

The Synthesis of Dicationic Extended Bis-Benzimidazoles

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Abstract: The synthesis of extended dicationic bis-benzimidazoles starting from *trans*-1,2-bis(4-cyanophenyl)ethene and *trans*-1,2-bis(4-cyanophenyl)cyclopropane is reported. The target diamidines show significant *in vitro* activity against *B. subtilis*.

Keywords: DIBAL reduction, benzimidazoles, amidines, *Bacillus subtilis*.

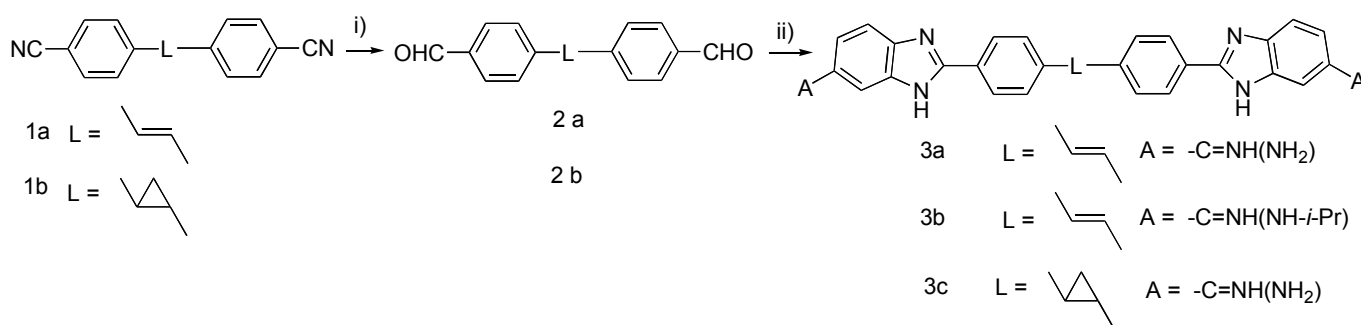
Introduction

Aromatic diamidines have a long history as antimicrobial agents [1]. Bis-benzimidazoles bearing amidino groups have been reported to show activity against a number of microorganisms. Flexible alkyl linked dicationic bis-benzimidazoles [2] and more ridged aryl linked bis-benzimidazoles are active *in vivo* against *Pneumocystis carinii* pneumonia in an immunosuppressed rat model [3]. The later type molecules also show promising *in vitro* activity against several fungal organisms [4,5]. As part of a general program of development of aromatic diamidines as antimicrobial agents [1,6] we report the synthesis and preliminary evaluation of new bis-benzimidazoles which are linked by diphenylethene and diphenylcyclopropane units that provide extended semi-rigid analogs.

Results and Discussion

The synthesis of the new bis-benzimidazoles, outlined in Scheme 1, begins with DIBAL reduction of the appropriate bis-nitriles (**1a** and **1b**) to provide the corresponding bis-aldehydes (**2a** and **2b**) in moderate yields. The bis-aldehydes **2a** and **2b** were converted into the target molecules **3a-3c** by 1,4-benzoquinone facilitated oxidative coupling of the aldehydes with the appropriate 3,4-diamino-benzamidines in 60-70% yields.

Scheme 1.



Reagents and conditions: i) DIBAL, CH₂Cl₂

ii) 3,4-diaminobenzamidine or 3,4-diamino-*N*-isopropylbenzamide, 1,4-benzoquinone, CH₂Cl₂-EtOH

Compounds **3a-3c** were screened against *B. subtilis* N10 and *E. coli* AB1157. The compounds were inactive against *E. coli*, but showed good anti-*Bacillus* activity as indicated in Table 1. The compounds were also tested for mammalian cell in vitro cytotoxicity using CV1 Vero cells with the XTT cytotoxicity test (Table 1) [7]. A desirable therapeutic index (2 log differential) was noted for **3c**.

Table 1. Antimicrobial Screening Results

Compound	MIC/MBC <i>B. subtilis</i> N10	Cell toxicity CV1 IC ₅₀	Therapeutic Index (IC ₅₀ /MIC)
3a	<0.4/ND	ND	-
3b	0.4/11	8.0	20
3c	0.4/>100	80.0	200

All values are in μM. ND = not done

Conclusions

We have presented a facile route for the synthesis of the benzimidazoles **3a-3c** and demonstrated that these molecules show significant in vitro activity versus *B. subtilis*.

Experimental

General

Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded employing a Varian GX400 instrument and chemical shifts (δ) are in ppm relative to TMS used as internal standard. Mass spectra were recorded on a VG analytical 70-SE spectrometer. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA. All chemicals and solvents were purchased from Aldrich Chemical Co. or Fisher Scientific.

trans-1,2-Bis(4-formylphenyl)ethane (**2a**). Diisobutyl aluminum hydride (12 mL, 12 mmol, 1M in dichloromethane) was slowly added under nitrogen to a stirred solution of *trans*-1,2-bis(4-cyanophenyl)ethene (**1a**) [8] (0.92 g, 4.0 mmol) in anhydrous dichloromethane (100 mL). The mixture was heated at reflux for 4 h, then treated with a mixture of ice and 10% sulfuric acid and extracted with dichloromethane. The organic layer was dried over sodium sulfate and the solvent was evaporated. The crude product was purified by flash chromatography using ethyl acetate and hexane (1:2) as the eluent. The solid obtained was crystallized from ethanol to yield 0.56 g (59 %) of **2a**, mp 167-169 °C (lit.[9] mp 165-170°C); MS: m/z : 236 (M^+); ^1H -NMR (DMSO- d_6) δ 10.01 (s, 2H), 7.93 (d, 4H, $J = 8.1$ Hz), 7.86 (d, 4H, $J = 8.1$ Hz), 7.54 (s, 2H); ^{13}C -NMR (DMSO- d_6) δ 192.4, 142.4, 135.1, 130.7, 130.0, 127.4; Anal. Calcd. for $\text{C}_{16}\text{H}_{12}\text{O}_2$: C, 81.33; H, 5.12. Found: C, 81.09; H, 5.436.

trans-1,2-Bis[4-(5-amidino-2-benzimidazolyl)phenyl]ethane hydrochloride (**3a**). A solution of *trans*-1,2-Bis(4-formylphenyl)ethane (**2a**) (0.21 g, 0.90 mmol) in dichloromethane (10 mL) was stirred until all the solid dissolved. Absolute ethanol (60 mL), 3,4-diaminobenzamidine hydrochloride [10] (0.34 g, 1.8 mmol), and 1,4-benzoquinone (0.20 g, 1.8 mmol) were added to this solution. The mixture was heated at reflux for 4 h under nitrogen. After cooling to 0 °C anhydrous ethyl ether was added and the resulting precipitate was filtered and washed with dry ether. The solid was dissolved in absolute ethanol (25 mL) and absolute ethanol saturated with hydrogen chloride gas (35 mL), and the mixture was heated at gentle reflux for 2 h. After cooling to 0 °C, addition of dry ether yielded a precipitate. The solid was filtered, washed with dry ether, and crystallized from ethanol to yield 0.40 (69 %) of desired product **3a**, mp > 300 °C; MS for $\text{C}_{30}\text{H}_{24}\text{N}_8$ (free base): 497.1 ($\text{M}+\text{H}$); ^1H -NMR (DMSO- d_6) δ 8.47 (d, 4H, $J = 7.6$ Hz), 8.27 (s, 2H), 7.92 (d, 4H, $J = 7.6$ Hz), 7.92 (d, 2H, $J = 7.6$ Hz), 7.82 (d, 2H, $J = 7.6$ Hz), 7.57 (s, 2H); ^{13}C -NMR (DMSO- d_6) δ 162.8, 150.2, 146.3, 140.8, 139.1, 138.6, 129.5,

128.4, 127.6, 126.9, 122.8, 120.1, 115.2; Anal. Calcd. for $C_{30}H_{28}N_8Cl_4 \cdot 1.5H_2O$: C, 53.82; H, 4.67; N, 16.74. Found: C, 53.86; H, 4.79; N, 16.49.

trans-1,2-Bis[4-(5-*N*-isopropylamidino-2-benzimidazolyl)phenyl]ethene hydrochloride (**3b**). Solutions of *trans*-1,2-bis(4-formylphenyl)ethene (**2a**, 0.21 g, 0.90 mmol) in CH_2Cl_2 (10 mL) and 3,4-diamino-*N*-isopropylbenzimidine hydrochloride [11] (0.42 g, 1.8 mmol) in ethanol (60 mL) and 1,4-benzoquinone (0.20 g, 1.8 mmol) were allowed to react and then worked up as described above for **3a** to yield 0.46 (71 %) mp > 300 °C; MS for $C_{36}H_{36}N_8$ (free base): 581.4 (M+H); 1H -NMR (DMSO- d_6) δ 8.50 (d, 4H, J = 8.4 Hz), 8.14 (s, 2H), 7.94 (d, 4H, J = 8.4 Hz), 7.91 (d, 2H, J = 8.4 Hz), 7.71 (d, 2H, J = 8.4 Hz), 7.61 (s, 2H), 4.11 (m, 2H), 1.31 (d, 12H, J = 6.4 Hz); ^{13}C -NMR (DMSO- d_6) δ 162.3, 153.7, 152.2, 149.6, 141.5, 138.8, 129.0, 128.5, 127.2, 127.0, 122.4, 121.9, 115.6, 44.9, 21.1; Anal. Calcd. for $C_{36}H_{40}N_8C_{14}$: C, 59.51; H, 5.55; N, 15.42. Found: C, 59.34; H, 5.64; N, 14.98.

trans-1,2-Bis(4-formylphenyl)cyclopropane (**2b**). A solution of *trans*-1,2-bis(4-bromophenyl)cyclopropane [12] (1.0 g, 2.8 mmol) and copper cyanide (2.1 g, 22 mmol) in *N,N*-dimethylformamide (10 mL) was heated at reflux for 24 h under nitrogen. The mixture was poured onto ice. The precipitate was filtered and then extracted three times with boiling chloroform (3x30 mL). The combined organic extracts were washed two times with hydrochloric acid (2x50 mL) and three times with water (3x50 mL), and dried over sodium sulfate. After evaporation of solvent, the bis-nitrile *trans*-1,2-bis(4-cyanophenyl)cyclopropane (0.30 g, 44 %) was obtained by crystallization from ethanol, mp 124–126 °C; MS: m/z: 244 (M^+); 1H -NMR (acetone- d_6) δ 7.69 (d, 4H, J = 8.1 Hz), 7.43 (d, 4H, J = 8.1 Hz), 2.48 (t, 2H, J = 7.8 Hz), 1.72 (t, 2H, J = 7.8 Hz); ^{13}C -NMR (DMSO- d_6) δ 147.9, 132.1, 126.5, 118.9, 108.4, 28.7, 19.9. Anal. Calcd. for $C_{17}H_{12}N_2$: C, 83.58; H, 4.95; N, 11.47. Found: C, 83.81; H, 4.90; N, 11.35.

The bis-nitrile (0.49 g, 2.0 mmol) in dry methylene chloride (50 mL) and diisobutylaluminum hydride (6.0 mL, 6.0 mmol, 1 M in dichloromethane) were allowed to react and worked up as described for **2a** to yield 0.32 g (64 %) of **2b**, mp = 145–146 °C; MS: m/z: 250 (M^+); 1H -NMR (acetone- d_6) δ 10.00 (s, 2H), 7.86 (d, 4H, J = 8.0 Hz), 7.44 (d, 4H, J = 8.0 Hz), 2.50 (t, 2H, J = 7.2 Hz), 1.74 (t, 2H, J = 7.2 Hz); ^{13}C -NMR (acetone- d_6) δ 192.4, 150.5, 135.9, 130.6, 127.3, 30.0, 20.6; Anal. Calcd. for $C_{17}H_{14}O_2$: C, 81.58; H, 5.64. Found: C, 81.51; H, 5.72.

trans-1,2-Bis[4-(5-amidino-2-benzimidazolyl)phenyl]cyclopropane hydrochloride (**3c**). Solutions of *trans*-1,2-bis(4-formylphenyl)cyclopropane (**2b**, 0.38 g, 1.5 mmol) in dichloromethane (10 mL), 3,4-diaminobenzimidine hydrochloride [10] (0.56 g, 3.0 mmol) in ethanol (80 mL) and 1,4-benzoquinone were allowed to react and worked up as describe for **3a** to yield 0.80 g (82 %) of **3c**, mp > 300 °C; MS for $C_{31}H_{26}N_8$ (free base): 511.1 (M+H); 1H -NMR (DMSO- d_6) δ 8.42 (d, 4H, J = 8.0 Hz), 8.28 (s, 2H), 7.94 (d, 2H, J = 8.8 Hz), 7.87 (d, 2H, J = 8.8 Hz), 7.54 (d, 4H, J = 8.0 Hz), 2.55 (t, 2H, J = 7.2 Hz), 1.80 (t, 2H, J = 7.2 Hz); ^{13}C -NMR (DMSO- d_6) δ 165.6, 152.3, 147.6, 137.8, 134.5, 128.2, 126.5, 124.1, 123.6, 122.5, 115.2, 114.4, 29.0, 20.0; Anal. Calcd. for $C_{31}H_{30}N_8Cl_4 \cdot 0.5H_2O$: C, 55.95; H, 4.70; N, 16.84. Found: C, 55.94; H, 4.73; N, 16.66.

MIC, MBC and in vitro cell toxicity determinations.

B. subtilis N10 and *E. coli* AB1157 were grown in LB broth at 35°C for liquid culture or plated on LB plates containing 1.5% agar [13]. *In vitro* cell toxicity tests were performed as described in the literature [7] using CV1 cells cultured in Glutamax RPMI medium (Invitrogen) plus 5% fetal bovine serum (Atlanta Biologicals), 100 U of penicillin G/mL, 100 µg streptomycin/mL and 0.25 µg of amphotericin B/mL. XTT was from Sigma. A rapid screen was initially performed to evaluate potential antimicrobial properties of the compounds. Approximately 0.1 mL of overnight cultures of several test strains grown in LB broth were spread on LB agar plates. After drying, a 2 µL spot of a 10 mM stock solution for the test compounds was placed on the plate. After overnight incubation at 35°C, zones of inhibition were measured with a ruler; compounds with inhibition zones greater than 3 mm were tested for their MIC and MBC values.

The minimum inhibitory concentration test (MIC) used a broth microdilution method in 96 well culture plates by placing 100 µL of LB broth in the wells. Test compounds were placed in the top row for a final concentration of 100 µM. Three-fold dilutions were performed down the wells of the plate with a final dilution of 0.4 µM. 100 µL of an overnight culture diluted to approximately 1×10^4 cells/mL was then placed in each well. After overnight incubation at 35°C, plates were scored for turbidity. The MIC was scored as the lowest concentration that appeared clear after overnight incubation.

The minimum bactericidal concentration (MBC) test was performed on wells that appeared clear after overnight incubation. 50 µL of the culture was spread on a LB agar plate and incubated overnight. The MBC was scored at the lowest concentration of test compound that resulted in no growth of the bacterial strain.

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