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Synthesis and antibacterial evaluation of new, unsymmetrical triaryl bisamidine compounds

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Abstract

Herein we describe the synthesis and antibacterial evaluation of a new, unsymmetrical triaryl bisamidine compound series, [Am]-[indole]-[linker]-[HetAr/Ar]-[Am], in which [Am] is an amidine or amino group, [linker] is a benzene, thiophene or pyridine ring, and [HetAr/Ar] is a benzimidazole, imidazopyridine, benzofuran, benzothiophene, pyrimidine or benzene ring. When the [HetAr/Ar] unit is a 5,6-bicyclic heterocycle, it is oriented such that the 5-membered ring portion is connected to the [linker] unit and the 6-membered ring portion is connected to the [Am] unit. Among the 34 compounds in this series, compounds with benzofuran as the [HetAr/Ar] unit showed the highest potencies. Introduction of a fluorine atom or a methyl group to the triaryl core led to the more potent analogs. Bisamidines are more active toward bacteria while the monoamidines are more active toward mammalian cells (as indicated by low CC₅₀ values). Importantly, we identified compound **P12a** (MBX 1887) with a relatively narrow spectrum against bacteria and a very high CC₅₀ value. Compound **P12a** has been scaled up and is currently undergoing further evaluations for therapeutic applications.

New therapies are urgently needed for treatment of Gram-negative bacterial infections. Due to the emergence of bacterial strains resistant to all classes of β -lactam antibiotics (penicillins, cephalosporins, and carbapenems), aminoglycosides, and quinolones we have

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reached a crisis in the availability of effective therapy.¹ Among the five novel antibiotics introduced since 2000 (linezolid, daptomycin, retapamulin, fidaxomicin and bedaquiline), none are effective against Gram-negative infections.² All of the newly approved antibiotics for Gram-negative infections are analogs of known drugs, e.g., β -lactams, fluoroquinolones, tetracyclines and macrolides.² To manage infections complicated by the continued development of resistance, new therapeutic approaches and rapid development of novel antibiotics are essential.

In collaboration with the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), we have developed a series of bisamidine compounds that show potent activities against prevalent Gram-negative and Gram-positive pathogens.³ Details on the synthesis and structure-activity relationship studies of compounds in the symmetrical head-to-head bisindole series (Figure 1) have been published elsewhere.⁴ In this report we describe the synthesis and biological evaluation of a new, unsymmetrical series, in which one of the indole rings in the triaryl core has been replaced with another aromatic unit (benzimidazole, imidazopyridine, benzofuran, benzothiophene, pyrimidine or benzene) and the central linker unit contains benzene, thiophene, or pyridine rings.

Our initial synthetic efforts focused on the synthesis of unsymmetrical compounds containing an indole, a phenyl linker, and a non-indole 5:6 heterocyclic system. These compounds were prepared from the following three indole derivatives: *N*-Boc-2-(4-bromophenyl)indole-6-carbonitrile (**5**), (4-(*N*Boc-6-cyanoindol-2-yl)phenyl)boronic acid pinacol ester (**6**), and (*N*-Boc-6-cyanoindol-2-yl)boronic acid (**7**) following path A, B or C as illustrated in Scheme 1. Piperidine-catalyzed condensation of 4-methyl-3-nitrobenzonitrile and 4-bromobenzaldehyde provided a mixture of *cis*- and *trans*-stilbenes **3**. Treatment of **3** with triethylphosphite led to reduction of the nitro group and formation of the indole **4**.⁵ Protection of the indole nitrogen with a Boc group provided the intermediate **5**, which could then be directly converted to the intermediate nitrile **9** by conducting a Suzuki-Miyaura cross-coupling⁶ with an aryl pinacolboronate (path A). Alternatively, borylation of the bromide **5** with bis(pinacolato)diboron in the presence of a palladium catalyst gave intermediate boronic acid ester **6**⁷ which could also be converted to **9** using palladium-catalyzed coupling methodology (path B). Intermediate **9** could also be synthesized by condensation of commercially available boronic acid **7** with aryl bromide **8** (path C). Subsequent treatment of compounds **9** with diamines in the presence of phosphorous pentasulfide provided the desired amidine products **10**.⁸

Preparation of benzimidazole and imidazopyridine containing analogs followed path C (Scheme 2). Condensation of 3,4-diaminobenzonitrile (**11**) and 4-bromobenzaldehyde (**2**) at reflux in the presence of sodium metabisulfite provided benzimidazole **12**.⁹ Treatment of 2-amino-5-cyanopyridine (**13**) and 2-amino-4-cyanopyridine (**14**) with 2,4'-dibromoacetophenone (**15**) provided the corresponding imidazopyridines **16** and **17**, respectively.¹⁰ Coupling of the diarylbromides **12**, **16** and **17** with (*N*-Boc-6-cyanoindol-2-yl)boronic acid (**7**), followed by amidine formation provided products **P1a** to **P3b**.

Preparation of benzofuran- and benzothiophene-containing analogs followed path A (Scheme 3). The preparation of (6-cyanobenzofuran-2-yl)boronic acid pinacol ester (**24**) has

been optimized and scaled up to hundreds of grams and involves no chromatographic purifications.¹¹ The synthesis began acid (**18**) to provide the iodide **19** in 51% yield.¹² Conversion of the carboxylic group of **19** to the corresponding nitrile was accomplished in 3 steps, and involved chlorination, amination and dehydration to provide the nitrile **20** in 51% overall yield.¹³ Sonogashira coupling of **20** with TMS-acetylene gave arylacetylene **21** in 89% yield.¹¹ Treatment of **21** with CuI and triethylamine provided benzofuran-6-carbonitrile (**22**) in 68% yield.¹¹ Iridium-catalyzed borylation of **22** and the commercially available benzofuran-5-carbonitrile (**23**) occurred selectively at the 2-position to provide (6-cyanobenzofuran-2-yl)boronic acid pinacol ester (**24**) and (5-cyanobenzofuran-2-yl)boronic acid pinacol ester (**25**) in 74% and 83% yields, respectively.¹¹ Cross-coupling of **24**, **25** and the commercially available (6-cyanobenzothiophen-2-yl)boronic acid (**26**) with the intermediate **5**, followed by amidine formation as described above, provided products **P4** to **P6f**.

Minimum inhibitory concentration (MIC) values for compounds **P1a-P6f** against a range of clinically important Gram-positive and Gram-negative pathogens, including those proficient and deficient in efflux mechanisms, as well as cytotoxic concentration (CC₅₀) values against human HeLa cells, are shown in Table 1. Compounds containing benzimidazole and imidazopyridine heterocyclic systems are more susceptible to efflux by *E. coli* than those containing benzothiophene and benzofuran systems, as the MIC values against the efflux deficient (TolC⁻) strains are substantially lower than those against the wild-type (TolC⁺) strains. Against the efflux-deficient (TolC⁻) strain, all compounds are very active (MIC <1 µg/mL). The MIC values against *K. pneumonia* closely resemble those against the *E. coli* wild type strain (differences within 2-fold). In contrast, their MIC values against *P. aeruginosa* varied widely, from <1 µg/mL (**P6c**) to >80 µg/mL (**P2a**, **P6e**). These values remained essentially the same against the efflux deficient strain PAO1 (mexAB-oprM), indicating that the reduced potency against *Pseudomonas* for some compounds was not due to efflux by the mexAB-oprM pump. Most of the compounds are broadly active against Gram-positive pathogens with the exception of compounds **P2a** and **P3a**. Notably, the tetrahydropyrimidine amidine homologs **P2b** and **P3b** are still very potent against MRSA, VRE and *B. anthracis*, following a trend that we found for the bisindoles series.⁴ The differences in MIC values caused by a simple change from a 5-membered ring to 6-membered ring amidine in these analogs are striking. Effects of the amidine group on activity were further examined in the indole-benzofuran containing scaffold. Again, the tetrahydropyrimidine amidine imparts the greatest activity; methylimidazoline, hydroxyl- or methoxytetrahydropyrimidine amidines all exhibit significantly lower potency against *P. aeruginosa*. The fluorotetrahydropyrimidine amidine makes compound **P6f** nearly as active as the corresponding norfluoro analog **P6c**, but less cytotoxic. Lastly, we noted that the position of the amidine on the imidazopyridine does not affect the activity but on the benzofuran it has a moderate effect, with the 6-position being generally more preferred. An exception is that **P5** is more potent than **P6c** against *B. anthracis*.

To determine whether both amidine groups are required for antibacterial activity, we prepared a number of analogs that have different amine-containing groups. Preparation of these analogs followed paths A and B as shown in Scheme 4. Coupling of intermediate **6**

with commercially available building blocks **30**, **31** and intermediate **5** with (commercially available) **34** followed by amidine formation provided products **P7a** to **P9b**.

MIC and CC₅₀ values for **P7a** to **P9b** are shown in Table 2. Overall, these compounds possess significantly lower antibacterial activities compared to the bisamidine compounds, and higher cytotoxicity against HeLa cells. Notably, **P7a** and **P8a** are potent against the efflux deficient strain of *E. coli* but are not active against the wild type strain, suggesting that they are strongly susceptible to efflux. Compounds **P7a**, **P8a**, **P9a** and **P9b** are active against Gram-positive pathogens. These activities sharply decline in the methylimidazoline isomers, **P7b** and **P8b**. Lastly, compounds **P9a** and **P9b** are highly cytotoxic. Thus, the data suggests that the bisamidine is important for activity against bacteria, especially Gram-negative bacteria, while monoamidines have more inhibitory effects on the growth of mammalian cells.

Because the benzofuran-containing analog **P6c** has the most potent antibacterial activity of this series and an acceptable level of cytotoxicity, we decided to explore the indole-aryl-benzofuran scaffold further by varying the central linker unit and the amidine substitutions. Preparation of these compounds followed a route similar to path A, as shown in Scheme 5. Syntheses of *N*-Boc-2-(4-bromo-3-fluorophenyl)indole-6-carbonitrile (**37**) and *N*-Boc-2-(5-bromothiophen-2-yl)indole-6-carbonitrile (**40**) were based on the route used previously for the preparation of **5**. Thus, condensation of 4-bromo-3-fluorobenzaldehyde (**35**) and 5-bromothiophene-2-carboxaldehyde with 4-methyl-3-nitrobenzonitrile (**1**), followed by treatment with triethylphosphite, and subsequent protection of the indole nitrogen with a Boc group, provided 6-cyanoindoles **37** and **40**, respectively. Coupling of **37**, **40** and **42**¹⁴ with (6-cyanobenzofuran-2-yl)boronic acid pinacol ester (**24**) followed by amidine formation provided products **P10** to **P13d**.

Compounds with a 3-methyl substituent on the indole were prepared as shown in Scheme 6, using the Fischer indole synthesis. Condensation of 3-cyanophenylhydrazine (**43**)¹⁵ with 4'-bromopropiophenone (**44**) provided a mixture of regioisomers **45** and **46**, which were carried on to the next step. Protection of the indole nitrogen with a Boc group followed by coupling of the product mixture with (6-cyanobenzofuran-2-yl)boronic acid pinacol ester (**24**) provided triaryl compounds **49** and **50**, which were separated by silica gel chromatography. Amidination of **49** provided the bisamidine **P14** as expected. Under the same conditions, only one nitrile group in **50** was converted to the corresponding amidine, leading to isolation of the monoamidine product **P15**. We speculate that the 4-cyanoindole functionality in bis-nitrile **50** is hindered with respect to the 6-cyanoindole functionality in bis-nitrile **49**, which is the reason for the production of monoamidated compound **P15**.

MIC and CC₅₀ values for the indole-benzofuran analogs with various linkers are summarized in Table 3. Introduction of methyl and fluoro groups to the core provided potent compounds (**P14**, **P10**), while the more polar diaminopropane functionality was less effective (**P11**). This pattern is consistent with what we observed in the first series (Table 1): addition of nitrogen atoms to the core produced compounds with higher levels of efflux. Replacing the phenyl linker with pyridine or thiophene led to compounds that were more susceptible to efflux and less active against Gram-negative bacteria. Notably, thiophene-

linked compound **P12a** showed very low cytotoxicity. This unique feature suggests that **P12a** could be useful for narrow-spectrum treatment of select pathogens. For example, we found that **P12a** is very active against *Francisella tularensis* (MIC 0.063–2.5 µg/mL across 13 strains tested). We have subsequently optimized the synthesis of **P12a** to the hundred-gram scale. Further evaluation of this compound is pending and will be reported in the future.

In summary, we have prepared 34 new unsymmetrical triaryl bisamidines and monoamidines compounds, featuring 15 new triaryl core structures, and evaluated them for antibacterial activities and cytotoxicity. We found that benzofuran can replace one indole ring from the original hit compound (MBX 1066) to deliver broad-spectrum compounds with improved potency such as **P5**, **P6c** and **P6f**. Introduction of a fluorine atom or a methyl group to the triaryl core led to the more potent analogs **P10** and **P14**, respectively. Bisamidines are more active toward bacteria while the monoamidines are more active toward mammalian cells (as indicated by low CC₅₀ values). Importantly, we have identified compound **P12a** with a relatively narrow spectrum of activity against bacteria and a very high CC₅₀ value. The synthesis of compound **P12a** has been optimized and scaled up, and the compound is currently under further evaluations for therapeutic applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Antibiotic Resistance Threats in the United States. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2013.
2. Butler MS, Blaskovich MA, Cooper MA. J. Antibiot. 2013; 66:571–591. [PubMed: 24002361]
- 3 (a). Panchal RG, Ulrich RL, Lane D, Butler MM, Houseweart C, Opperman T, Williams JD, Peet NP, Moir DT, Nguyen T, Gussio R, Bowlin T, Bavari S. Antimicrob. Agents Chemother. 2009; 53:4283–4291. [PubMed: 19635954] (b) Opperman TJ, Williams JD, Houseweart C, Panchal RG, Bavari S, Peet NP, Moir DT, Bowlin TL. Bioorg. Med. Chem. 2010; 18:2123–2130. [PubMed: 20188576] (c) Butler MM, Williams JD, Peet NP, Moir DT, Panchal RG, Bavari S, Shinabarger DL, Bowlin TL. Antimicrob. Agents Chemother. 2010; 54:3974–3977. [PubMed: 20625154] (d) Jacobs MR, Bajaksouzian S, Good CE, Butler MM, Williams JD, Peet NP, Bowlin TL, Endimiani A, Bonomo RA. Diagnostic Microbiology and Infectious Disease. 2011; 69:114–116. [PubMed: 21146724] (e) Panchal RG, Lane D, Boshoff HI, Butler MM, Moir DT, Bowlin TL, Bavari S. J. Antibiot. 2012:1–3.
4. Williams JD, Nguyen ST, Gu S, Ding X, Butler MM, Tashjian TF, Opperman TJ, Panchal RG, Bavari S, Peet NP, Moir DT, Bowlin TL. Bioorg. Med. Chem. 2013; 21:7790–7806. [PubMed: 24239389]

- 5 (a). Humphrey GR, Kuethe JT. Chem. Rev. 2006; 106:2875–2911. [PubMed: 16836303] (b) Sundberg RJ. J. Org. Chem. 1965; 30:3604–3610. (c) Majgier-Baranowska H, Williams JD, Li B, Peet NP. Tetrahedron Lett. 2012; 53:4785–4788.
6. Miyaura N, Suzuki A. Chem. Rev. 1995; 95:2457–2483.
7. Ishiyama T, Murata M, Miyaura N. J. Org. Chem. 1995; 60:7508–7510.
8. Li B, Pai R, Cardinale SC, Butler MM, Peet NP, Moir DT, Bowlin TL, Bavari S. J. Med. Chem. 2010; 53:2264–2276. [PubMed: 20155918]
9. Yamashita T, Tanaka H, Yamada S, Yamazaki Y. Synth. Comm. 2009; 39:2982–2988.
10. El Kazzouli S, Griffon Du Bellay A, Berteina-Raboin S, Guillaumet G, Delagrangé P, Caignard D-H. Eur. J. Med. Chem. 2011; 46:4252–4257. [PubMed: 21764185]
11. For small scale synthesis of **24**, see: Williams JD, Ding X, Nguyen ST, Vines KK, Peet NP. Syn. Comm. 2013; 43:1974–1979.
12. Masayuki, S.; Kazumi, T.; Kuniya, S.; Kazuyuki, S.; Kohichi, F.; Shunji, T.; Takashi, K. US2003/109547. 2003. p. A1
13. Takayanagi, M.; Nakagawa, T.; Yamanashi, M.; Takehana, S.; Takahashi, M.; Shoji, M.; Fukuda, Y.; Kayahara, T.; Sagi, K. EP976722. 2000. p. A1
14. For synthesis of **42**, see ref [4].
15. Alexander RS, Bai SA, Clark CG, Fevig JM, Glemmo RA, He K, Knabb RM, Lam PYS, Li R, Luetzgen JM, Orwat MJ, Pinto DJP, Rossi KA, Smallwood AM, Teleha CA, Wexler RR, Wong PC, Wright MR. J. Med. Chem. 2003; 46:4405–4418. [PubMed: 14521405]
16. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, M7-A7. Approved Standard-Seventh Edition. Clinical and Laboratory Standards Institute; Wayne, PA: 2006.
17. Marshall NJ, Goodwin CJ, Holt SJ. Growth Regul. 1995; 5:69–84. [PubMed: 7627094]

Figure 1.
Unsymmetrical and symmetrical scaffolds

Scheme 1.

General methods for compound preparation. Reagents and conditions: a) piperidine, 125 °C; b) P(OEt)₃, reflux; c) Boc₂O, DMAP, THF; d) PdCl₂(dppf), B₂pin₂, NaOAc, dioxane, 80 °C; e) P₂S₅, diamine, 120-130 °C.

Scheme 2.

Preparation of benzimidazole and imidazopyridine containing analogs. Reagents and conditions: a) $\text{Na}_2\text{S}_2\text{O}_5$, EtOH, DMSO, H_2O , 100 °C; b) **7**, $\text{Pd}(\text{Ph}_3\text{P})_4$ or $\text{Pd}(\text{OAc})_2/2$ -(Biphenyl)*t*-Bu₂P, K_2CO_3 , EtOH, toluene, 70 °C; c) $\text{CF}_3\text{CO}_2\text{H}$, MeOH, CH_2Cl_2 ; d) P_2S_5 , $\text{NH}_2\text{CH}_2(\text{CH}_2)_n\text{NH}_2$, 120 °C; e) EtOH, reflux.

Scheme 3.

Preparation of benzofuran and benzothiophene containing compounds. Reagents and conditions: a) ICl, AcOH, 45 °C; b) EtCO₂Cl, Et₃N, THF; c) NH₄OH; d) (CF₃CO)₂O, pyridine, CH₂Cl₂; e) TMS-acetylene, Pd(PPh₃)₂Cl₂, CuI, Et₃N, THF, 40 °C; f) CuI, Et₃N, EtOH, reflux; g) B₂pin₂, [Ir(OMe)COD]₂, hexanes; h) **5**, Pd(PPh₃)₄, Na₂CO₃, EtOH, toluene; i) P₂S₅, NH₂CHR(CH₂)_nNH₂, 120 °C; j) P₂S₅, NH₂CH₂CHR'CH₂NH₂, 120 °C.

Scheme 4.

Preparation of monoamidine compounds. Reagents and conditions: a) **6**, Pd(PPh₃)₄, Na₂CO₃, DME, H₂O, 75 °C; b) P₂S₅, NH₂-R'-CH₂NH₂, 120 °C; c) **5**, Pd(PPh₃)₄, Na₂CO₃, EtOH, toluene, 75 °C; d) P₂S₅, NH₂CH₂(CH₂)_nNH₂, 120 °C.

Scheme 5.

Preparation of compounds with the indole-aryl-benzofuran scaffold. Reagents and conditions: a) piperidine, sulfolane, 145 °C; a') piperidine, 110 °C; b) $P(OEt)_3$, 130 °C; c) Boc_2O , DMAP, THF; d) **24**, $Pd(PPh_3)_4$, Na_2CO_3 , EtOH, toluene, 70 °C; e) P_2S_5 , $NH_2(CH_2)_3NH_2$, 120 °C; f) **24**, $Pd(dppf)Cl_2$, Na_2CO_3 , MeCN, 70 °C; g) P_2S_5 , $NH_2CHR^2CH_2NH_2$, 120 °C; h) P_2S_5 , $NH_2CH_2CHR^3CH_2NH_2$, 120 °C

Scheme 6.

Synthesis of 3-methylindole containing compounds. Reagents and conditions: a) *p*-TsOH•H₂O, toluene, reflux; b) Boc₂O, DMAP, DMF; c) Pd(PPh₃)₄, Na₂CO₃, DME, H₂O, 85 °C; d) P₂S₅, NH₂(CH₂)₃NH₂, 120 °C.

Table 1

MIC and CC₅₀ values for compounds P1a – P6f

Cpd #	Structures		MIC (μg/mL) ^{a,b,c}								CC ₅₀ ^d	
	5,6-HetAr	Am	E.col+	E.col-	Paer	P.aer	K.pne	B.sub	MRSA	VRE	B.anth	HeLa
1066			1.25	0.16	7.5	2.5	0.24	0.12	0.16	0.16	0.31	32.5
P1a			10	0.31	20	40	20	0.16	1.25	0.63	5	8.6
P1b	''		2.5	0.31	2.5	2.5	5.0	0.08	0.31	0.31	0.31	27.6
P2a			40	0.63	>80	>80	80	0.31	80	20	40	-

Cpd #	Structures		MIC (μg/mL) ^{a,b,c}							CC ₅₀ ^d		
	5,6-HetAr	Am	E.col+	E.col−	P.aer	P.ae-	K.pne	B.sub	MRSA	VRE	B.anth	HeLa
P2b	"		5.0	0.31	5.0	5.0	5.0	0.08	0.63	0.63	1.25	19.7
P3a			2.5	0.31	40	>80	5.0	2.5	>80	>80	>80	2.5
P3b	"		2.5	0.31	40	>80	5.0	0.16	2.5	1.25	1.25	20.9
P4			0.63	0.31	10	10	1.25	0.10	0.31	0.31	0.16	5.9
P5			0.63	0.39	2.5	2.5	1.25	0.08	0.31	0.08	0.08	8.5
P6a			0.47	0.24	10.0	15.0	0.94	0.16	0.16	0.16	0.31	9.7

Cpd #	Structures		MIC (µg/mL) ^{a,b,c}							CC ₅₀ ^d		
	5,6-HetAr	Am	E.col+	E.col–	P.aer	P.ae-	K.pne	B.sub	MRSA	VRE	B.anth	HeLa
P6c	"		0.63	0.16	0.63	0.47	0.55	0.14	0.10	0.39	5.0	26
P6b	"		1.09	0.63	80.0	80.0	5.0	0.35	0.55	0.63	0.63	7.6
P6b-iso	"		0.63	0.63	60.0	80.0	2.5	0.20	0.31	0.39	0.31	5.7
P6d	"		2.5	0.16	6.3	15.0	1.09	0.08	0.31	0.31	0.55	11.9
P6e	"		2.5	0.24	>80	>80	1.25	0.08	0.31	0.63	0.31	6.0
P6f	"		1.25	0.27	5.0	2.5	0.63	0.16	0.31	0.31	0.31	47.5

^a MIC values obtained by the broth dilution method;¹⁶

^b bacterial strains: Ecol+ = *Escherichia coli* 700 TolC+, Ecol- = *E. coli* TolC-, P.aer = *Pseudomonas aeruginosa* PAO 1, P.ae- = *P. aeruginosa* PAO 1 (mexAB-oprM), K. pne = *Klebsiella pneumoniae* 13882, B. subs = *Bacillus subtilis* BD54, MRSA = methicillin-resistant *Staphylococcus aureus* 1094, VRE = vancomycin-resistant *Enterococcus faecalis* ATCC 51575, B. anth = *Bacillus anthracis* Sterne;

^c MIC 1: green; 1 < MIC 5: blue; 5 < MIC 20: yellow; MIC > 20: pink;

^d cytotoxicity determined by using the vital stain MTS,¹⁷ units in $\mu\text{g/mL}$.

Table 2

MIC and CC₅₀ values for compounds P7a – P9b^a

Cpd #	Structures	MIC (µg/mL)							CC ₅₀	
		E.col+ ^a	E.col- ^a	P.aer	K.pne	B. sub	MRSA	VRE	B. anth	HeLa
P7a		35	0.63	>80	70	0.78	7.5	3.75	0.55	2.0
P7b		>80	60.0	>80	>80	10.0	>80	>80	22.5	1.3
P8a		>80	0.63	>80	>80	0.63	2.5	5.0	0.63	5.7
P8b		>80	40.0	>80	>80	40.0	40.0	>80	40.0	1.1
P9a		>80	>80	>80	>80	0.63	1.04	3.3	2.1	0.63
P9b		>80	>80	>80	>80	0.63	1.09	3.1	0.47	0.63

^a bacterial strains: Ecol+ = *E. coli* 700 TolC+, Ecol- = *E. coli* TolC-, P.aer = *P. aeruginosa* PAO 1, K. pne = *K. pneumoniae* 13882, B. sub = *B. subtilis* BD54, MRSA = methicillin-resistant *S. aureus* 1094, VRE = vancomycin-resistant *E. faecalis* ATCC 51575, B. anth = *B. anthracis* Sterne; MIC 1: green; 1 < MIC 20: yellow; MIC >20: pink; CC₅₀ units in µg/mL

Table 3

MIC and CC₅₀ values for compounds P10 – P15^a

Cpd #	Structures		MIC (µg/mL)								CC ₅₀	
	linker	Am	E.col+	E.col-	P.aer	K.pne	B.sub	MRSA	VRE	B. anth	HeLa	
P10			0.63	0.31	2.5	0.63	0.08	0.16	0.16	0.08	14.3	
P11			2.5	0.63	2.5	1.25	0.24	0.63	1.25	0.63	4.6	
P12a			2.80	0.27	66.7	60.0	0.16	0.94	1.56	1.09	182.2	
P12b	..		1.25	0.63	26.7	20.0	0.31	1.04	0.63	0.52	14.5	

Cpd #	Structures		MIC (μg/mL)							CC ₅₀	
	linker	Am	E.col+	E.col−	P.aer	K.pne	B.sub	MRSA	VRE	B. anth	HeLa
P12c	''		0.63	0.31	10.0	2.5	0.08	0.31	0.31	0.16	8.7
P12d	''		5.0	0.47	40.0	15.0	0.43	1.88	2.5	1.25	12.7
P12e	''		1.25	0.31	53.3	2.5	0.16	0.63	0.63	0.31	22.5
P13a			1.25	0.16	1.25	1.56	0.08	0.31	0.39	0.16	15.6
P13b	''		1.88	0.27	5.0	2.5	0.08	0.31	0.63	0.63	2.2
P13c	''		80	0.63	25	8.8	3.75	2.5	5.0	1.09	16.7
P13d	''		8.3	0.31	8.3	2.5	0.16	0.83	0.63	0.63	19.6
P14	See scheme 6		0.31	0.31	1.25	0.31	0.08	0.16	0.16	0.08	1.5
P15	See scheme 6		20.0	10.0	40.0	20.0	2.5	5.0	5.0	2.5	-

^a bacterial strains: Ecol+ = *E. coli* 700 TolC+, Ecol- = *E. coli* TolC-, Paer = *P. aeruginosa* PAO 1, K, pne = *K. pneumoniae* 13882, B, subs = *B. subtilis* BD54, MRSA = methicillin-resistant *S. aureus* 1094, VRE = vancomycin-resistant *E. faecalis* ATCC 51575, B, anth = *B. anthracis* Sterne; MIC 1: green; 1 < MIC 5: blue; 5 < MIC 20: yellow; MIC > 20: pink; CC50 units in µg/mL