Bioorganic & Medicinal Chemistry 21 (2013) 7790–7806

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Potent and broad-spectrum antibacterial activity of indole-based bisamidine antibiotics: Synthesis and SAR of novel analogs of MBX 1066 and MBX 1090

John D. Williams [a,*], Son T. Nguyen [a], Shen Gu [a], Xiaoyuan Ding [a], Michelle M. Butler [a], Tommy F. Tashjian [a], Timothy J. Opperman [a], Rekha G. Panchal [b], Sina Bavari [b], Norton P. Peet [a], Donald T. Moir [a], Terry L. Bowlin [a]

[a] Microbiotix, Inc., 1 Innovation Drive, Worcester, MA 01604, USA
[b] United States Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Frederick, MD 21702, USA

ARTICLE INFO

Article history:
Received 27 August 2013
Revised 3 October 2013
Accepted 11 October 2013
Available online 25 October 2013

Keywords:
Antibiotic
Antibacterial
Broad-spectrum
Indole
Cadogan–Sundberg reaction
Reissert indole synthesis
McMurry reductive homocoupling reaction
Amidine
Imidazoline
Tetrahydropyrimidine

ABSTRACT

The prevalence of drug-resistant bacteria in the clinic has propelled a concerted effort to find new classes of antibiotics that will circumvent current modes of resistance. We have previously described a set of bisamidine antibiotics that contains a core composed of two indoles and a central linker. The first compounds of the series, MBX 1066 and MBX 1090, have potent antibacterial properties against a wide range of Gram-positive and Gram-negative bacteria. We have conducted a systematic exploration of the amidine functionalities, the central linker, and substituents at the indole 3-position to determine the factors involved in potent antibacterial activity. Some of the newly synthesized compounds have even more potent and broad-spectrum activity than MBX 1066 and MBX 1090.

1. Introduction

It has become accepted doctrine in the past several years that resistance to known antibiotic classes is a looming health care crisis.[1–5] The prevalence of drug-resistant bacterial strains such as MRSA and VRE has been steadily increasing,[6,7] and new strains of Gram-negative bacteria that are resistant to virtually all antibiotics have been identified.[4,8] Because of the limitations of known drug classes for treating resistant bacterial strains, the identification of new drug classes is imperative.[9,10] Although recent efforts to identify inhibitors of specific essential bacterial enzymes has had limited success,[11] phenotypic screening remains a viable alternative.

We recently identified a set of potent antibacterial compounds using a high-throughput cell-based screen that utilized the rescue of macrophages from *Bacillus anthracis*-mediated cell death.[12] The most potent compounds identified in this screen had the common structural feature of linked indole-based aromatic core structures flanked by two cyclic amidine functionalities (see Fig. 1). Further examination of these compounds demonstrated that in addition to the potent antibacterial activity against *B. anthracis*, the compounds were active against a wide range of both Gram-positive and Gram-negative pathogens.[12,13]

Previous studies have shown that these compounds inhibit DNA synthesis[12] and new results suggest they accomplish this by binding to the minor groove of DNA.[14] Subsequent testing of the four compounds in a murine model of *Staphylococcus aureus* infection showed promising activity for the two 'head-to-head' compounds, MBX 1066 and MBX 1090;[12] the two 'head-to-tail' compounds MBX 1113 and MBX 1128, however, were less protective at similar doses.[15] Because the 'head-to-head' compounds were more potent in the mouse assay, we decided to undertake a more rigorous study of their structure and activity against a range of bacterial strains. Described herein is the account of the modification of the central linker region of MBX 1066/1090, the amidine functionality, and substituents at the 3-position of the indole, and their effect on the antibacterial potency of the resulting compounds.

* Corresponding author. Tel.: +1 508 757 2800.
E-mail address: jwilliams@microbiotix.com (J.D. Williams).

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.10.014

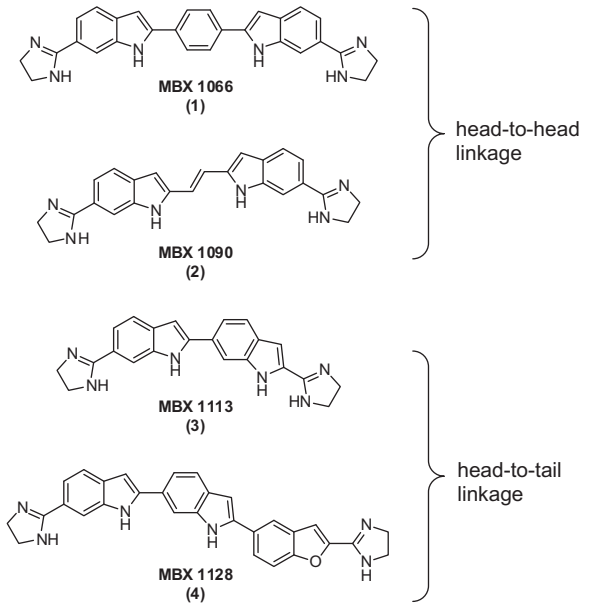


Figure 1. Bisamidine antibiotics identified in HTS of macrophage rescue assay.

2. Materials and methods

2.1. Chemistry

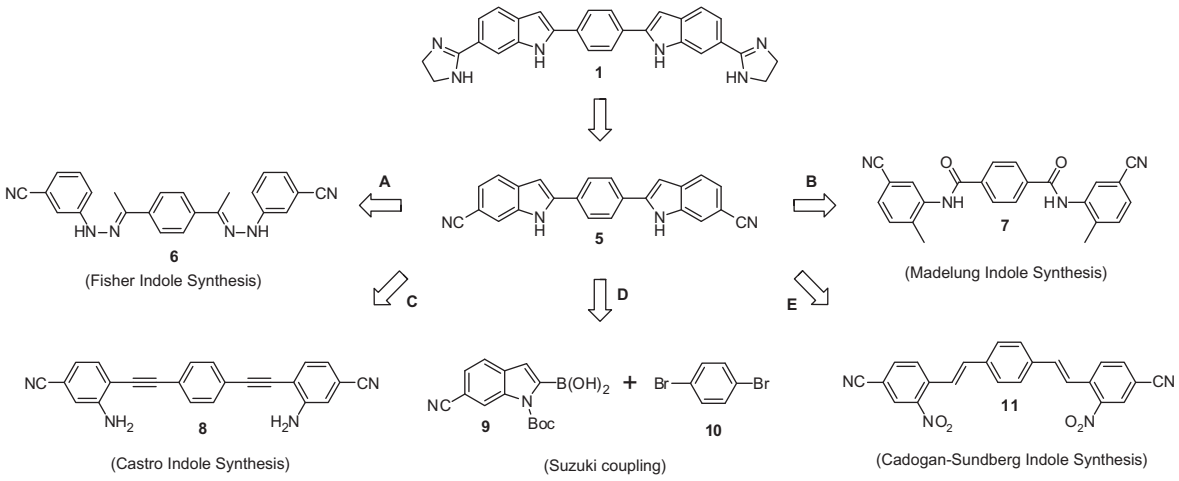
Upon initial discovery of MBX 1066 and MBX 1090, the first task was to find a suitable gram-scale synthesis for the compounds so that additional in vitro and in vivo assays could be performed. It was immediately evident that the synthesis of the desired bisamidines would require the construction of the corresponding dinitriles. The requisite dinitrile (**5**) for the final synthesis of phenyl-linked bisindole MBX 1066 (**1**) is shown in Scheme 1. We were presented with a wide array of potential entries into the triaryl system **5**; because of the ubiquity of indoles in natural products and pharmaceutically interesting compounds, many different strategies for synthesizing substituted indoles have been documented.[16–18]

Our initial attempt for the synthesis of **5** relied upon the venerable Fisher indole synthesis (Scheme 1, Path A).[19] Although the requisite diacetylbenzene is available, and the nitrile-substituted phenylhydrazine could be easily prepared, the cyclization reaction (via unisolated intermediate **6**) produced a low yield and an intractable mixture of isomers resulting from the two potential isomers formed by each reaction. We quickly turned our attention to reactions in which the regiochemistry was preselected by the choice of substrate. Attempts to use the Madelung synthesis[20,21] (Path B) with diamide **7** resulted only in liberation of the corresponding aniline. The Castro indole synthesis[22] (Path C) was considered, but the construction of acetylenic substrate **8** could not be accomplished under Sonogashira conditions[23,24] with the corresponding 4-bromo-3-nitrobenzonitrile. Although we could use Suzuki coupling reactions[25] (Path D) to join two preformed indole moieties to 1,4-dibromobenzene,[26,27] we expected the yield would be low due to deboronylation of the α-heteroatom boronic acid,[28] and the requisite boronic acid was expensive.

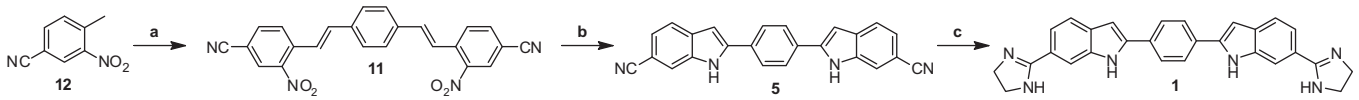
Finally, inspired by the work of Dann et al.,[29,30] we employed the Cadogan–Sundberg reaction[31,32] (Path E) to simultaneously form both indoles (Scheme 2). Thus, the bis(stilbene) intermediate **11** was constructed in an efficient manner from the piperidine-catalyzed condensation of 4-cyano-2-nitrotoluene (**12**) and terephthaldehyde.[29,33] By refluxing **11** in triethyl phosphite, **5** was produced in good yield and large quantities without requiring chromatography for purification. The dinitrile was then smoothly converted to MBX 1066 by treatment of the dinitrile with catalytic phosphorous pentasulfide in hot ethylenediamine.[34]

To synthesize the alkene-linked core for MBX 1090, (i.e., dinitrile **13**; Scheme 3), we initially relied upon the literature synthesis provided by Dann et al., who used a Wittig strategy to form the critical double bond (Path A).[30] However, we were unable to reproduce these results, and phosphonium salt **15** could not be isolated. We were successful, however, in synthesizing aldehyde **14** via a classical Reissert indole synthesis[35] (see Scheme 4 below).[36] Using this substrate, we saw the potential to directly synthesize **13** using a McMurry-type reductive homocoupling reaction (Path B).[37]

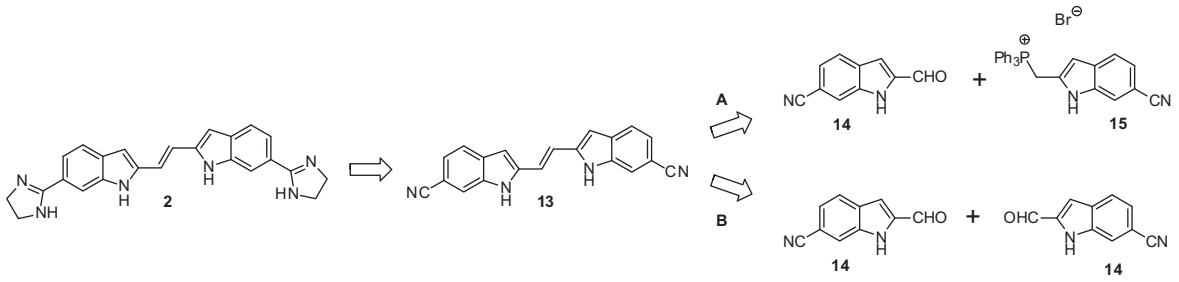
Thus, we first constructed the ketoester **16** from nitrotoluene **12** by base-catalyzed condensation with diethyl oxalate[38] (Scheme 4). Reductive cyclization was accomplished by using zinc in acetic acid to form indole-2-carboxylic acid ester **17**. The ester **17** was first reduced to the alcohol **18** with lithium borohydride,[39] then oxidized to the aldehyde **14** with manganese dioxide.[36] Importantly, we were able to convert the aldehyde **14** directly to dinitrile **13** in one step by treating the aldehyde with low-valent titanium formed in situ from titanium trichloride and lithium wire.[40] To our delight, not only was the requisite dinitrile



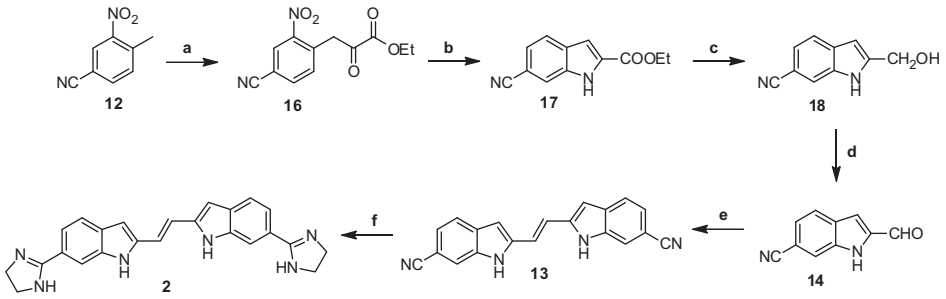
Scheme 1. Potential retrosyntheses for MBX 1066 (**1**).



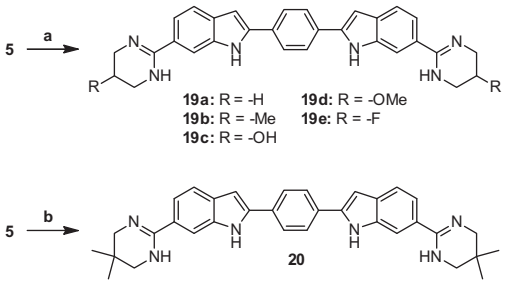
Scheme 2. Synthesis of MBX 1066. Reagents and conditions: (a) terephthaldehyde, piperidine, sulfolane, 150 °C; (b) $P(OEt)_3$ reflux; (c) ethylenediamine, P_2S_5, 120 °C.



Scheme 3. Retrosyntheses for MBX 1090 (**2**).



Scheme 4. Synthesis of MBX 1090. Reagents and conditions: (a) diethyl oxalate, NaOEt, EtOH; (b) Zn/AcOH; (c) $LiBH_4$, THF; (d) MnO_2, acetone; (e) $TiCl_3$, Li wire, DME, reflux then **14**, reflux; (f) ethylenediamine, P_2S_5, 120 °C.



Scheme 5. Synthesis of MBX 1066 analogs with six-membered ring cyclic amidines. Reagents and conditions: (a) $H_2NCH_2CH(R)CH_2NH_2$, P_2S_5, 120 °C; (b) $H_2NCH_2C(Me)_2CH_2NH_2$, P_2S_5, 120 °C.

formed in good yield, but there was no evidence of the corresponding *Z*-isomer in the reaction.[1]

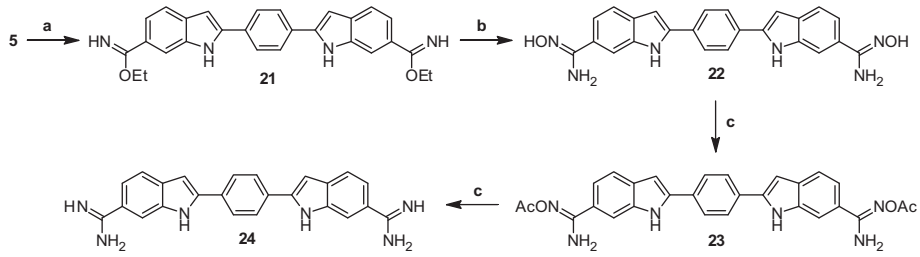
As with the above example, the imidazoline functionalities were installed efficiently by treating the dinitrile with phosphorous pentasulfide in hot ethylenediamine, to produce MBX 1090 (**2**) in good overall yield.[34]

The phenyl-linked dinitrile core **5** was found to be substantially easier to produce than the corresponding ethylene-lined dinitrile **13**. Additionally, the phenyl-linked bisamidine MBX 1066 (**1**) was also found to be a more potent antibacterial than was MBX 1090 (**2**). Therefore, we used intermediate **5** to further explore the effect of other basic and non-basic substituents on each end of the molecule. We began by exploring the cyclic amidine portion of the molecule, and determining whether different ring sizes or ring substituents could influence the antibacterial activity of the compounds (Scheme 5). Using the same phosphorous pentasulfide-catalyzed reaction that was used to synthesize the imidazoline hit compounds,[34] we treated the intermediate dinitrile **5** with different substituted 1,3-diaminopropanes to produce the disubstituted analogs **19a**–**e**, and the tetrasubstituted analog **20**.

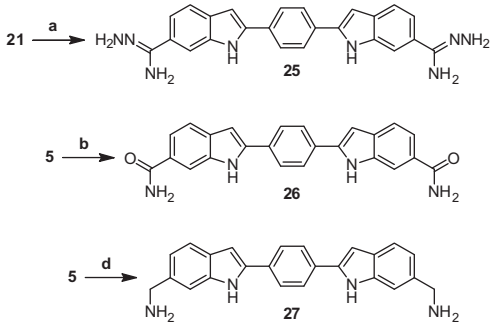
Another important consideration with regard to the activity of the compounds was whether the unsubstituted amidine would behave in the same manner as the imidazoline and tetrahydropyrimidine above. Our initial attempts to synthesize unsubstituted amidine **24** via direct substitution of the corresponding imidate **21**, formed by acid-mediated alcoholysis of nitrile **5**, with either aqueous or anhydrous ammonia were not successful. However, the imidate **21** could be converted efficiently to the carboxamidrazone **22** (Scheme 6). Although carboxamidoximes can sometimes be directly converted to amidines by reductive deoxygenation,[41] the low solubility of **22** hampered our efforts to do so. By acetylating **22** with acetic anhydride, however, we were able to provide a substrate (**23**) that could be cleanly reduced[42] to our desired amidine **24** by catalytic hydrogenation.

In addition to imidate **21**, we were also interested in other amidine-like groups that would test whether the basicity of the

[1] Because **2** is symmetrical, there is no proton–proton coupling with which to assign the configuration of the double bond. In the original synthesis of **2** (Ref. 30), Dann et al. noted that the *E*-isomer of intermediate dinitrile **13** had a high melting point (>360 °C) and fluoresced under sunlight, while the *Z*-isomer had a lower melting point (287–290 °C) and did not similarly fluoresce. The intermediate **13** synthesized under McMurry coupling conditions had a high melting point (>300 °C) and clearly fluoresced under UV light; it was thus assigned as the *E*-isomer.



Scheme 6. Synthesis of unsubstituted diamidine **24**. Reagents and conditions: (a) HCl (g), EtOH, 45 °C; (b) NH_2OH, $EtOH/H_2O$; (c) Ac_2O, AcOH; (d) H_2, Pd/C, AcOH/EtOH.



Scheme 7. Synthesis of carboxamidrazone, amide, and amine analogs of MBX 1066. Reagents and conditions: (a) hydrazine, EtOH; (b); TFA, H_2SO_4; (c) HCl (g), EtOH, 45 °C; (d) H_2, Raney Ni, EtOH/EtOAc.

amidines of MBX 1066 was critical to the antibacterial activity (Scheme 7). Carboxamidrazone **25** was synthesized using imidate **21** after we determined that direct nucleophilic addition of hydrazine to nitrile **5** in various solvents did not provide the desired material. Acid-catalyzed hydrolysis of nitrile **5** using a TFA/sulfuric acid system[43] produced diamide **26** in good yield. Finally, hydrogenation of both nitrile groups in **5** using Raney nickel[44] produced the diamine **27**.

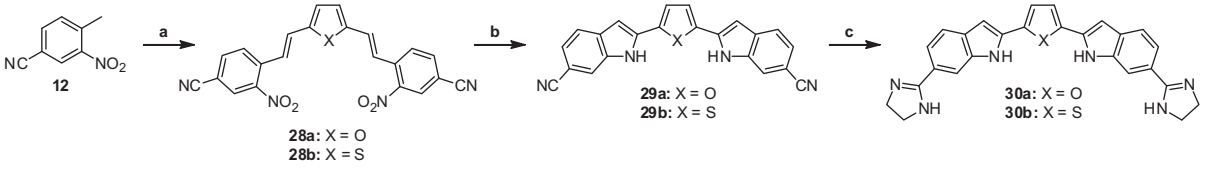
We next turned our attention to the linker portion of the molecule. From our experiences with MBX 1066 and MBX 1090, we knew that some amount of linker modification would be tolerated, and we wanted to determine the limits of structural changes that might be ultimately beneficial. We first used two additional commercially available aromatic dialdehydes to construct MBX 1066 analogs with five-member linker moieties. In a manner directly analogous to the synthesis above, furan-2,5-dicarboxaldehye and thiophene-2,5-dicarboxaldehyde were both converted to the intermediate nitrostyrenes **28a** and **28b** (Scheme 8). These nitrostyrenes were treated with hot triethyl phosphite to effect the Cadogan–Sundberg cyclization, and the intermediate nitriles (**29a** and **29b**) were converted to the corresponding bisamidine compounds **30a** and **30b**.

To synthesize the corresponding pyridine-linked or pyrazine-linked compounds would have required analogous dialdehydes that are not commercially available. However, an alternative procedure using the versatile Suzuki coupling methodology[25] did prove to be a viable option (Scheme 9). Synthesis of bromopyridine **31** was accomplished by coupling one equivalent of the commercially available boronic acid **9** to 3-bromo-6-iodopyridine. A second indole moiety was then installed using a similar process to provide the dinitrile **32**, which was subsequently converted to the imidazoline **33** using the same procedure. A similar strategy was pursued for the synthesis of pyrazine-linked compound **35**. In this case, however, we realized that isolating the intermediate indole-substituted bromopyrazine was unnecessary; coupling two equivalents of boronic acid **9** with 2,5-dibromopyrizine produced the desired nitrile **34** in fair yield. Nitrile **34** was then converted to **35** in the same manner used previously.[34]

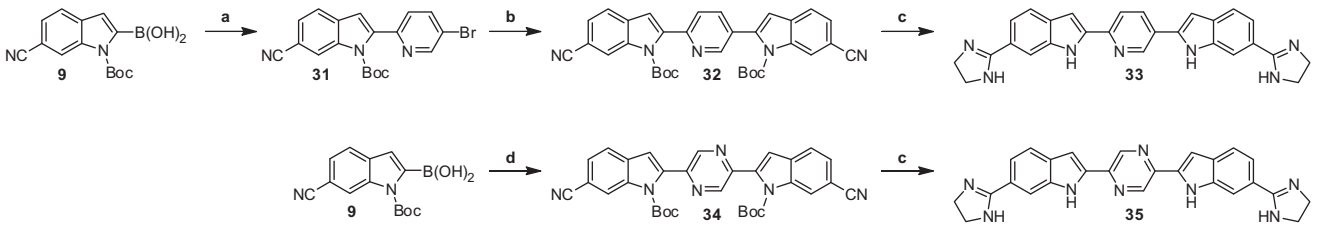
To determine whether the linker portion of the molecule must include a π-system (aryl, heteroaryl, or vinylic), or could be a simple saturated linker; we next synthesized a series of compounds in which the indole cores were attached by hydrocarbon chains of varying length. We realized that the most efficient synthesis of ethyl-linked compound **37** (Scheme 10) would use the previously synthesized alkene-containing intermediate **13**. Simple hydrogenation of the double bond produced the dinitrile **36**, which was then converted to bisamidine **37** with phosphorous pentasulfide in 1,3-diaminopropane.[34]

The synthesis of propyl- and butyl-linked compounds (**44a** and **44b**, respectively) necessitated a different synthetic plan, as no corresponding alkene-linked material was available (Scheme 11). We viewed the Cadogan–Sundberg reaction as the most promising route to these compounds, and thus constructed the corresponding alkyl-linked bis(nitrostyrene) compounds **39a** and **39b** as key intermediates from aldehyde **38**, which were synthesized according to literature precedent.[45] A Wittig reaction using aldehyde **38** and the appropriate alkyl-bis(phosphonium) bromide then provided the desired intermediates **39a** and **39b**. Much to our surprise, however, refluxing these intermediates with triethyl phosphite provided mixtures highly enriched in *N*-ethoxyindoles[32,46] (i.e., **40a**/**41a** and **40b**/**41b**), with very little of the expected unsubstituted bisindoles being produced. Because of the difficulty in separating these mixtures, we next constructed the tetrahydropyrimidine moieties using the previous conditions to form the bisamidines **42a**/**43a** and **42b**/**43b**. These bisamidines were then reduced with Raney nickel in the presence of hydrogen gas to produce the desired bisamidines **44a** and **44b**.

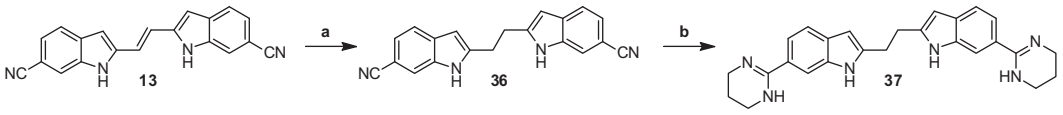
Because the tetrahydropyrimidine analog **19a** showed the most potent antibacterial potency of the compounds we studied, we also investigated whether the same modification (i.e., imidazoline to



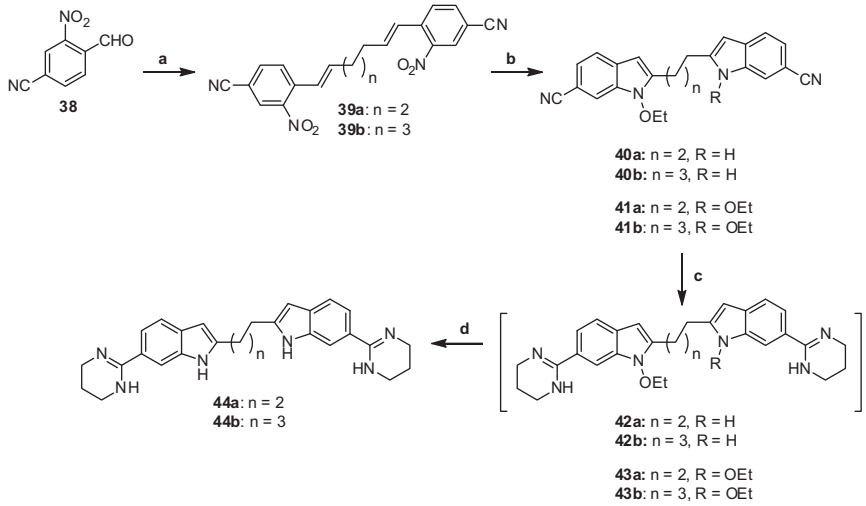
Scheme 8. Synthesis of furan- and thiophene-linked MBX 1066 analogs. Reagents and conditions: (a) furan-2,5-dicarboxaldehyde or thiophene-2,5-dicarboxaldehyde, piperidine, sulfolane, 150 °C; (b) $P(OEt)_3$ reflux; (c) ethylenediamine, P_2S_5, 120 °C.



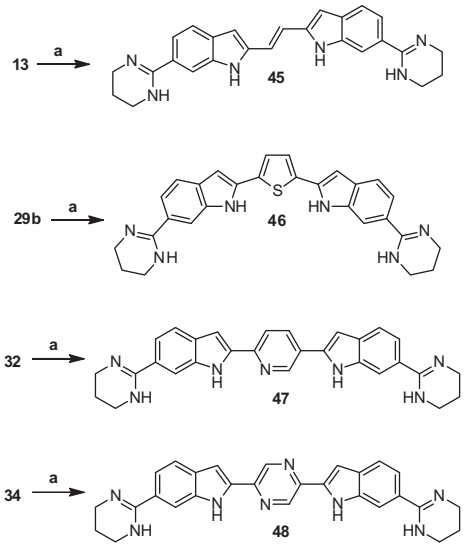
Scheme 9. Synthesis of pyridine-linked and pyrazine-linked MBX 1066 analogs. Reagents and conditions: (a) 3-bromo-6-iodopyridine, $Pd(PPh_3)_4$, aq Na_2CO_3, toluene/EtOH, reflux; (b) compound **9**, $Pd(PPh_3)_4$, aq Na_2CO_3, toluene/EtOH, 80 °C; (c) ethylenediamine, P_2S_5, 120 °C; (d) 2,5-dibromopyrazine, $Pd(PPh_3)_4$, aq Na_2CO_3, DME, reflux.



Scheme 10. Synthesis of an ethyl-linked bisamidine analog. Reagents and conditions: (a) H_2, Pd/C, EtOH; (b) 1,3-diaminopropane, P_2S_5, 120 °C.



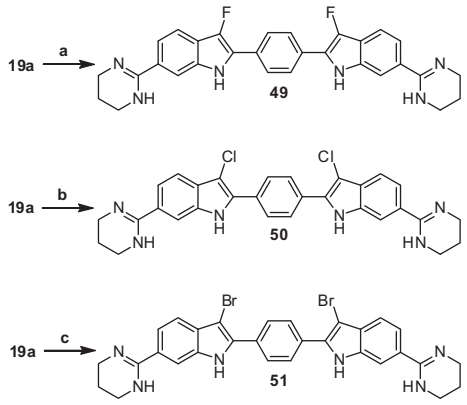
Scheme 11. Synthesis of propyl-linked and butyl-linked bisamidine analogs. Reagents and conditions: (a) pentane-1,5-bis(triphenylphosphonium) bromide or hexane-1,5-bis(triphenylphosphonium) bromide, NaHMDS, THF; (b) $P(OEt)_3$ reflux; (c) 1,3-diaminopropane, P_2S_5, 120 °C; (d) H_2, Raney Ni, MeOH.



Scheme 12. Synthesis of tetrahydropyrimidine analogs of **2**, **30b**, and **36**. Reagents and conditions: (a) 1,3-diaminopropane, P_2S_5, 120 °C.

tetrahydropyrimidine) would have similar beneficial effects using other core structures. The tetrahydropyrimidines **45**, **46**, **47**, and **48** were successfully synthesized from their corresponding dinitriles using the 1,3-diaminopropane/phosphorous pentasulfide reagent conditions[34] used to produce **19a** (Scheme 12).

Lastly, we wanted to determine what effect substituents at the 3-position of the indole would have on the antibacterial properties of the series. Indoles will undergo facile substitution at the 3-position when exposed to a variety of electrophiles; we were particularly interested in halogenation at that position, so we constructed a series of compounds bearing 3-halo substituents (Scheme 13). Although the dinitrile **5** underwent efficient halogenation with *N*-bromosuccinimide at the 3-position of both indoles, the subsequent formation of the tetrahydropyrimidine led to dehalogenation; the dehalogenated compound **19a** was the only product obtained (reaction not shown). To avoid this complication, we subsequently used **19a** itself as a substrate for the halogenation. Although the bisamidine compound **19a** (as its TFA salt) is substantially less soluble than the dinitrile **5**, it did undergo halogenation with *N*-halosuccinimides in DMF at room temperature to form the 3-chloro analog **50**, and the 3-bromo analog **51** although in moderate or low yields. The fluorination of **19a** was also accomplished by using Selectfluor™ as a halogenating agent,[47] to form the 3-fluoro analog **49**.



Scheme 13. Synthesis of 3-substituted analogs of **19a**. Reagents and conditions: (a) Selectfluor™, DMF; (b) NCS, DMF; (c) NBS, DMF.

2.2. Antibacterial activity assays (MIC)

CLSI protocols were used to determine the antibacterial activities of bisamidine compounds against representative Gram-positive and -negative pathogens.[48] Ciprofloxacin was used as a positive control. Each assay was performed at least 3 times and the average Minimum Inhibitory Concentration (MIC) value (in μg/ml) was calculated as the lowest concentration that prevented bacterial growth. Bacterial strains included: *Staphylococcus aureus*, ATCC 25923; Methicillin-resistant *S. aureus*, clinical isolate 1094; *Enterococcus faecalis*, ATCC 29212; Vancomycin-resistant *E. faecalis*, ATCC 51575; *Bacillus anthracis*, Sterne; *Pseudomonas aeruginosa*, PAO1, ATCC BAA-47; *P. aeruginosa* PAO1 ΔmexAB-oprM (efflux pump knockout); *Escherichia coli*, K12 lab strain; *E. coli*, K12 ΔTolC (efflux pump knockout); *Klebsiella pneumoniae*, ATCC 13882; *Haemophilus influenzae*, ATCC 10211; *Acinetobacter baumannii*, ATCC 19606.

3. Results and discussion

The bisamidines were tested for their antibacterial activity against a range of both Gram-positive and Gram-negative bacterial pathogens, and the results are reported in Tables 1–3. The MIC (in μg/mL) is reported for twelve pathogens (8 species) that are representative of clinically important bacteria, including drug-resistant and efflux-deficient strains. The Gram-positive pathogens we assayed include two strains of *Staphylococcus aureus* (methicillin-sensitive and methicillin-resistant), two strains of *Enterococcus faecalis* (vancomycin-sensitive and vancomycin-resistant), and *Bacillus anthracis* Sterne (an attenuated lab strain). In addition, we assayed the compounds against seven different Gram-negative pathogens, including wild-type and efflux-deficient *Pseudomonas aeruginosa*, wild-type and efflux-deficient *Escherichia coli*, and ATCC strains of *Klebsiella pneumoniae*, *Haemophilus influenzae*, and *Acinetobacter baumannii*. Again, these bacteria represent a substantial cross-section of clinically important pathogens. The efflux-deficient *P. aeruginosa* and *E. coli* strains are isogenic with their corresponding efflux-capable strains; because these strains are identical except for their ability to efflux exogenous substances, a large difference between the antibacterial activity of the compounds strongly suggests that efflux, and not other resistance mechanisms, is the reason for low antibacterial activity against the wild-type bacteria.

Several trends are evident in the resulting biological data (Tables 1–3). As expected, the compounds are generally more active against Gram-positive bacterial species than Gram-negative species, although most of the compounds do inhibit *P. aeruginosa* and *E. coli* growth in the low μg/mL range. The MIC values for *A. baumanii* are particularly high, reflecting the intrinsic resistance of the pathogen, and only a few compounds (**19a**, **30b**, **46,** and **49**–**51**) inhibit this species at levels at or below 10 μg/mL.

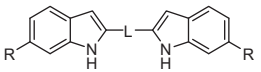
Our initial explorations into the effect of the specific amidine functionalities on the activity of the bisamidine antibiotics led us to synthesize the tetrahydropyrimidine **19a**. The antibacterial potency of this compound against Gram-positive bacteria was very similar to the imidazoline parent compound MBX 1066 (**1**, Table 1), with MIC values for Gram-positive organisms improving by a factor of about 2. Much to our surprise, however, the antibacterial activity of **19a** was much greater against several Gram-negative strains. Thus, even though **1** is a moderate inhibitor of *P. aeruginosa* (7.5 μg/mL) and *E. coli* (1.25 μg/mL), **19a** is >20-fold more potent against *P. aeruginosa* and almost 10 fold more potent against *E. coli*. Further modification of the tetrahydropyrimidine ring (i.e., compounds **19b**–**e**, **20**), however, did not further improve activity. Compound **19b** was nearly as potent as **19a** against most strains, with the exception of *H. influenzae* and *A. baumanii*. The more highly substituted analog, compound **20,** was the least active, with MIC values increasing by between 4 and >32-fold over those for **19a**.

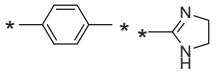
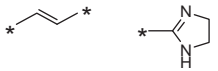
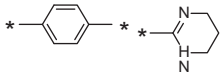
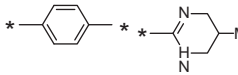
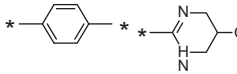
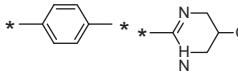
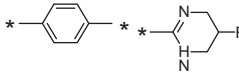
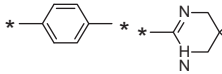
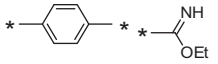
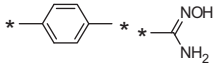
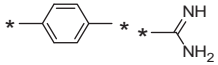
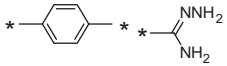
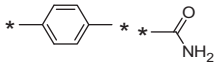
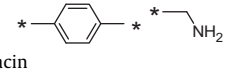
Interestingly, part of the improved activity seen against Gram-negative bacteria seems to be related to the capacity of the bacterial efflux systems to remove the compounds from intracellular compartments. A large difference can be seen between the MIC value of MBX 1066 (**1**, Table 1) against efflux-competent and efflux-deficient *P. aeruginosa* and *E. coli* strains. In this case the difference is about sixfold for the former and about eightfold for the latter. In sharp contrast, however, the potency of **19a** against either strain is completely unaffected by the presence or absence of the relevant efflux systems. This difference strongly suggests that the bacterial efflux pumps are unable to remove **19a**, while **1** is still a substrate for the transport process.

In contrast to the improvement of activity found by increasing the ring size of the substituted amidine, removing the alkyl substituent had little effect on the potency of the compound. In most cases, the MIC values of **24** were within twofold of the parent compound, MBX 1066 (**1**). One notable exception was the very potent inhibition of *E. faecalis* (0.04 μg/mL), the most potent inhibition of any bacterial strain by any bisamidine antibiotic in the current study. Interestingly, other modifications involving the amidine moiety also resulted in lower antibacterial potency. Imidate **21** and carboxamidoxime **22** both had moderate potency against Gram-positive pathogens, but were devoid of activity against the Gram-negative strains (with the exception of the efflux-defective *E. coli* strain). Carboxamidrazone **25** and aminomethyl compound **27** had moderate antibacterial activities against the assayed strains when compared to **1**, but the activity of the amide **26** was much lower. This trend strongly suggests that the most potent compounds require highly basic functionalities, such as amidines, on the ends of the molecule to exert their antibacterial effect. The less basic amine and amide functionalities of **27** and **26**, respectively, are not basic enough for the compounds to exert optimal bacterial growth inhibition.

Next, we examined the role of the central linkers in the antibacterial properties of the bisamidines (Table 2). Given that our initial compounds, MBX 1066 (**1**) and MBX 1090 (**2**) had two distinct linker types (phenyl and ethylene, respectively), it was reasonable to surmise that other linkers would be tolerated. Substituting other aromatic species such as furan (**30a**), thiophene (**30b**), pyridine (**33**), or pyrazine (**35**) for the phenyl linker produced compounds with a range of activities. The thiophene-linked compound **30b** was nearly as potent against most strains as MBX 1066, and was more potent against *A. baumanii*. This is not surprising given the structural similarities between phenyl rings and thiophenes. Interestingly, unlike **19a**, thiophene **30b** was subject to efflux, as determined by the activity against efflux-efficient and efflux-deficient *P. aeruginosa*.

Table 1
Antibacterial properties of bisamidines with modification of the amidine moiety



Compound	L	R	MIC[a] (μg/mL)											
			Staphylococcus aureus[b]	*Staphylococcus aureus* (MRSA)[b]	*Enterococcus faecalis*[b]	*Enterococcus faecalis* (VRE)[b]	*Bacillus anthracis*[b]	*Pseudomonas aeruginosa* (efflux +)[b]	*Pseudomonas aeruginosa* (efflux −)[b]	*Escherichia coli* (efflux +)[b]	*Escherichia coli* (efflux −)[b]	*Klebsiella pneumoniae*[b]	*Haemophilus influenzae*[b]	*Acinetobacter baumanii*[b]
1			0.16	0.16	0.16	0.16	0.31	7.5	1.25	1.25	0.16	0.31	0.63	>80
2			0.63	0.63	0.31	0.63	0.31	25	25	0.63	0.16	1.25	2.5	>80
19a			0.08	0.08	0.08	0.08	0.08	0.31	0.31	0.16	0.16	0.31	0.31	2.5
19b			0.08	0.08	0.16	0.08	0.08	0.63	0.63	0.31	0.16	0.31	1.25	40
19c			0.31	0.31	0.16	0.63	0.16	1.25	0.31	1.25	0.63	0.63	1.25	>80
19d			0.16	0.16	0.16	0.16	0.16	5	2.5	5	0.31	0.63	N.D.[c]	>80
19e			0.08	0.16	0.16	0.08	0.08	1.25	0.31	0.63	0.31	0.31	N.D.[c]	40
20			0.31	0.31	0.31	0.63	0.31	10	40	0.63	0.63	0.63	2.5	>80
21			0.63	5	2.5	10	2.5	>80	>80	N.D.[c]	N.D.[c]	>80	N.D.[c]	N.D.[c]
22			0.31	1.25	2.5	1.25	0.16	>80	>80	>80	0.63	>80	N.D.[c]	N.D.[c]
24			0.16	0.31	0.04	0.16	0.08	10	N.D.[c]	0.63	0.16	0.63	N.D.[c]	40
25			0.63	1.25	0.63	0.63	0.31	10	N.D.[c]	2.5	0.63	1.25	N.D.[c]	>80
26			2.5	>80	10	>80	>80	>80	>80	>80	5	>80	N.D.[c]	>80
27			0.63	1.25	1.25	1.25	1.25	40	40	5	1.25	2.5	N.D.[c]	>80
Ciprofloxacin			0.31	20	1.25	0.63	0.08	0.63	⩽0.02	⩽0.02	⩽0.02	0.04	10	1.25

[a] Lowest concentration that prevented bacterial growth.
[b] Bacterial strains: *Staphylococcus aureus*, ATCC 25923; Methicillin-resistant *S. aureus*, clinical isolate 1094; *Enterococcus faecalis*, ATCC 29212; Vancomycin-resistant *E. faecalis*, ATCC 51575; *Bacillus anthracis*, Sterne; *Pseudomonas aeruginosa*, PAO1, ATCC BAA-47; *P. aeruginosa* PAO1 ΔmexAB-oprM (efflux pump knockout); *Escherichia coli*, K12 lab strain; *E. coli*, K12 ΔTolC efflux knockout; *Klebsiella pneumoniae*, ATCC 13882; *Haemophilus influenzae*, ATCC 10211; *Acinetobacter baumannii*, ATCC 19606.
[c] Not determined.

In contrast to the potency of thiophene-linked compound **30b**, the furan-linked bisamidine **30a** had very little antibacterial potency, and pyrazine-linked compound **36** had greatly attenuated activity. Pyridine-linked compound **33** showed an intermediate level of antibacterial activity, but was clearly more potent against Gram-positive bacteria than the Gram-negative strains we tested. We are unable at this time to completely rationalize these findings; there appears, however, to be a correlation between the DNA-binding properties of these compounds, which is dependent on their structure, and antibacterial activity when efflux pumps have been inactivated. For example, the furan-linked compound **30** exhibits low affinity for DNA in a binding assay and has very little antibacterial activity, while compounds **1** and **2** have a higher binding affinity for DNA and are much more potent as antibacterial agents.[14]

Having determined that substituting the imidazoline tail units with tetrahydropyrimidines was beneficial for antibacterial activity, we chose to synthesize tetrahydropyrimidine analogs of several more compounds to determine whether the trend was general, or specific to one core structure. By comparing the antibacterial activity of five imidazoline-based compounds (i.e., **1**, **2**, **30b**, **34**, **36**) to the corresponding tetrahydropyrimidine-based compounds (i.e., **19a**, **45**, **46**, **47**, **48**), we determined that there was general improvement of antibacterial potency. Only imidazoline **2** showed a degree of mixed results; it was more potent than the tetrahydropyrimidine **45** against four bacterial species. The difference was not large however, and the corresponding tetrahydropyrimidine was much more potent against *P. aeruginosa* and somewhat more potent against seven other strains. Given these findings, subsequent compounds were synthesized using tetrahydropyrimidine tail units.

The next series of compounds, containing saturated alkyl linkers (i.e., **37**, **44a**, **44b**; Table 2), also showed a range of activity based on their structure. Interestingly, compounds **37** and **44b**, which have even-numbered carbon linkers, were substantially more potent than **44a**, which has an odd number of carbons in the linker; the cause of this difference might also be related to the ability of the compounds to adopt a confirmation that favors DNA binding. Because none of these compounds were as potent as the corresponding phenyl-linked compound **19a**, no attempt was made to rigorously prove this hypothesis.

Lastly, compounds that are halogenated in the 3-position of the indole all retained most of their antibacterial activity (Table 3). The 3-chloro compound **50** was the most potent overall, although the potency against *H. influenzae* was poor. Compound **50** was, however, slightly more potent against *S. aureus* and *A. baumanii*.

All of the above bisamidine compounds can be converted to their corresponding acid salt forms. In general, trifluoroacetic salts are more soluble in DMSO and other organic solvents, while acetic acid salts have higher aqueous solubility. Hydrochloric acid salts tend to have lower solubility in either aqueous or organic solvent systems. The solubility of the free bases varies greatly with the structure of the molecule; those compounds that have aromatic linkers have much lower solubility than those with more flexible linkers. Regardless of the salt form, the biological activity of the compounds is consistent for all compounds (data not shown) to the limits of solubility. Because the salt form does not affect the biological activity of the compounds, we have omitted the salts from the schemes and tables. The experimental section does, however, reference the salt forms, if applicable.

4. Conclusion

Beginning from a high-throughput screen of antibacterial compounds, we identified four related compounds that were potent inhibitors of bacterial DNA replication. We devised scalable syntheses for two of the compounds and rigorously characterized their antibacterial spectrum. We further explored the structure to determine important features with respect to antibacterial activity. Basic moieties, such as amidines, are required on each end of the molecule, and tetrahydropyrimidines are preferred to imidazolines. Altering the linker portion of the molecule produced widely varying results; molecules containing phenyl, thiophene, or alkene linkers were more potent, while molecules containing furan, pyrazine, and alkyl linkers were, on the whole, less potent. Finally, addition of halogens at the 3-position of the indoles slightly decreased activity in the case of fluorine, chlorine, and bromine, and more significantly in the case of iodine. Further investigation of the compounds, including in vivo efficacy against a wider range of pathogens, is underway.

5. Experimental

5.1. Biology

5.1.1. Determination of minimum inhibitory concentration (MIC)

MICs were determined by the broth microdilution method outlined in the CLSI (formerly NCCLS) guidelines.[48] Briefly, log-phase bacterial cultures were grown in cation-adjusted Mueller–Hinton broth (MHB). Diluted bacterial cultures from log-phase growth were seeded into 96-well plates at a concentration of 1×10^5 CFU/mL, and plates were incubated at 37 °C for 16–20 h with shaking (or, in the case of *H. influenza*, Haemophilus Test Medium and 37 °C, 5% CO_2 was used). Test compound stock solutions in DMSO were diluted into media, to achieve a final concentration of 1% DMSO. Cell growth was determined by measuring optical density (600 nm) in a microplate reader (Dynex Technologies, Chantilly, VA). MIC values for antimicrobial compound-treated cultures were calculated as the lowest concentration of drug at which growth was not apparent, as measured by optical density at 600 nm.

5.2. Chemistry

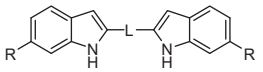
5.2.1. General

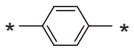
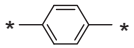
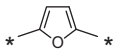
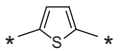
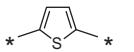
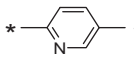
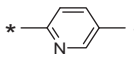
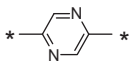
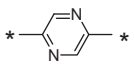
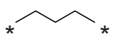
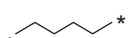
Reagents and solvents were obtained from commercial sources and used without additional purification. Evaporation of solvents was accomplished under reduced pressure (40–60 mmHg), at less than 40 °C, unless otherwise noted. Chromatography solvent systems are expressed in v/v ratios or as% vol. Melting points were taken on EZ-Melt automated melting point apparatus (Stanford Research Systems, Inc.) in manual mode, and are uncorrected. Thin-layer chromatography was performed on silica gel GHLF plates from Analtech (Newark, DE), and the chromatograms were visualized under UV light at 254 nm. ^{1}H NMR spectra were obtained at 300 MHz on a Bruker DPX300 spectrometer; chemical shift values for ^{1}H were determined relative to an internal tetramethylsilane standard (0.00 ppm). Mass spectrometry was performed by CreaGen Biosciences (Woburn, MA). Analytical HPLC was performed by Averica Discovery Services (Marlborough, MA). All target compounds were found to be $\geqslant$95% pure by analytical HPLC unless otherwise noted.

5.2.2. 1,4-Bis(4-cyano-2-nitrostyryl)benzene (11)

To a 3-L flask fitted with a reflux condenser charged with 4-cyano-2-nitrotoluene (**12**; 200 g, 1.24 mol), terephthaldehyde (83.0 g, 0.62 mol), and sulfolane (500 mL) was added piperidine (53.0 g, 0.63 mol). The resulting mixture was heated at 150 °C for 1 h, forming an orange precipitate. The reaction was cooled to 20 °C, and diluted with $CHCl_3$ (1 L). The mixture was stirred for 30 min at 20 °C, then the precipitate was filtered and rinsed with additional $CHCl_3$ (600 mL). The resulting solids were dried to

Table 2
Antibacterial properties of bisamidines with modified linkers and tetrahydropyrimidines



Compound	L	R	MIC[a] (μg/mL)											
			Staphylococcus aureus[b]	*Staphylococcus aureus* (MRSA)[b]	*Enterococcus faecalis*[b]	*Enterococcus faecalis* (VRE)[b]	*Bacillus anthracis*[b]	*Pseudomonas aeruginosa* (efflux +)[b]	*Pseudomonas aeruginosa* (efflux −)[b]	*Escherichia coli* (efflux +)[b]	*Escherichia coli* (efflux −)[b]	*Klebsiella pneumoniae*[b]	*Haemophilus influenzae*[b]	*Acinetobacter baumanii*[b]
1			0.16	0.16	0.16	0.16	0.31	7.5	1.25	1.25	0.16	0.31	0.63	>80
19a			0.08	0.08	0.08	0.08	0.08	0.31	0.31	0.16	0.16	0.31	0.31	2.5
2			0.63	0.63	0.31	0.63	0.31	25	25	0.63	0.16	1.25	2.5	>80
45			0.31	0.31	1.25	1.25	0.16	2.5	2.5	1.25	0.16	0.63	5	20
30a			40	40	>80	>80	5	>80	>80	20	0.63	>80	N.D.[c]	>80
30b			0.16	0.31	1.25	1.25	0.16	10	1.25	0.63	0.31	0.63	0.63	10
46			0.08	0.16	0.16	0.16	0.16	5	1.25	0.63	0.31	0.63	N.D.[c]	5
33			0.31	0.31	0.31	0.31	0.31	>80	>80	2.5	0.63	2.5	N.D.[c]	>80
47			0.16	0.16	0.63	0.16	0.16	2.5	2.5	0.63	0.16	5	2.5	20
35			5	5	20	10	2.5	>80	>80	20	0.63	10	N.D.[c]	>80
48			0.31	0.31	0.31	0.31	0.16	5	5	2.5	0.31	1.25	N.D.[c]	>80
37			0.63	1.25	5	2.5	0.63	10	10	2.5	0.31	N.D.[c]	N.D.[c]	80
44a			20	20	80	0.63	20	>80	>80	80	1.25	N.D.[c]	N.D.[c]	>80
44b			0.63	1.25	2.5	0.31	1.25	40	40	1.25	0.16	N.D.[c]	N.D.[c]	>80
Ciprofloxacin			0.31	20	1.25	0.63	0.08	0.63	⩽0.02	⩽0.02	⩽0.02	0.04	10	1.25

[a] Lowest concentration that prevented bacterial growth.
[b] Bacterial strains: *Staphylococcus aureus*, ATCC 25923; Methicillin-resistant *S. aureus*, clinical isolate 1094; *Enterococcus faecalis*, ATCC 29212; Vancomycin-resistant *E. faecalis*, ATCC 51575; *Bacillus anthracis*, Sterne; *Pseudomonas aeruginosa*, PAO1, ATCC BAA-47; *P. aeruginosa* PAO1 ΔmexAB-oprM (efflux pump knockout); *Escherichia coli*, K12 lab strain; *E. coli*, K12 ΔTolC efflux knockout; *Klebsiella pneumoniae*, ATCC 13882; *Haemophilus influenzae*, ATCC 10211; *Acinetobacter baumannii*, ATCC 19606.
[c] Not determined.

Table 3
Antibacterial properties of bisamidines with 3-substituents

Compound	X	MIC[a] (μg/mL)										
		Staphylococcus aureus[b]	*Staphylococcus aureus* (MRSA)[b]	*Enterococcus faecalis*[b]	*Enterococcus faecalis* (VRE)[b]	*Bacillus anthracis*[b]	*Pseudomonas aeruginosa* (efflux +)[b]	*Pseudomonas aeruginosa* (efflux −)[b]	*Escherichia coli* (efflux +)[b]	*Escherichia coli* (efflux −)[b]	*Klebsiella pneumoniae*[b]	*Haemophilus influenzae*[b]	*Acinetobacter baumanii*[b]
19a	H	0.08	0.08	0.08	0.08	0.08	0.31	0.31	0.16	0.16	0.31	0.31	2.5
49	F	0.16	0.08	0.08	0.16	0.08	2.5	1.25	0.63	0.63	0.63	5	2.5
50	Cl	0.04	0.04	0.63	0.63	0.08	1.25	1.25	0.63	0.63	0.63	>80	1.25
51	Br	0.08	0.04	1.25	0.63	0.08	1.25	1.25	0.63	0.63	N.D.[c]	N.D.[c]	1.25
Ciprofloxacin		0.31	20	1.25	0.63	0.08	0.63	⩽0.02	⩽0.02	⩽0.02	0.04	10	1.25

[a] Lowest concentration that prevented bacterial growth.
[b] Bacterial strains: *Staphylococcus aureus*, ATCC 25923; Methicillin-resistant *S. aureus*, clinical isolate 1094; *Enterococcus faecalis*, ATCC 29212; Vancomycin-resistant *E. faecalis*, ATCC 51575; *Bacillus anthracis*, Sterne; *Pseudomonas aeruginosa*, PAO1, ATCC BAA-47; *P. aeruginosa* PAO1 ΔmexAB-oprM (efflux pump knockout); *Escherichia coli*, K12 lab strain; *E. coli*, K12 ΔTolC efflux knockout; *Klebsiella pneumoniae*, ATCC 13882; *Haemophilus influenzae*, ATCC 10211; *Acinetobacter baumannii*, ATCC 19606.
[c] Not determined.

constant weight to yield 207 g (79%) of product as an orange powder: mp >300 °C; compound is too insoluble for TLC or NMR.

5.2.3. 1,4-Bis(6-cyanoindol-2-yl)benzene (5)

To a 3-L flask fitted with a reflux condenser charged with 1,4-bis(4-cyano-2-nitrostyryl)benzene (**11**; 165 g, 0.39 mol) was added triethyl phosphite (1.5 L). The mixture was heated to reflux for 2 days, during which time the orange suspension turned to a gold-yellow suspension. The reaction was cooled to 40–50 °C, and chloroform (800 mL) was added. The suspension was stirred for 30 min at 20 °C, then the solids were filtered and rinsed with additional $CHCl_3$ (400 mL). The resulting solids were dried to constant weight to yield 93 g (66%) of product as gold-yellow powder: mp >300 °C; R_f 0.52 (1:1 hexane/EtOAc); ^{1}H NMR (DMSO-d_6): δ 12.24 (s, 2H), 8.06 (s, 4H), 7.88 (s, 2H), 7.73 (d, 2H), 7.35 (d, 2H), 7.18(s, 2H).

5.2.4. 1,4-Bis[6-(4,5-dihydro-1*H*-imidazol-2-yl)indol-2-yl] benzene (1; MBX 1066; as trifluoroacetic acid salt)

To a suspension of 1,4-bis(6-cyanoindole-2-yl)benzene (**5**; 1.79 g, 5.0 mmol) in ethylenediamine (25 mL) was added phosphorous pentasulfide (360 mg, 1.6 mmol). The suspension was heated in a sealed vessel to 130 °C (oil bath) for 16 h, then cooled to room temperature and poured into water (300 mL). The resulting yellow suspension was stirred for 30 min, then filtered. The solids were rinsed with water (200 mL), then dried under vacuum to yield a yellow powder. The powder was dissolved in trifluoroacetic acid (100 mL) and MeOH (100 mL) with heating. The dark yellow solution was dissolved rapidly with Et_2O (500 mL). The resulting solids were filtered, rinsed with Et_2O (100 mL), and dried to provide 3.13 g (97%) of product as a yellow powder: mp >300 °C; R_f 0.04 (80:18:2 $CHCl_3$:MeOH:aq $MeNH_2$); ^{1}H NMR (DMSO-d_6) δ 12.40 (s, 2H), 10.42 (s, 2H), 8.13 (s, 4H), 8.07 (s, 2H), 7.80 (d, 2H), 7.58 (d, 2H), 7.23 (s, 2H), 4.03 (s, 8H); *m*/*z* expected 444.2, found 445.6 $(M+H)^+$.

5.2.5. Ethyl 3-(4-cyano-2-nitrophenyl)-2-oxopropionate (16)

Sodium metal (6.0 g, 260 mmol) was dissolved completely in absolute ethanol (300 mL), then 4-cyano-2-nitrotoluene (**12**; 15.0 g, 93 mmol) and diethyl oxalate (100 mL, 740 mmol) were added. The resulting dark solution was stirred at room temperature for 16 h. The ethanol was then removed under vacuum, and the residual dark oil was dissolved in EtOAc (300 mL). The dark solution was washed with 0.5 M aqueous HCl (400 mL) and brine (100 mL), then dried over $MgSO_4$, filtered, and evaporated. The residual diethyl oxalate was then distilled (kugelrohr; 10 mmHg, 120 °C), and the remaining syrup adsorbed onto silica gel (800 mL unpacked) by evaporation from CH_2Cl_2. The product was eluted with $CHCl_3$ (3 L) until no more product was obtained. The filtrate was then evaporated and the crude material recrystallized from boiling $CHCl_3$/hexane to provide 22.05 g (91%) of product as a pale yellow solid as a mixture of enol and keto tautomers: mp 142–144 °C; R_f 0.24 (2:1 hexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 11.0–10.6 (s, br, 0.7H), 8.64 (d, 0.3H), 8.50 (d, 0.7H), 8.38 (d, 0.7H), 8.24 (dd, 0.3H), 8.15 (dd, 0.7H), 7.75 (d, 0.3H), 6.60 (s, 0.7H), 4.70 (s, 0.6H), 4.34–4.27 (overlapping q, 2H), 1.34–1.28 (overlapping t, 3H).

5.2.6. Ethyl 6-cyanoindole-2-carboxylate (17)

To a solution of ethyl 3-(4-cyano-2-nitrophenyl)-2-oxopropanoate (**16**; 21.90 g, 89 mmol) in glacial acetic acid (350 mL and water (50 mL) was added zinc dust (100 g, 1.5 mol). The mixture was stirred for 25 min, and the clumps of Zn metal were broken up as the reaction proceeded. The hot AcOH solution was filtered through Celite, and the solids were rinsed with hot glacial acetic acid (100 mL). The filtrate was evaporated to ~250 mL total volume and diluted with water (500 mL). The resulting solids were

filtered, rinsed with water (100 mL), and dried, then recrystallized from $CHCl_3$/hexane to provide 13.27 g (70%) of product as a pale yellow solid: mp 175–176 °C; R_f 0.56 (2:1 hexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 12.44 (s, 1H), 7.90 (s, 1H), 7.86 (d, 1H), 7.41 (dd, 1H), 7.27 (d, 1H), 4.38 (q, 2H), 1.36 (t, 3H).

5.2.7. 2-Hydroxymethylindole-2-carbonitrile (18)

To a solution of ethyl 6-cyanoindole-2-carboxylate (**17**; 14.0 g, 65 mmol) in THF (300 mL) was added a solution of lithium borohydride in THF (2.0 M, 75 mL, 150 mmol). The resulting solution was heated to reflux for 16 h, then cooled to room temperature. Concentrated aqueous HCl was added in ~1 mL portions until no more vigorous reaction occurs upon addition, and pH was ~7 (paper). The reaction was diluted with brine (300 mL), and extracted with EtOAc (200 mL × 2). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to provide a tacky yellow solid. The solid was recrystallized from boiling $CHCl_3$/MeOH to provide 6.75 g (60%) of product as a pale yellow, crystalline solid: mp 177–179 °C; R_f 0.42 (10% MeOH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 11.63 (s, 1H), 7.79 (d, 1H), 7.64 (d, 1H), 7.29 (dd, 1H), 6.44 (s, 1H), 5.45 (s, 1H), 4.67 (d, 2H).

5.2.8. 6-Cyanoindole-2-carboxaldhyde (14)

To a solution of 2-hydroxymethylindole-2-carbonitrile (**18**; 2.10 g, 12.2 mmol) in acetone (50 mL) was added manganese dioxide (3.15 g, 36 mmol). The resulting suspension was stirred for 16 h at room temperature, then additional manganese dioxide (1.05 g, 12 mmol) was added, and the reaction stirred an additional 24 h. The mixture was then filtered through Celite to remove the remaining solids, and the solids were washed with acetone (20 mL). The filtrate was evaporated and the resulting crude material was adsorbed onto silica gel (~100 mL unpacked) by evaporation from CH_2Cl_2. The product was eluted with EtOAc (250 mL) until no more product was obtained. The filtrate was then evaporated and the crude material recrystallized from boiling CH_2Cl_2/hexane to provide 1.14 g (55%) of olive-colored powder: R_f 0.45 (2:1 hexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 12.50 (s, 1H), 9.97 (s, 1H), 7.96–7.91 (m, 2H), 7.52–7.42 (m, 2H).

5.2.9. (*E*)-1,2-Bis(6-cyanoindol-2-yl)ethylene (13)

To a suspension of titanium(III) chloride (4.58 g, 29.7 mmol) in dry DME (75 mL) was added lithium wire (630 mg, 90.8 mmol), broken into small pieces. The suspension was heated to reflux (120 °C oil bath) with very vigorous stirring for 2 h. The reaction was cooled to just below the boiling point, then 6-cyanoindole-2-carboxaldhyde (**14**, 1.00 g, 5.9 mmol) was added in one portion. The reaction was heated to reflux (120 °C oil bath) for 10 min, until TLC showed complete consumption of starting material. The reaction was then cooled to room temperature and poured into ice water (800 mL), and stirred until any residual Li metal was consumed. To the aqueous suspension was added EtOAc (800 mL) and Celite (250 mL). The mixture was filtered through Celite, and the remaining solids were washed with EtOAc (200 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated to yield a yellow-orange solid. The solid was dissolved in CH_2Cl_2 and adsorbed onto silica gel (200 mL) by evaporation. The product was eluted through additional silica gel (200 mL) with EtOAc until no more product was obtained. The filtrate was evaporated to yield 0.80 g (88%) of dark yellow powder: mp >300 °C; R_f 0.29 (2:1 hexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 12.08 (s, 2H), 7.83 (s, 2H), 7.70 (d, 2H), 7.40 (s, 2H) 7.34 (d, 2H), 6.80 (s, 2H).

5.2.10. (*E*)-1,2-Bis[6-(4,5-dihydro-1*H*-imidazol-2-yl)indol-2-yl] ethylene (2; MBX 1090; as trifluoroacetic acid salt)

To a solution of (*E*)-1,2-bis(6-cyanoindol-2-yl)ethylene (**13**; 616 mg, 2.0 mmol) in ethylenediamine (10 mL) was added phosphorous pentasulfide (120 mg, 0.54 mmol). The resulting red-orange solution was heated to 130 °C in a sealed tube for 2 h, then cooled to room temperature and poured into water (200 mL). The suspension was stirred vigorously for 15 min, then refrigerated overnight. The resulting solids were filtered, rinsed with cold water (50 mL), and dried to provide an orange-brown solid. The solid was dissolved in trifluoroacetic acid (10 mL), and MeOH (10 mL). Et_2O (150 mL) was added, and the mixture was allowed to stand 2 h. The solids were filtered, rinsed with Et_2O (100 mL), and dried to provide 739 mg (60%) of yellow-orange powder: mp >300 °C; R_f 0.14 (80:18:2 $CHCl_3$:MeOH:aq $MeNH_2$); ^{1}H NMR (DMSO-d_6) δ 12.54 (s, 2H), 10.40 (s, 4H), 8.01 (s, 2H), 7.76 (d, 2H), 7.55 (d, 2H), 7.47 (s, 2H), 6.83 (s, 2H), 4.03 (s, 8H); *m*/*z* expected 394.2, found 395.6 $(M+H)^+$.

5.2.11. 1,4-Bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl] benzene (19a; as trifluoroacetic acid salt)

To a suspension of 1,4-bis(6-cyanoindole-2-yl)benzene (**5**; 717 mg, 2.0 mmol) in 1,3-diaminopropane (25 mL) was added phosphorous pentasulfide (120 mg, 0.54 mmol). The suspension was heated in a sealed vessel to 120 °C (oil bath) for 2.5 h, then cooled to room temperature and poured into water (200 mL). The resulting yellow suspension was stirred for 30 min, then filtered. The solids were rinsed with water (100 mL), then dried under vacuum to yield a yellow powder. The powder was dissolved in trifluoroacetic acid (10 mL) and MeOH (10 mL) with heating. The yellow solution was diluted rapidly with Et_2O (150 mL). The resulting solids were filtered, rinsed with Et_2O (50 mL), and dried to provide 1.27 g (98%) of product as a yellow powder: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.30 (s, 2H), 9.85 (s, 4H), 8.10 (s, 4H), 7.81 (s, 2H), 7.76 (d, 2H), 7.35 (d, 2H), 7.18 (s, 2H), 3.53 (s, 8H), 2.02 (s, 4H); *m*/*z* expected 472.2, found 237.4 $(M+2H^+)/2$.

5.2.12. 1,4-Bis[6-(5-methyl-3,4,5,6-tetrahydropyrimidin-2-yl) indol-2-yl]benzene (19b; as trifluoroacetic acid salt)

To a suspension of 1,4-bis(6-cyanoindole-2-yl)benzene (**5**; 200 mg, 0.56 mmol) in 2-methyl-1,3-diaminopropane (1 mL) was added phosphorous pentasulfide (70 mg, 0.32 mmol). The suspension was heated in a sealed vessel to 120 °C (oil bath) for 3 h, then cooled to room temperature and poured into cold water (5 mL). The resulting yellow suspension was stirred for 30 min, then filtered. The solids were rinsed with water (1 mL), and EtOAc (1 mL) then dried under vacuum to yield a yellow powder. The powder was subjected to preparative HPLC purification with a gradient of 0–50% CH_3CN/H_2O, each containing 0.2% TFA. Product-containing fractions were pooled and allowed to stand 4 days, after which time the product had precipitated. The yellow solid was filtered any dried to provide 140 mg (34%) of product as a yellow, microcrystalline solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.26 (s, 2H), 9.88 (s, 4H), 8.20 (s, 4H), 7.81 (s, 2 h), 7.76 (d, 2H), 7.35 (d, 2H), 7.20 (s, 2H), 3.58 (m, 4H), 3.15 (m, 4H), 2.18 (m, 2H), 1.07 (d, 6H); *m*/*z* expected 500.3, found 251.6 $(M+2H^+)/2$.

5.2.13. 1,4-Bis[6-(5-hydroxy-3,4,5,6-tetrahydropyrimidin-2-yl) indol-2-yl]benzene (19c; as trifluoroacetic acid salt)

To a suspension of 1,4-bis(6-cyanoindole-2-yl)benzene (**5**; 360 mg, 1.0 mmol) in 1,3-diamino-2-propanol (5 mL) was added phosphorous pentasulfide (60 mg, 0.27 mmol). The suspension was heated in a sealed vessel to 120 °C (oil bath) for 24 h, then cooled to room temperature and poured into water (100 mL). The resulting yellow suspension was stirred for 30 min, then filtered. The solids were rinsed with water (20 mL), then dried under vacuum to yield a yellow powder. The powder was dissolved in trifluoroacetic acid (5 mL) and MeOH (5 mL) with heating. The yellow solution was diluted rapidly with Et_2O (100 mL). The resulting solids were filtered, rinsed with Et_2O (20 mL), and dried to provide

652 mg (89%) of product as a yellow powder: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.28 (s, 2H), 9.80 (s, 4H), 8.10 (s, 4H), 7.81 (s, 2H), 7.78 (d, 2H), 7.35 (d, 2H), 7.20 (s, 2H), 5.62 (s, 2H), 4.28 (s, 2H), 3.59 (d, 4H), 3.40 (d, 4H); *m*/*z* expected 504.2, found 253.3 (M+2H$^+$)/2.

5.2.14. 1,4-Bis[6-(5-methoxy-3,4,5,6-tetrahydropyrimidin-2-yl) indol-2-yl]benzene (19d; as trifluoroacetic acid salt)

To a suspension of 1,4-bis(6-cyanoindole-2-yl)benzene (**5**; 110 mg, 0.31 mmol) in 1,3-diamino-2-methoxypropane (1.0 g) was added phosphorous pentasulfide (64 mg, 0.29 mmol). The suspension was heated in a sealed vessel to 130 °C (oil bath) for 4 h, then cooled to room temperature. Water (10 mL) was added, then the suspension was stirred for 16 h and filtered. The solid was rinsed with water (20 mL), then dried under vacuum to provide 164 mg (100%) of product as yellow powder: mp >300 °C; ^{1}H NMR (DMSO+1% TFA-d) δ 12.25 (br s, 2H), 9.81 (br s, 4H), 8.08 (s, 4H), 7.79 (s, 2H), 7.76 (d, 2H), 7.33 (dd, 2H), 7.18 (s, 1H), 4.00 (m, 2H), 3.61 (m, 8H), 3.38 (s, 6H); *m*/*z* expected 532.3, found 267.7 (M+2H$^+$)/2.

5.2.15. 1,4-Bis[6-(5-fluoro-3,4,5,6-tetrahydropyrimidin-2-yl) indol-2-yl]benzene (19e; as trifluoroacetic acid salt)

To a suspension of 1,4-bis(6-cyanoindole-2-yl)benzene (**5**; 100 mg, 0.28 mmol) and 1,3-diamino-2-fluoropropane (240 mg) in triethylamine (1.5 mL) was added phosphorous pentasulfide (80 mg, 0.36 mmol). The suspension was heated in a sealed vessel to 130 °C (oil bath) for 1 h, then cooled to room temperature. Water (10 mL) was added, and the resulting yellow suspension was stirred for 30 min, then filtered and evaporated. The resulting solid was subjected to preparative HPLC purification with a gradient of 10–100% CH_3CN/H_2O, each containing 0.2% TFA. Product-containing fractions were pooled, and the CH_3CN removed under vacuum. The remaining aqueous solution was lyophilized to provide 36 mg (16%) of product as yellow powder: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.30 (br s, 2H), 10.04 (br s, 4H), 8.10 (s, 4H), 7.83 (s, 2H), 7.79 (d, 2H), 7.37 (d, 2H), 7.21 (s, 2H), 5.48 (d, 2H), 3.83–3.67 (m, 8H); *m*/*z* expected 508.2, found 255.4 (M+2H$^+$)/2.

5.2.16. 1,4-Bis[6-(5,5-dimethyl-3,4,5,6-tetrahydropyrimidin-2-yl) indol-2-yl]benzene (20; as trifluoroacetic acid salt)

To a suspension of 1,4-bis(6-cyanoindole-2-yl)benzene (**5**; 360 mg, 1.0 mmol) in 2,2-dimethyl-1,3-diaminopropane (5 mL) was added phosphorous pentasulfide (60 mg, 0.27 mmol). The suspension was heated in a sealed vessel to 120 °C (oil bath) for 4 h, then cooled to room temperature and poured into water (100 mL). The resulting yellow suspension was stirred for 30 min, then filtered. The solids were rinsed with water (20 mL), then dried under vacuum to yield a yellow-brown powder. The powder was dissolved in trifluoroacetic acid (5 mL) and MeOH (5 mL) with heating. The yellow solution was diluted rapidly with Et_2O (100 mL). The resulting solids were filtered, rinsed with Et_2O (20 mL), and dried to provide 644 mg (85%) of product as a orange powder: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.26 (s, 2H), 9.94 (s, 4H), 8.10 (s, 4H), 7.82 (s, 2H), 7.78 (d, 2H), 7.37 (d, 2H), 7.20 (s, 2H), 3.25 (s, 8H), 1.08 (s, 12H); *m*/*z* expected 528.3, found 265.6 (M+2H$^+$)/2.

5.2.17. 1,4-Bis{6-[ethoxy(imino)methyl]indol-2-yl}benzene (21; as hydrochloric acid salt)

1,4-Bis(6-cyanoindole-2-yl)benzene (**5**; 210 mg, 0.5860 mmol) was suspended in dry ethanol (200 mL) and treated with HCl gas at 0 °C for 30 min. The resulting mixture was heated to 50 °C (oil bath) in a sealed tube 4 days. The ethanol and HCl were then evaporated in vacuo to provide 306 mg (100%) of product as a dark green solid (79% purity by analytical HPLC): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.54 (s, 2H), 11.64 (s, 2H), 10.87 (s, 2H), 8.23 (s, 2H), 8.15 (s, 4H), 7.77 (d, 4H), 7.25 (s, 2H), 4.65 (q, 4H), 1.54 (t, 6H); *m*/*z* expected 450.2, found 451.5 (M+H)$^+$.

5.2.18. 1,4-Bis[6-(*N*′-hydroxycarbamimidoyl)indol-2-yl]benzene (22)

To a suspension of 1,4-bis{6-[ethoxy(imino)methyl]indol-2-yl}benzene (**21**; 160 mg, 0.31 mmol) in EtOH (20 mL) was added a solution of hydroxylamine hydrochloride (200 mg, 2.9 mmol) and sodium hydroxide, (1.0 M aqueous; 3.5 mL, 3.5 mmol) in EtOH, 2 mL). The reaction mixture was stirred at room temperature for 3 days, then poured into cold water (100 mL). The mixture was stirred for 30 min, then the solids were filtered, rinsed with water (20 mL), and dried to provide 123 mg (95%) of product as a brown powder (94% purity by analytical HPLC): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 11.67 (s, 2H), 9.50 (s, 2H), 7.98 (s, 4H), 7.71 (s, 2H), 7.50 (s, br, 2H), 7.38 (s, br, 2H), 7.00 (s, 2H), 5.77 (s, 4H); *m*/*z* expected 424.2, found 425.6 (M+H)$^+$.

5.2.19. 1,4-Bis[6-(*N*′-acetoxycarbamimidoyl)indol-2-yl]benzene (23)

To a solution of 1,4-bis[6-(*N*′-hydroxycarbamimidoyl)indol-2-yl]benzene (**22**; 191 mg, 0.45 mmol) in acetic acid (4.0 mL) was added acetic anhydride (160 μL, 1.7 mmol). The mixture was stirred at room temperature for 20 h, then the solvent was removed under reduced pressure to provide 282 mg (100%) of product as a yellow solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 11.96 (s, 2H), 11.80 (s, 2H), 8.01 (s, 4H), 7.80 (s, 2H), 7.59 (d, 2H), 7.38 (d, 2H), 7.06 (s, 2H), 6.75 (s, 4H), 2.16 (s, 6H), 1.91 (s, 6H).

5.2.20. 1,4-Bis[6-(carbamimidoyl)indol-2-yl]benzene (24; as hydrochloric acid salt)

To a suspension of 1,4-bis[6-(*N*′-acetoxycarbamimidoyl)indol-2-yl]benzene (**23**; 78 mg, 0.15 mmol) in a 3:1 solution of EtOH/acetic acid (8 mL), was added 5% palladium on carbon (10 mg). The mixture was hydrogenated at 45 psi in a Parr apparatus for 3 days. The resulting solution was filtered through Celite and rinsed with EtOH (10 mL). To the filtrate was added aqueous HCl (1.0 M, 1.0 mL, 1.0 mmol). The filtrate was then evaporated under vacuum to provide 71 mg (100%) of product as a yellow powder: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.37 (s, 2H), 9.27 (s, 4H), 8.85 (s, 4H), 8.12 (s, 4H), 7.94 (s, 2H), 7.77 (d, 2H), 7.45 (d, 2H), 7.21 (s, 2H); *m*/*z* expected 392.2, found 393.6 (M+H)$^+$.

5.2.21. 1,4-Bis[6-(carbamohydrazonoyl)indol-2-yl]benzene (25; as hydrochloric acid salt)

To a solution of 1,4-bis{6-[ethoxy(imino)methyl]indol-2-yl}benzene hydrochloride (**21**; 160 mg, 0.31 mmol) in absolute ethanol (20 mL) was added hydrazine (195 μL, 0.61 mmol). The reaction mixture was allowed to stand at room temperature for 4 days, then ether (20 mL) was added to reaction mixture. The resulting brown solid was filtered and washed with water to afford 115 mg (89%) of product as a brown solid (88% purity by analytical HPLC): mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.38 (s, 2H), 10.90 (s, 2H), 9.38 (s, 2H), 8.73 (s, 2H), 8.12 (s, 4H), 7.84 (s, 2H), 7.75 (d, 2H), 7.35 (d, 2H), 7.181 (s, 2H), 5.28 (s, br, 4H); *m*/*z* expected 422.2, found 423.5 (M+H)$^+$.

5.2.22. 1,4-Bis[6-(carbamoyl)indol-2-yl]benzene (26)

A solution of 1,4,-bis(6-cyanoindol-2-yl)benzene (**5**; 169 mg, 0.47 mmol) in 4:1 TFA/H_2SO_4 (2.5 mL) was stirred at room temperature for 17 h. The resulting mixture was then poured into cold water (50 mL). The resulting solids were filtered, rinsed with water (10 mL), and dried to provide 163 mg (88%) of product as a brown powder (87% purity by analytical HPLC): mp >300 °C; ^{1}H NMR

(DMSO-d_6) δ 11.85 (s, 2H), 8.03 (s, 4H), 8.0–7.9 (s, br, 2H), 7.96 (s, 2H), 7.57 (s, 4H), 7.25–7.15 (s, br, 2H), 7.06 (s, 2H); *m*/*z* expected 394.1, found 395.1 $(M+H)^+$.

5.2.23. 1,4-Bis[6-(aminomethyl)indol-2-yl]benzene (27; as trifluoroacetic acid salt)

To a solution of 1,4,-bis(6-cyanoindol-2-yl)benzene (**5**; 300 mg, 0.84 mmol) in EtOH (50 mL) and EtOAc (25 mL) was added Raney nickel (~4 mL of aqueous slurry). The mixture was hydrogenated at 40 psi in a Parr apparatus for 3 days. The resulting yellow suspension was carefully decanted, and the solvent was removed under vacuum. The crude material was subjected to C18-reverse phase flash chromatography with a gradient of 30–100% CH_3CN/H_2O, each containing 0.2% TFA. Product-containing fractions were pooled and evaporated to provide 150 mg (30%) of product as a red solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 11.78 (s, br, 2H), 8.21 (s, br, 6H), 8.07 (s, 4H), 7.59 (d, 2H), 7.52 (s, 2H), 7.11 (d, 2H), 7.02 (s, 2H), 4.13 (dd, 4H); *m*/*z* expected 366.2, found 350.8 $(M-NH_2)^-$.

5.2.24. 2,5-Bis(4-cyano-2-nitrostyryl)furan (28a)

Furan-2,5-dicarboxaldehyde (197 mg, 1.6 mmol) and 4-cyano-2-nitrotoluene (**12**; 600 mg, 3.7 mmol) were dissolved in sulfolane (1 mL) to which was added piperidine (200 μL, 2.0 mmol). The mixture was heated to 150 °C in a sealed tube for 30 min, producing a dark suspension. The reaction was cooled to room temperature, and MeOH (5 mL) and Et_2O (5 mL) were added. The solids were triturated, filtered, rinsed with Et_2O, and dried to yield 417 mg (64%) of product as a brown powder. Material is too insoluble for ^{1}H NMR evaluation.

5.2.25. 2,5-Bis(4-cyano-2-nitrostyryl)thiophene (28b)

Thiophene-2,5-dicarboxaldehyde (700 mg, 5.0 mmol) and 4-cyano-2-nitrotoluene (**12**; 1.65 g, 5.0 mmol) were dissolved in sulfolane (2 mL) to which was added piperidine (250 μL, 2.5 mmol). The mixture was heated to 150 °C in a sealed tube for 30 min, producing a dark suspension. The reaction was cooled, and MeOH (50 mL) was added. The solids were triturated with 1:1 MeOH/Et_2O (50 mL), filtered, rinsed with Et_2O, and dried to yield 1.76 g (82%) of product as a brown powder. Material is too insoluble for ^{1}H NMR evaluation.

5.2.26. 2,5-Bis(6-cyanoindol-2-yl)furan (29a)

2,5-Bis(4-cyano-2-nitrostyryl)furan (**28a**; 390 mg, 0.95 mmol) was suspended in triethyl phosphite (15 mL) and heated to gentle reflux (170 °C oil bath) for 16 h. The excess phosphite was removed under vacuum, and the resulting dark residue was adsorbed onto silica gel (100 mL) by evaporation from CH_2Cl_2. The product was placed onto additional clean silica gel (100 mL), and eluted from the silica gel with 8:1 acetone/EtOAc until no more product was obtained. The filtrate was evaporated, and the residue was recrystallized from boiling CH_3CN/acetone to yield 121 mg (37%) of product as an orange-brown solid: mp >300 °C; R_f 0.43 (1:1 hexane/EtOAc) ^{1}H NMR (DMSO-d_6) δ 12.24 (s, 2H), 7.88 (s, 2 h), 7.75 (d, 2H), 7.38 (s, 2H), 7.21 (d, 2H), 7.05 (s, 2H).

5.2.27. 2,5-Bis(6-cyanoindol-2-yl)thiophene (29b)

2,5-Bis(4-cyano-2-nitrostyryl)thiophene (**28b**; 1.76 g, 4.1 mmol) was suspended in triethyl phosphite (50 mL) and heated to gentle reflux (170 °C oil bath) for 16 h. The excess phosphite was removed under vacuum, and the resulting dark residue was adsorbed onto silica gel (200 mL) by evaporation from CH_2Cl_2. The product was placed onto additional clean silica gel (100 mL), and eluted from the silica gel with 4:1 acetone/EtOAc until no more product was obtained. The filtrate was evaporated, and the residue was recrystallized from boiling CH_3CN/acetone to yield 397 mg (26%) of product as an orange-brown solid: mp >300 °C; R_f 0.42 (1:1 hexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 12.28 (s, 2H), 7.84 (s, 2 h), 7.71 (d, 2H), 7.70 (s, 2H), 7.36 (d, 2H), 6.93 (s, 2H).

5.2.28. 2,5-Bis[6-(4,5-dihydro-1*H*-imidazol-2-yl)indol-2-yl] furan (30a; as trifluoroacetic acid salt)

To a solution of 2,5-bis(6-cyanoindol-2-yl)furan (**29a**; 96 mg, 0.28 mmol) in ethylenediamine (2 mL) was added phosphorous pentasulfide (12 mg, 0.05 mmol). The mixture was heated in a sealed tube at 120 °C for 3 h, then cooled to room temperature and poured into water (50 mL). The resulting solid was rinsed with water (50 mL) and dried under vacuum. The solids thus obtained were complete dissolved in trifluoroacetic acid (2 mL) with gentle warming. The yellow solution was diluted with MeOH (2 mL), then poured into Et_2O (40 mL). The resulting yellow precipitate was filtered and dried under vacuum to constant weight to provide 163 mg (95%) of product as a yellow powder: mp dec >220 °C; R_f 0.13 (80:18:2 $CHCl_3$:MeOH:aq $MeNH_2$); ^{1}H NMR (DMSO-d_6) δ 12.43 (s, 2H), 10.43 (s, 4H), 8.06 (s, 2H), 7.82 (d, 2H), 7.59 (d, 2H), 7.28 (s, 2H), 7.09 (s, 2H), 4.03 (s, 8H); *m*/*z* expected 434.2, found 435.0 $(M+H)^+$.

5.2.29. 2,5-Bis[6-(4,5-dihydro-1*H*-imidazol-2-yl)indol-2-yl] thiophene (30b; as trifluoroacetic acid salt)

To a solution of 2,5-bis(6-cyanoindol-2-yl)thiophene (**29b**; 100 mg, 0.27 mmol) in ethylenediamine (2 mL) was added phosphorous pentasulfide (12 mg, 0.05 mmol). The mixture was heated in a sealed tube at 120 °C for 4 h, then cooled to room temperature and poured into water (50 mL). The resulting solid was rinsed with water (50 mL) and dried under vacuum. The solids thus obtained were complete dissolved in trifluoroacetic acid (2.5 mL) with gentle warming. The yellow solution was diluted with MeOH (2.5 mL), then poured into Et_2O (40 mL). The resulting yellow precipitate was filtered and dried under vacuum to constant weight to provide 173 mg (95%) of product as a yellow-orange powder: mp >300 °C ^{1}H NMR (DMSO-d_6) δ 12.48 (s, 2H), 10.42 (s, 4H), 8.04 (s, 2H), 7.78 (d, 2H), 7.77 (s, 2H), 7.57 (d, 2H), 6.96 (s, 2H), 4.03 (s, 8H); *m*/*z* expected 450.2, found 226.5 $(M+2H^+)/2$.

5.2.30. *N*-Boc-2-(5-bromopyridin-2-yl)indole-6-carbonitrile (31)

To a solution of *N*-Boc-6-cyanoindole-2-boronic acid (**9**; 25.0 g, 87.4 mmol) and 2-iodo-5-bromopyridine (24.73 g, 87.4 mmol) in toluene (850 mL) and ethanol (80 mL) were added an aqueous solution of sodium carbonate (2.0 M, 87.5 mL, 175 mmol), and tetrakis(triphenylphosphine)palladium (2.52 g, 2.2 mmol. The resulting mixture was refluxed for 5 h under an argon atmosphere, then cooled to room temperature and diluted with water (250 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (250 mL). The combined organic extracts were washed with aqueous sodium carbonate (125 mL), water (250 mL × 2), and brine (250 mL), then dried over sodium sulfate, and filtered. The filtrate was evaporated to provide a residue which was subjected to flash chromatography on silica gel with 5% EtOAc/hexane. Product-containing fractions were pooled and evaporated to provide 20.7 g (62%) of product as a white solid: R_f 0.41 (3:1 hexane/EtOAc); ^{1}H NMR (DMSO-d_6) δ 8.73 (s, 1H), 8.50 (s, 1H), 7.92 (dd, 1H), 7.64 (d, 1H), 7.49 (d, 1H), 7.42 (d, 1H), 6.80 (s, 1H), 1.38 (s, 9H).

5.2.31. 2,5-Bis(*N*-Boc-6-cyanoindol-2-yl)pyridine (32)

To solution of *N*-Boc-2-(5-bromopyridin-2-yl)indole-6-carbonitrile (**31**; 269 mg, 0.68 mmol) and *N*-Boc-6-cyanoindole-2-boronic acid (**9**; 387 mg, 1.35 mmol) dissolved in toluene (14 mL) were added tetrakis(triphenylphosphine)palladium (0) (79 mg, 0.07 mmol) and aqueous sodium carbonate (1.0 M, 4.0 mL,

4.0 mmol). The resulting mixture was heated to 80 °C for 7 h, then cooled to room temperature and poured into EtOAc (10 mL) and water (5 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic extracts were evaporated, and the residue was subjected to flash chromatography on silica gel with 0–75% EtOAc/hexane. Product-containing fractions were pooled and evaporated to provide 146 mg (38%) of product as a pale yellow solid: R_f 0.23 (1:1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.77 (s, 1H), 8.56 (s, 1H), 8.51 (s, 1H), 7.89 (dd, 1H), 7.70–7.62 (m, 3H), 7.56–7.51 (m, 2H), 6.88 (s, 1H), 6.74 (s, 1H), 1.48 (s, 9H), 1.43 (s, 9H).

5.2.32. 2,5-Bis[6-(4,5-dihydro-1*H*-imidazol-2-yl)indol-2-yl] pyridine (33; as acetic acid salt)

To a solution of 2,5-bis(*N*-Boc-6-cyanoindol-2-yl)pyridine (**32**; 116 mg, 0.21 mmol) in ethylenediamine (2.0 mL) was added phosphorous pentasulfide (46 mg, 0.21 mmol). The reaction mixture was heated to 120 °C in a sealed tube for 3 h, the cooled to room temperature and poured into water (10 mL). The mixture was stirred for 30 min, then the solids were filtered, washed with water (5 mL), and dried to provide a yellow solid. The solid was dissolved in water (100 mL) and acetic acid (10 mL). The yellow solution was lyophilized to provide 90 mg (68%) of product as a yellow powder: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 9.20 (d, 1H), 8.38 (d, 1H), 8.16 (d, 1H), 7.98 (s, 1H), 7.93 (s, 1H), 7.63–7.51 (m, 4H), 7.27 (s, 1H), 7.18 (s, 1H), 3.67 (s, 4H), 3.66 (s, 4H), 1.88 (s, 9H); *m*/*z* expected 445.2, found 224.1 (M+2H$^+$)/2.

5.2.33. 2,5-Bis(*N*-Boc-6-cyanoindol-2-yl)pyrazine (34)

To a solution of *N*-Boc-6-cyanoindole-2-boronic acid (**9**; 0.31 g, 1.08 mmol), in 1,2-DME (12 mL) were added 2,5-dibromopyrazine (0.10 g, 0.42 mmol), $Pd(Ph_3P)_4$ (243 mg, 0.21 mmol), Na_2CO_3 (268 mg, 2.53 mmol), and water (3 mL). The mixture was stirred and heated to 78 °C for 6 h, the cooled to 5 °C overnight, and filtered. The resulting solid was rinsed with water (10 mL) and dried to provide 0.24 g (100%) as pale yellow solid: R_f 0.17 (3:1 hexane/EtOAc). This crude material was used for the next step without further purification.

5.2.34. 2,5-Bis[6-(imidazolin-2-yl)indol-2-yl]pyrazine (35; as acetic acid salt)

To a solution of 2,5-Bis(*N*-Boc-6-cyanoindol-2-yl)pyrazine (**34**; 116 mg, 0.21 mmol) in ethylenediamine (2 mL) was added phosphorous pentasulfide (28 mg, 0.13 mmol). The mixture was heated in a sealed tube at 130 °C for 4 h, then cooled to room temperature and water (5 mL) was added. The suspension was filtered. The solid was rinsed with water (20 mL) and dried in vacuum to provide 90 mg yellow powder. The powder was suspended in water (14 mL) and acetic acid (40 μL) was added. After stirring for 24 h, the cloudy solution was filtered through a 0.45 μm pore sized PTFE membrane. The filtrate was lyophilized to provide 38 mg (32%) of product as a yellow powder: mp >300 °C ^{1}H NMR (DMSO-d_6) δ 12.14 (br s, 2H), 9.34 (s, 2H), 7.99 (s, 2H), 7.65 (d, 2H), 7.55 (d, 2H), 7.44 (s, 2H), 3.65 (s, 8H), 1.88 (s, 6H, AcOH); *m*/*z* expected 446.2, found 224.5 (M+2H$^+$)/2.

5.2.35. 1,2-Bis(6-cyanoindol-2-yl)ethane (36)

To a solution of (*E*)-1,2-bis(6-cyanoindol-2-yl)ethylene (**13**; 200 mg, 0.65 mmol) in EtOH (40 mL) was added 5% palladium on carbon (300 mg). The mixture was hydrogenated at 30 psi in a Parr apparatus for 7 h. The mixture was filtered through Celite, and the solids rinsed with additional EtOH (10 mL). The filtrate was evaporated to dryness to provide 205 mg (100%) of crude product which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 11.67 (br, 2H); 7.77 (s, 2H); 7.55 (d, 2H); 7.26 (d, 2H); 6.37 (s, 2H); 3.24 (s, 4H).

5.2.36. 1,2-Bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl] ethane (37; as trifluoroacetic acid salt)

To a solution of 1,2-bis(6-cyanoindol-2-yl)ethane (**36**; 205 mg, 0.65 mmol) in 1,3-diaminopropane (3 mL) was added phosphorous pentasulfide (164 mg, 0.74 mmol). The mixture was heated to 120 °C in a sealed tube for 2 h, then cooled to room temperature and poured into cold water (5 mL). The mixture was centrifuged, and the aqueous layer decanted. The solids were resuspended and centrifuged again. After decanting the liquid, the resulting red-brown solid was subjected to preparative HPLC purification with a gradient of 20–100% CH_3CN/H_2O, each containing 0.2% TFA. Product-containing fractions were pooled, and the CH_3CN removed under vacuum. The remaining aqueous solution was lyophilized to provide 21 mg (5%) of product as a yellow powder: ^{1}H NMR (DMSO-d_6) δ 11.75 (br, 1H), 9.72 (br, 4H), 7.70 (s, 2H), 7.60 (d, 2H), 7.25 (d, 2H), 6.36 (s, 2H), 3.49 (t, 8H), 3.27 (s, 4H); 1.99 (quint, 4H); *m*/*z* expected 424.2, found 213.7 (M+2H$^+$)/2.

5.2.37. 1,7-Bis-(4-cyano-2-nitrophenyl)-1,6-heptadiene (39a)

To a solution of of pentamethylene bis(triphenylphosphonium) bromide (1.18 g, 1.56 mmol) in dry THF (20 mL) cooled to 0 °C was added a solution of sodium hexamethyldisilazide in THF (2.0 M, 1.56 mL 3.12 mmol), dropwise over 10 min. The solution was stirred at 0 °C for 35 min, then cooled to −78 °C. A solution of 2-nitro-4-cyano-benzaldehyde[45] (**38**; 0.55 g, 3.12 mmol) in dry THF (5.0 mL) was added dropwise over 5 min. The resulting brown solution was stirred at −78 °C for 30 min, then warmed to room temperature and stirred an additional 16 h. The reaction mixture was poured into an aqueous solution of citric acid (10%, 30 mL) and product was extracted with dichloromethane (2 × 30 mL). The combined organic extracts were filtered through a pad of silica gel, and rinsed with CH_2Cl_2 (20 mL). The filtrate was evaporated and the residue subjected to flash chromatography on silica gel with 1:1 hexane/EtOAc. Product-containing fractions were pooled and evaporated to provide 0.33 g (60%) of product as yellow solid: R_f 0.70 (1:1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.27–8.15 (m, 2H), 7.85–7.42 (m, 4H), 6.95–6.63 (m, 2H), 6.44–6.21 (m, 1H), 6.00–5.80 (m, 1H), 2.48–2.00 (m, 4H), 1.79–1.58 (m, 2H).

5.2.38. 1,8-Bis-(4-cyano-2-nitrophenyl)-1,7-octadiene (39b)

To a solution of hexamethylene bis(triphenylphosphonium) bromide (4.0 g, 5.22 mmol) in dry THF (50 mL) cooled to 0 °C was added a solution of sodium hexamethyldisilazide in THF (2.0 M, 5.23 mL, 10.5 mmol), dropwise over 10 min. The solution was stirred at 0 °C for 35 min, then cooled to −78 °C. A solution of 2-nitro-4-cyano-benzaldehyde[45] (**38**; 1.84 g, 10.45 mmol) in dry THF (10 mL) was added dropwise over 5 min. The resulting brown solution was stirred at −78 °C for 30 min, then warmed to room temperature and stirred an additional 16 h. The reaction mixture was poured into an aqueous solution of citric acid (10%, 30 mL) and product was extracted with dichloromethane (2 × 50 mL). The combined organic extracts were filtered through a pad of silica gel, and rinsed with CH_2Cl_2 (50 mL). The filtrate was evaporated and the residue subjected to flash chromatography on silica gel with 1% MeOH/$CHCl_3$. Product-containing fractions were pooled and evaporated to provide 1.55 g (55%) of product as yellow solid: R_f 0.71 (1% MeOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 1H), 8.17 (s, 1H), 7.85-7.69 (m, 3H), 7.52–7.45 (m, 1H), 6.90–6.66 (m, 2H), 6.46–6.29 (m, 1H), 6.00–5.85 (m, 1H), 2.35–1.92 (m, 4H), 1.60–1.35 (m, 4H).

5.2.39. 1-(6-Cyanoindol-2-yl)-3-(*N*-ethoxy-6-cyanoindol-2-yl) propane (40a) and 1,3-bis(*N*-ethoxy-6-cyanoindol-2-yl)propane (41a)

A solution of 1,7-bis-(4-cyano-2-nitrophenyl)-1,6-heptadiene (**39a**; 84 mg, 0.22 mmol) in triethyl phosphite (3 mL) was heated

at 160 °C for 18 h in a sealed tube, then cooled to room temperature. The solvent was evaporated, and the residue was subjected to flash chromatography on silica gel with 4:1 toluene/EtOAc. Product-containing fractions were pooled and evaporated to provide 21 mg (20%) of **40a** and 24 mg (21%) of **41a** as yellow solids: **40a**: R_f 0.70 (1:1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.50 (s, br, 1H), 7.64–7.50 (m, 4H), 7.31–7.14 (m, 2H), 6.38 (s, 1H), 6.22 (s, 1H), 4.20 (q, 2H), 2.88 (m, 4H), 2.21 (m, 2H), 1.35 (t, 3H). **41a**: R_f 0.60 (1:1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.60 (s, 2H), 7.47 (d, 2H), 7.23 (dd, 2H), 6.16 (s, 2H), 4.15 (q, 4H), 2.86 (t, 4H), 2.16 (quint, 2H), 1.37 (t, 6H).

5.2.40. 1-(6-Cyanoindol-2-yl)-4-(*N*-ethoxy-6-cyanoindol-2-yl) butane (40b) and 1,4-bis(*N*-ethoxy-6-cyanoindol-2-yl)butane (41b)

A solution of 1,8-bis-(4-cyano-2-nitrophenyl)-1,6-octadiene (**39b**; 310 mg, 0.77 mmol) in triethyl phosphite (4 mL)was heated at 160 °C for 18 h in a sealed tube, then cooled to room temperature. The solvent was evaporated, and the residue was subjected to flash chromatography on silica gel with hexane (1 CV) then toluene (1 CV), then 1% MeOH/$CHCl_3$. Product-containing fractions were pooled and evaporated to provide 77 mg (22%) of **41b** and 51 mg (16%) of **40b** as yellow solids: **40b**: R_f 0.60 (1% MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD) δ 7.77 (s, 1H), 7.65 (s, 1H), 7.60–7.53 (m, 2H), 7.31–7.22 (m, 2H), 6.33 (s, 1H), 6.24 (s, 1H), 4.27 (q, 2H), 2.91 (t, 4H), 1.90 (quint, 4H), 1.38 (t, 3H). **41b**: R_f 0.70 (1% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 2H), 7.49 (d, 2H), 7.25 (dd, 2H), 6.15 (s, 2H), 4.20 (q, 4H), 2.90 (m, 4H), 1.91 (m, 4H), 1.42 (t, 6H).

5.2.41. 1,3-Bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl] propane (44a)

To a solution of 1-(6-cyanoindol-2-yl)-3-(*N*-ethoxy-6-cyanoindol-2-yl)propane (**40a**; 21 mg, 0.06 mmol) and 1,3-bis(*N*-ethoxy-6-cyanoindol-2-yl)propane (**41a**; 16 mg, 0.04 mmol) in 1.3-diaminopropane (1.5 mL) was added phosphorous pentasulfide (25 mg, 0.11 mmol). The mixture was heated to 145 °C in a sealed tube for 3 h, then cooled to room temperature. The mixture was poured into cold water (3 mL), then centrifuged for 15 min. The aqueous supernatant was carefully decanted, the solids resuspended, and the process repeated two more times. The remaining solids were dissolved in MeOH (3 mL), to which was added Raney nickel (~1 mL of aqueous slurry). The mixture was hydrogenated at atmospheric pressure and 55 °C for 3 h. The suspension was filtered through Celite, and rinsed with MeOH (10 mL). The filtrate was evaporated to provide 7.5 mg (42%) of the product as a yellow solid: mp >300 °C; ^{1}H NMR (CD_3OD) δ 7.68 (s, 2H), 7.56 (d, 2H), 7.27 (d, 2H), 6.33 (s, 2H), 3.55 (t, 8H), 2.89 (t, 4H), 2.21 (m, 2H), 2.06 (quint, 4H); *m*/*z* expected 438.3, found 220.6 ($M+2H^+$)/2.

5.2.42. 1,4-Bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl] butane (44b)

To a solution of 1-(6-cyanoindol-2-yl)-3-(*N*-ethoxy-6-cyanoindol-2-yl)propane (**40b**; 51 mg, 0.10 mmol) and 1,3-bis(*N*-ethoxy-6-cyanoindol-2-yl)propane (**41b**; 77 mg, 0.14 mmol) in 1.3-diaminopropane (2.0 mL) was added phosphorous pentasulfide (100 mg, 0.45 mmol). The mixture was heated to 140 °C in a sealed tube for 3.5 h, then cooled to room temperature. The mixture was poured into cold water (3 mL), then centrifuged for 15 min. The aqueous supernatant was carefully decanted, the solids resuspended, and the process repeated two more times. The remaining solids were dissolved in MeOH (8 mL), to which was added Raney nickel (~3 mL of aqueous slurry). The mixture was hydrogenated at atmospheric pressure and 55 °C for 15 h. The suspension was filtered through Celite, and rinsed with MeOH (25 mL). The filtrate was evaporated to provide 90 mg (90%) of the product as a yellow solid: mp >300 °C; ^{1}H NMR (CD_3OD) δ 7.66 (s, 2H), 7.57 (d, 2H), 7.25 (d, 2H), 6.29 (s, 2H), 3.58 (t, 8H), 2.83 (t, 4H), 2.20 (quint, 4H), 1.86 (quint, 4H); *m*/*z* expected 452.3, found 227.7 ($M+2H^+$)/2.

5.2.43. (*E*)-1,2-Bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl] ethylene (45; as trifluoroacetic acid salt)

To a solution of (*E*)-1,2-bis(6-cyanoindol-2-yl)ethylene (**13**; 100 mg, 0.32 mmol) in 1,3-diaminopropane (1.0 mL) was added phosphorous pentasulfide (20 mg, 0.09 mmol). The mixture was heated to 110 °C for 30 min in a microwave reaction station, then allowed to cool to room temperature. The mixture was poured into cold water (1 mL), then centrifuged for 15 min. The aqueous supernatant was carefully decanted, the solids resuspended, and the process repeated two more times. The remaining solids were subjected to preparative HPLC purification with a gradient of 10–100% CH_3CN/H_2O over 10 min; each solvent contained 0.2% TFA. Product-containing fractions were pooled, and the CH_3CN removed under vacuum. The remaining aqueous solution was lyophilized to provide 49 mg (23%) of product as a brown solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.74 (s, 2H); 7.69 (d, 2H); 7.30 (dd, 2H); 7.29 (s, 2H); 6.73 (s, 2H); 3.62 (t, 8H); 2.15 (quint, 4H); *m*/*z* expected 422.2, 212.3 ($M+2H^+$)/2.

5.2.44. 2,5-Bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl] thiophene (46; as acetic acid salt)

To a suspension of 2,5-bis(6-cyanoindol-2-yl)thiophene (**29b**; 0.57 g, 1.6 mmol) in 1,3-diaminopropane (10 mL) was added phosphorous pentasulfide (120 mg, 0.54 mmol). The mixture was heated in a sealed tube at 120 °C for 2 h. then cooled to room temperature. The mixture was poured into cold water (200 mL), then centrifuged for 15 min. The aqueous supernatant was carefully decanted, the solids resuspended, and the process repeated two more times. The remaining solids were dissolved with boiling AcOH (5.0 mL) and MeOH (5.0 mL). The liquid was filtered, then poured into Et_2O (150 mL). The resulting suspension was stirred 30 min, then filtered, rinsed with Et_2O (40 mL), and dried to provide 0.38 g (34%) of product as a yellow powder: mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 7.89 (s, 2H), 7.71 (d, 4H), 7.34 (d, 2H), 6.90 (s, 2H), 3.51 (s, br, 8H), 1.98 (s, 4H), 1.77 (s, 12H); *m*/*z* expected 478.2, found 240.6 ($M+2H^+$)/2.

5.2.45. 2,5-Bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl] pyridine (47; as acetic acid salt)

To a suspension of 2,5-bis(*N*-Boc-6-cyanoindol-2-yl)pyridine (**32**; 130 mg, 0.26 mmol) in 1,3-diaminopropane (10 mL) was added phosphorous pentasulfide (45 mg, 0.20 mmol). The suspension was heated in a sealed vessel to 120 °C (oil bath) for 2 h, then cooled to room temperature and poured into water (200 mL). The resulting yellow suspension was stirred for 30 min, then filtered. The solids were rinsed with water (100 mL), then dried under vacuum to yield a yellow powder. The powder was dissolved in acetic acid (8 mL) and MeOH (4 mL) with heating. The yellow solution was diluted rapidly with Et_2O (100 mL). The resulting solids were filtered, rinsed with Et_2O (50 mL), and dried to provide 219 mg g (85%) of product as a yellow powder: mp >300 °C; ^{1}H NMR (DMSO-d_6/D_2O) δ 9.26 (s, 1H), 8.45 (d, 1H), 8.22 (d, 1H), 7.91 (d, 2H), 7.81 (t, 2H), 7.38–7.29 (m, 4H), 3.53 (s, br, 8H), 2.01 (s, br, 4H), 1.80 (s, 12H); *m*/*z* expected 473.2, found 238.2 ($M+2H^+$)/2.

5.2.46. 2,5-Bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl]pyrazine (48; as acetic acid salt)

To a solution of 2,5-Bis(*N*-Boc-6-cyanoindol-2-yl)pyrazine (**34**; 116 mg, 0.21 mmol) in 1,3-diaminopropane (2 mL) was added

phosphorous pentasulfide (28 mg, 0.13 mmol). The mixture was heated in a sealed tube at 130 °C for 4 h, then cooled to room temperature and water (5 mL) was added. The suspension was filtered and the solids rinsed with water (20 mL) and dried in vacuum to provide a yellow powder. The powder was suspended in water (14 mL) and acetic acid (40 μL) was added. After stirring for 24 h, the cloudy solution was filtered through a 0.45 μm pore sized PTFE membrane. The filtrate was lyophilized to provide 46 mg (37%) of product as a yellow powder: mp >300 °C ^{1}H NMR (MeOd-d_4) δ 9.24 (s, 2H),7.89 (s, 2H), 7.83 (d, 2H), 7.39 (s, 2H), 7.35 (d, 2H), 3.62 (t, 8H), 2.14 (pent, 4H), 1.90 (s, 6H); *m*/*z* expected 474.2, found 238.5 (M+2H$^+$)/2.

5.2.47. 2,5-Bis[3-fluoro-6-(3,4,5,6-tetrahydropyrimidin-2-yl) indol-2-yl]benzene (49; as trifluoroacetic acid salt)

To a stirred slurry of 2,5-bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl]benzene (**19a**; 200 mg, 0.42 mmol) in DMF (2 mL) was added Selectfluor® (300 mg, 0.85 mmol). The slurry was stirred at room temperature for 1.5 h, then added to cold brine (5 mL). The suspension was allowed to stand for 20 min, and was then centrifuged for 15 min. The aqueous supernatant was carefully decanted, the solids resuspended, and the process repeated two more times. The remaining solids were subjected to preparative HPLC purification with a gradient of 30–90% CH_3CN/H_2O, each containing 0.2% TFA. Product-containing fractions were pooled, and the CH_3CN removed under vacuum. The remaining aqueous solution was lyophilized to provide 24 mg (8%) of product as a yellow-green powder: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.35 (s, br, 2H), 10.00 (s, br, 4H), 8.38 (s, 4H), 7.87 (s, 2H), 7.85 (d, 2H), 7.46 (d, 2H), 3.90 (br, 8H), 2.08 (br, 4H); *m*/*z* expected 508.3, found 255.6 (M+2H$^+$)/2.

5.2.48. 2,5-Bis[3-chloro-6-(3,4,5,6-tetrahydropyrimidin-2-yl) indol-2-yl]benzene (50; as trifluoroacetic acid salt)

To a stirred slurry of 2,5-bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl]benzene (**19a** [as TFA salt]; 100 mg, 0.14 mmol) in DMF (2 mL) was added *N*-chlorosuccinimide (42 mg, 0.31 mmol). The slurry was stirred at room temperature for 29 h, then added to cold water (2 mL). The suspension was allowed to stand for 20 min, and was then centrifuged for 15 min. The aqueous supernatant was carefully decanted, the solids resuspended, and the process repeated two more times. The remaining solids were dried under vacuum to provide 66 mg (60%) of product as a yellow powder: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.60 (s, br, 2H), 9.93 (s, br, 4H), 8.23 (s, 4H), 7.86 (s, 2H), 7.78 (d, 2H), 7.49 (d, 2H), 3.54 (t, 8H), 2.02 (t, 4H); *m*/*z* expected 540.2, found 271.5 (M+2H$^+$)/2.

5.2.49. 2,5-Bis[3-bromo-6-(3,4,5,6-tetrahydropyrimidin-2-yl) indol-2-yl]benzene (51; as trifluoroacetic acid salt)

To a stirred slurry of 2,5-bis[6-(3,4,5,6-tetrahydropyrimidin-2-yl)indol-2-yl]benzene (**19a** [as TFA salt]; 100 mg, 0.14 mmol) in DMF (2 mL) was added *N*-bromosuccinimide (56 mg, 0.31 mmol). The slurry was stirred at room temperature for 5 h, then added to cold water (2 mL). The suspension was allowed to stand for 20 min, and was then centrifuged for 15 min. The aqueous supernatant was carefully decanted, the solids resuspended, and the process repeated two more times. The remaining solids were dried under vacuum to provide 85 mg (69%) of product as a yellow powder: mp >300 °C; ^{1}H NMR (CD_3OD) δ 8.15 (s, 4H), 7.89 (s, 2H), 7.75 (d, 2H), 7.48 (d, 2H), 3.66 (t, 8H), 2.17 (t, 4H); *m*/*z* expected 628.1, found 316.4 (M+2H$^+$)/2.

Acknowledgments

Research reported in this article was generously supported by the National Institute of Allergy and Infectious Disease of the National Institutes of Health under award number U01AI082052. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.10.014.

References and notes

1. Spellberg, B.; Guidos, R.; Gilbert, D.; Bradley, J.; Boucher, H. W.; Scheld, W. M.; Bartlett, J. G.; Edwards, J., Jr. *Clin. Infect. Dis.* **2008**, *46*, 155.
2. Nikaido, H. *Annu. Rev. Biochem.* **2009**, *78*, 119.
3. Wellington, E. M.; Boxall, A. B.; Cross, P.; Feil, E. J.; Gaze, W. H.; Hawkey, P. M.; Johnson-Rollings, A. S.; Jones, D. L.; Lee, N. M.; Otten, W.; Thomas, C. M.; Williams, A. P. *Lancet Infect. Dis* **2013**, *13*, 155.
4. Magiorakos, A. P.; Srinivasan, A.; Carey, R. B.; Carmeli, Y.; Falagas, M. E.; Giske, C. G.; Harbarth, S.; Hindler, J. F.; Kahlmeter, G.; Olsson-Liljequist, B.; Paterson, D. L.; Rice, L. B.; Stelling, J.; Struelens, M. J.; Vatopoulos, A.; Weber, J. T.; Monnet, D. L. *Clin. Microbiol. Infect.* **2012**, *18*, 268.
5. Boucher, H. W.; Talbot, G. H.; Benjamin, D. K., Jr.; Bradley, J.; Guidos, R. J.; Jones, R. N.; Murray, B. E.; Bonomo, R. A.; Gilbert, D. *Clin. Infect. Dis.* **2013**, *56*, 1685.
6. Klevens, R. M.; Edwards, J. R.; Tenover, F. C.; McDonald, L. C.; Horan, T.; Gaynes, R. *Clin. Infect. Dis.* **2006**, *42*, 389.
7. Gaynes, R.; Edwards, J. R. *Clin. Infect. Dis.* **2005**, *41*, 848.
8. Tsioutis, C.; Kritsotakis, E. I.; Maraki, S.; Gikas, A. *Eur. J. Clin. Microbiol. Infect. Dis.* **2010**, *29*, 301.
9. Moir, D. T.; Opperman, T. J.; Butler, M. M.; Bowlin, T. L. *Curr. Opin. Pharmacol.* **2012**, *12*, 535.
10. Coates, A. R.; Halls, G.; Hu, Y. *Br. J. Pharmacol.* **2011**, *163*, 184.
11. Payne, D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L. *Nat. Rev. Drug Disc.* **2007**, *6*, 29.
12. Panchal, R. G.; Ulrich, R. L.; Lane, D.; Butler, M. M.; Houseweart, C.; Opperman, T.; Williams, J. D.; Peet, N. P.; Moir, D. T.; Nguyen, T.; Gussio, R.; Bowlin, T.; Bavari, S. *Antimicrob. Agents Chemother.* **2009**, *53*, 4283.
13. Butler, M. M.; Williams, J. D.; Peet, N. P.; Moir, D. T.; Panchal, R. G.; Bavari, S.; Shinabarger, D. L.; Bowlin, T. L. *Antimicrob. Agents Chemother.* **2010**, *54*, 3974.
14. Opperman, T. J.; Li, J. B.; Lewis, M. A.; Houseweart, C.; Aiello, D.; Williams, J. D.; Peet, N. P.; Moir, D. T.; Long, E. C.; Bowlin, T. L. *Abstract of papers*, 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA; American Society for Microbiology, Washington, DC, 2009; Abstract F1, 1976.
15. Butler, M. M., personal communication.
16. Gribble, G. W. *Contemp. Org. Synth.* **1994**, *1*, 145.
17. Gribble, G. W. *J. Chem. Soc., Perkin Trans. I* **2000**, 1045.
18. Humphrey, G. R.; Kuethe, J. T. *Chem. Rev.* **2006**, *106*, 2875.
19. Robinson, B. *The Fischer Indole Synthesis*; Wiley-Interscience: New York, NY, 1982.
20. Madelung, W. *Chem. Ber.* **1912**, *45*, 1128.
21. Houlihan, W. J.; Parrino, V. A.; Uike, Y. *J. Org. Chem.* **1981**, *46*, 4511.
22. Castro, C. E.; Gaughan, E. J.; Owsley, D. C. *J. Org. Chem.* **1966**, *31*, 4071.
23. Alfonsi, M.; Arcadi, A.; Aschi, M.; Bianchi, G.; Marinelli, F. *J. Org. Chem.* **2005**, *70*, 2265.
24. Price, D. W., Jr; Dirk, S. M.; Maya, F.; Tour, J. M. *Tetrahedron* **2003**, *59*, 2497.
25. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
26. Kuduk, S. D.; Chang, R. K.; Wai, J. M. C.; Di Marco, C. N.; Cofre, V.; DiPardo, R. M.; Cook, S. P.; Cato, M. J.; Jovanovska, A.; Urban, M. O.; Leitl, M.; Spencer, R. H.; Kane, S. A.; Hartman, G. D.; Bilodeau, M. T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4059.
27. Li, B.; Pai, R.; Cardinale, S. C.; Butler, M. M.; Peet, N. P.; Moir, D. T.; Bavari, S.; Bowlin, T. L. *J. Med. Chem.* **2010**, *53*, 2264.
28. Robbins, D. W.; Hartwig, J. F. *Org. Lett.* **2012**, *14*, 4266.
29. Dann, O.; Ruff, J.; Wolff, H.-P.; Grießmeier, H. *Liebigs Ann. Chem.* **1984**, 409.
30. Dann, O.; Wolff, H.-P.; Schlee, R.; Ruff, J. *Liebigs Ann. Chem.* **1986**, 2164.
31. Cadogan, J. I. G.; Cameron-Wood, M.; Mackie, R. K.; Searle, R. J. G. *J. Chem. Soc. (Resumed)* **1965**, 4831.
32. Sundberg, R. J. *J. Org. Chem.* **1965**, *30*, 3604.
33. Ruggli, P.; Wolff, E. *Helv. Chim. Acta* **1936**, *19*, 5.
34. Anastassiadou, M.; Danoun, S.; Crane, L.; Baziard-Mouysset, G.; Payard, M.; Caignard, D. H.; Rettori, M. C.; Renard, P. *Bioorg. Med. Chem.* **2001**, *9*, 585.
35. Reissert, A. *Berichte* **1897**, *30*, 1030–1053.
36. Dann, O.; Char, H.; Fleischmann, P.; Fricke, H. *Liebigs Ann. Chem.* **1986**, *1986*, 438.
37. McMurry, J. E. *Chem. Rev.* **1989**, *89*, 1513.
38. Sall, D. J.; Arfsten, A. E.; Bastian, J. A.; Denney, M. L.; Harms, C. S.; McCowan, J. R.; Morin, J. M.; Rose, J. W.; Scarborough, R. M.; Smyth, M. S.; Um, S. L.; Utterback, B. G.; Vasileff, R. T.; Wikel, J. H.; Wyss, V. L.; Jakubowski, J. A. *J. Med. Chem.* **1997**, *40*, 2843.
39. Nystrom, R. F.; Chaikin, S. W.; Brown, W. G. *J. Am. Chem. Soc.* **1949**, *71*, 3245.
40. McMurry, J. E.; Fleming, M. P.; Kees, K. L.; Krepski, L. R. *J. Org. Chem.* **1978**, *43*, 3255.

41. Anbazhagan, M.; Boykin, D. W.; Stephens, C. E. *Synthesis* **2003**, *2003*, 2467.
42. Ismail, M. A.; Brun, R.; Wenzler, T.; Tanious, F. A.; Wilson, W. D.; Boykin, D. W. *J. Med. Chem.* **2004**, *47*, 3658.
43. Moorthy, J. N.; Singhal, N. *J. Org. Chem.* **1926**, *2005*, 70.
44. Huber, W. *J. Am. Chem. Soc.* **1944**, *66*, 876.
45. Patrick, D. A.; Bakunov, S. A.; Bakunova, S. M.; Kumar, E. V. K. S.; Lombardy, R. J.; Jones, S. K.; Bridges, A. S.; Zhirnov, O.; Hall, J. E.; Wenzler, T.; Brun, R.; Tidwell, R. R. *J. Med. Chem.* **2007**, *50*, 2468.
46. Majgier-Baranowska, H.; Williams, J. D.; Li, B.; Peet, N. P. *Tetrahedron Lett.* **2012**, *53*, 4785.
47. Nyffeler, P. T.; Duron, S. G.; Burkart, M. D.; Vincent, S. P.; Wong, C. H. *Angew. Chem. (International ed. in English)* **2004**, *44*, 192.
48. CLSI. In M7–A7; Approved Standard–7th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, 2006.