) in sterile water (10 mL) to provide a working concentration of 200 $\mu$g $mL^{-1}$. The test wells on each 96 well microtiter plate were initially inoculated with culture medium (100 $\mu$L) using Mueller−Hinton broth for antibacterial assays and Sabourand broth for antifungal assays. A solution of each test sample (100 $\mu$L) was added to one row of each plate, and a series of doubling dilutions was made for successive rows. Incubation was at 37 °C for antibacterial assays and 25 °C for antifungal assays. Plates were inspected visually for growth, and Resazurin was added to each well; a distinct color change from blue to red indicated that growth had occurred in an individual well. From the observed pattern of color, the MIC was determined. All tests included sterility and growth controls.

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**Supporting Information Available:** Abbreviations used in experimental section, instrumentation, purification methods, synthesis of precursor compounds, synthesis of minor groove binders, acid chloride method, DCC method, formylation, HBTU method, amidine-linked minor groove binders, and references to Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Dervan, P. B. Molecular recognition of DNA by small molecules. *Bioorg. Med. Chem.* **2001**, *9*, 2215−2235.
(2) White, S.; Szewczyk, J. W.; Turner, J. M.; Baird, E. E.; Dervan, P. B. Recognition of the four Watson−Crick base pairs in the DNA minor groove by synthetic ligands. *Nature* **1998**, *391*, 468−71.
(3) (a) Dickinson, L. A.; Burnett, R.; Melander, C.; Edelson, B. S.; Arora, P. S.; Dervan, P. B.; Gottesfeld, J. M. Arresting cancer proliferation by small-molecule gene regulation. *Chem. Biol.* **2004**, *11*, 1583−1594. (b) Mapp, A. K.; Ansari, A. Z.; Ptashne, M.; Dervan, P. B. Activation of gene expression by small molecule transcription factors. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3930−3935.
(4) James, P. L.; Merkina, E. E.; Khalaf, A. I.; Suckling, C. J.; Waigh, R. D.; Brown, T; Fox, K. R. DNA sequence recognition by an isopropyl substituted thiazole polyamide. *Nucleic Acids Res.* **2004**, *32*, 3410−3417.
(5) Hampshire, A. J.; Khairallah, H.; Khalaf, A. I.; Ebrahimabadi, A. H.; Waigh, R. D.; Suckling, C. J.; Brown, T.; Fox, K. R. DNA sequence recognition by an imidazole-containing isopropyl-substituted thiazole polyamide (thiazotropsin B). *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3469−3474.

(6) Anthony, N. G.; Fox, K. R.; Johnston, B. F.; Khalaf, A. I.; Mackay, S. P.; McGroarty, I. S.; Parkinson, J. A.; Skellern, G. G.; Suckling, C. J.; Waigh, R. D. DNA binding of a short lexitropsin. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1353−1356.
(7) Haq, I.; Ledbury, J. E.; Chowdrhy, B. Z.; Jenkins, T. C.; Chaires, J. B. Specific binding of Hoechst 33258 to the $(CGCAAATTTGCG)_2$ duplex: calorimetric and spectroscopic studies. *J. Mol. Biol*. **1997**, *271*, 244−257.
(8) Kirschner, D. Timebomb: the global epidemic of multi-drug resistant tuberculosis. *Nat. Med.* **2001**, *7*, 1173.
(9) Applebaum, P. C. MRSA−the tip of the iceberg *Clin. Microbiol. Infect.* **2006**, *12*, 3−10, Suppl. 2.
(10) Suckling, C. J. Minor groove binders 1998−2004: a review of the patent literature. *Expert Opin. Ther. Pat.* **2004**, *14*, 1693−1724.
(11) 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. DNA binding ligands targeting drug-resistant bacteria: structure, activity, and pharmacology. *J. Med. Chem.* **2003**, *46*, 3914−3929.
(12) Bürli, W.; R.; Ge, Y.; White, S.; Touami, S. M.; Taylor, M.; Kaizerman, J. A.; Moser, H. E. DNA binding ligands with excellent antibiotic potency against drug-resistant Gram-positive bacteria. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2591−2594.
(13) Bürli, R. W.; Mcminn, D.; Kaizerman, J. A.; Hu, W.; Ge, Y.; Pack, Q.; Jiang, V.; Gross, M.; Garcia, M.; Tanaka, R.; Moser, H. E. DNA binding ligands targeting drug-resistant Gram-positive bacteria. Part 1: Internal benzimidazole derivatives. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1253−1257.
(14) Bürli, R. W.; Jones, P.; Mcminn, D.; Le, Q.; Duan. J.-X.; Kaizerman, J. A.; Difuntorum, S.; Moser, H. E. DNA binding ligands targeting drug resistant Gram-positive bacteria. Part 2: C-terminal benzimidazoles and derivatives. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1259−1263.
(15) Bürli, R. W.; Kaizerman, J. A; Duan, J.-X.; Jones, P.; Johnson, K. W.; Iwamoto, M.; Truong, K.; Hu, W.; Stanton, T.; Chen, A.; Touami, S.; Gross, M.; Jiang, V.; Ge, Y.; Moser, H. E. DNA binding ligands with in vivo efficacy in murine models of bacterial infection: optimization of internal aromatic amino acids. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2067−2072.
(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. Minor groove binders as antibacterial agents. 1. Pyrrole tetraamides are potent antibacterial agents against vancomycin resistant *Enterococci* and methicillin resistant *Staphylococcus aureus. J. Med. Chem.* **2002**, *45*, 805−817.
(17) (a) Khalaf, A. I.; Pitt, A. R.; Scobie, M.; Suckling, C. J.; Urwin, J.; Waigh, R. D.; Young, S. C.; Fishleigh, R. V.; Fox, K. R. Design and synthesis of extended lexitropsins with good solubility and potentially improved tissue penetration characteristics. *J. Med. Chem.* **2000**, *43*, 3257−3266. (b) Khalaf, A. I.; Suckling, C. J.; Waigh, R. D. Novel hydrophobic minor groove agents. U.K. Pat. Appl. 0619325.4, 2006.
(18) Anthony, N. G.; Johnston, B. F.; Khalaf, A. I.; Mackay, S. P.; Parkinson, J. A.; Suckling, C. J.; Waigh, R. D. A short lexitropsin that recognizes the DNA minor groove at 5-ACTAGT-3: Understanding the role of isopropyl-thiazole. *J. Am. Chem. Soc.* **2004**, *126*, 11338−11349.
(19) Yamamoto, Y.; Kimachi, T.; Kanaoka, Y.; Kato, S.; Bessho, K. Synthesis and DNA binding properties of amide bond-modified analogues related to distamycin. *Tetrahedron Lett.* **1996**, *37*, 7801−7804.
(20) Nuhn, P.; Buege, A.; Harenberg, P.; Lettau, H.; Moschner, K.; Bauchmaul, M.; Schneider, R. Synthesis of lipoxygenase inhibitors. 1. Synthesis of open amidrazones *Pharmazie* **1993**, *48*, 340−342.
(21) Walczynski, K.; Timmerman, H.; Zuiderveld, O. P.; Zhang, M. Q.; Glinka, R. Histamine H-1 receptor ligands Part I. Novel thiazol-4-ylethanamine derivatives: synthesis and in vitro pharmacology. *Farmaco* **1999**, *54*, 533−541.
(22) Reynaud, P.; Moreau, R. C.; Thu, N. H. A property of primary thioamides. *C. R. Sci.* **1961**, *253*, 1968−1970.
(23) Freydank, A. C.; Humphrey, M. G.; Friedrich, R. W.; Luther-Davies, B. Synthesis and optical characterization of unsymmetrical oligophenylenevinylenes. *Tetrahedron* **2002**, *58*, 1425−1432.
(24) Lehrfeld, J.; Burkman, A. M.; Gearien, J. E. Synthesis of 6-substituted nicotinic acid derivatives as analogues of ergot alkaloids. *J. Med. Chem.* **1964**, *7*, 150−154.
(25) Khalaf, A. I.; Ebrahimabadi, A. H.; Drummond, A. J.; Anthony, N. G.; Mackay, S. P.; Suckling, C. J.; Waigh, R. D. Synthesis and antimicrobial activity of some netropsin analogues *Org. Biomol. Chem*. **2004**, *2*, 3119−3127.
(26) (a) Turin, L. M.; Pitt, A. R.; Suckling, C. J.; Waigh, R. W. Covalently linked dimeric DNA binding molecules. Genelabs Tech., Inc. WO0119792, 2001. (b) Zhang, W.; Liehr, S. J. R.; Velligan, M. D.; Dyatkina, N. B.; Botyanszki, J.; Shi, D.-F.; Roberts, C. D.; Khorlin, A.; Nelson, P. H.; Muchowski, J. M. Genelabs Tech., Inc. WO0200650, 2002.
(27) Khalaf, A. I.; Waigh, R. D.; Drummond, A. J.; Pringle, B.; McGroarty, I.; Skellern, G. G.; Suckling, C. J. Distamycin analogues with enhanced lipophilicity. *J. Med. Chem.* **2004**, *47*, 2133−2156.
(28) Fedier, A.; Fowst, C.; Tursi, J.; Geroni, C.; Haller, U.; Marchini, S.; Fink, D. Brostacillin (PNU-166196)−a new DNA minor groove binder retains sensitivity in DNA mismatch repair-deficient tumour cells. *Br. J. Cancer* **2003**, *89*, 1559−1565.
(29) ten Tije, A. J.; Verweij, J.; Sparreboom, A.; van der Gaast, A.; Fowst, C.; Fiorentini, F.; Tursi, J.; Antonellini, A.; Mantel, M.; Hartman, C. M.; Stoter, G.; Planting, A. S. T.; De Jonge, M. J. A. Phase I and pharmacokinetic study of brostacillin (PNU-166196), a new DNA minor groove binder, administered intravenously every 3 weeks to adult patients with metastatic cancer. *Clin. Cancer Res*. **2003**, *9*, 2957−2964.
(30) Lansiaux, A.; Tanious, F.; Mishal, Z.; Dassonneville, L.; Kumar, A.; Stephens, C. E.; Hu, Q.; Wilson, W. D.; Boykin, D. W.; Bailly, C. Distribution of furamidine analogues in tumor cells: targeting of the nucleus or mitochondria depending on the amidine substitution. *Cancer Res.* **2002**, *62*, 7219−7229.
(31) Taylor, A.; Webster, K. A.; Gustafson, T. A.; Kedes, L. The anticancer agent distamycin A displaces essential transcription factors and selectively inhibits myogenic differentiation distamycin. *Mol. Cell Biochem.* **1997**, *169*, 61−72.
(32) Ellis, E.; Ford, G.; Hunter, I. S. Unpublished results.
(33) Gemmell, C. G.; Goutcher, S. C.; Reid, R.; Sturrock, R. D. Role of certain virulence factors in a murine model of *Staphylococcus aureus* arthritis. *J. Med. Microbiol*. **1997**, *46*, 208−213.
(34) Drummond, A. J.; Waigh, R. D. The development of microbiological methods for phytochemical screening. *Recent Res. Devel. Phytochem.* **2000**, *4*, 143−152.
(35) Khalaf, A. I.; Pitt, A. R.; Scobie, M.; Suckling, C. J.; Urwin, J.; Waigh, R. D.; Young, S. C.; Fishleigh, R. V.; Wylie, W. A. The synthesis of some head to head linked minor groove binders. *Tetrahedron* **2000**, *56*, 5225−5239.
(36) Gupton, J. T.; Krolikowski, D. A.; Yu, R. H.; Riesinger, S. W.; Sikorski, J. A. Application of 2-substituted vinamidinium salts to the synthesis of 2,4-disubstituted pyrroles *J. Org. Chem.* **1990**, *55*, 4735−4740.
(37) Grehn, L. Preparative aspects of the nitration of 1-methylpyrrole derivatives−C-13 NMR of some nitropyrroles. *Chemica Scripta*, **1979**, *13*, 67−77.
(38) Inami, K.; Shiba, T. Total synthesis of antibiotic Althiomycin. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 352.
(39) Li, W-D. Z.; Wei, K. Intramolecular alkylative arylation of oxabicylic alkene: a potential diene approach for the synthesis of estrone and analogous steroid structures. *Org. Lett.* **2004**, *6*, 1333−1335.
(40) Cluzan, R.; Katz, L. Esters of substituted nicotinic acids. The Boots Company Ltd. U.S. 4,009,174, 1977.
(41) Nielsen, C. B.; Johnsen, M.: Arnbierg, J.; Pittelkow, M.; McIlroy, S. P.; Ogilby, P. R.; Jorgensen, M. Synthesis and characterisation of water-soluble pH enylene-vinylene-based singlet oxygen sensitizers for two photon excitation. *J. Org. Chem.* **2005**, *70*, 7065−7079.
(42) Uno, H.; Kurokawa, M.; Masuda, Y. Syntheses of 2-sulfamoylmethylbenzoxazole derivatives and determination of their anticonvulsant activity. *Chem. Pharm. Bull*. **1981**, *29*, 2359−2361.
(43) Kosaka, T.; Wakabayashi, T. Synthesis of 2-(4-aryl-1*E*,3*E*-butadienyl)benzoxazoles by the Horner−Wadsworth−Emmons reaction. *Heterocycles* **1995**, *41*, 477−486.
(44) Ramana, D. V.; Krishna, N. V. S. R. Ortho interactions and intramolecular aromatic substitutions in 2-(styryl)benzazoles under electron-impact. *Indian J. Chem., Sect. B* **1990**, *21*, 247−252.
(45) Rao, K. E.; Bathini, Y.; Lown, J. W. Synthesis of novel thiazole-containing DNA minor groove binding oligopeptides related to the antibiotic distamycin. *J. Org. Chem.* **1990**, *55*, 728−737.
(46) Grammaticakis, P. Medium ultraviolet and visible absorbsion of *N*-aroylarylamines. *Bull. Soc. Chim. France* **1964**, 924−935.
(47) Drefahl, G.; Gerlach, E. $\gamma$-Stilbazole-4′-aldehyde and $\gamma$-stilbazole-4′-carboxylic acid. *J. Prakt. Chem.* **1958**, *6*, 72−79.

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