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, \( \text{CH}_2\text{Cl}_2 \)
ii) 3,4-diaminobenzamidine or 3,4-diamino-\( \text{N} \)-isopropylbenzamidine, 1,4-benzoquinone, \( \text{CH}_2\text{Cl}_2 \)-\( \text{EtOH} \)

Compounds 3a-3c were screened against \( B. \text{subtilis} \) N10 and \( E. \text{coli} \) AB1157. The compounds were inactive against \( E. \text{coli} \), but showed good anti-\( \text{Bacillus} \) 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**

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC/MBC ( \text{B. subtilis N10} )</th>
<th>Cell toxicity ( \text{CV1 IC}_{50} )</th>
<th>Therapeutic Index (( \text{IC}_{50}/\text{MIC} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>&lt;0.4/ND</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>3b</td>
<td>0.4/11</td>
<td>8.0</td>
<td>20</td>
</tr>
<tr>
<td>3c</td>
<td>0.4/&gt;100</td>
<td>80.0</td>
<td>200</td>
</tr>
</tbody>
</table>

All values are in \( \mu \text{M} \). 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. $^1$H 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$^+$); $^1$H-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 C$_{16}$H$_{12}$O$_2$: C, 81.33; H, 5.12. Found: C, 81.09; H, 54.36.

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-diaminobenzamide 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 C$_{30}$H$_{24}$N$_8$ (free base): 497.1 (M+H); $^1$H-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,
trans-1,2-Bis[4-(5-N-isopropylamidino-2-benzanidazolyl)phenyl]ethene hydrochloride (3b). Solutions of trans-1,2-bis(4-formylphenyl)ethene (2a, 0.21 g, 0.90 mmol) in CH$_2$Cl$_2$ (10 mL) and 3,4-diamino-N-isopropylbenzamidine hydrochloride [11] (0.42 g, 1.8 mmol) in ethanol (60mL) 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); $^1$H-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); 13C-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$_8$Cl$_4$: 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-cyano-phenyl)cyclopropane (0.30 g, 44 %) was obtained by crystallization from ethanol, mp 124-126 °C; MS: m/z: 244 (M $^+$); $^1$H-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 $^+$); $^1$H-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-diaminobenzamidine hydrochloride [10] (0.56 g, 3.0 mmol) in ethanol (80mL) 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); $^1$H-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$_8$Cl$_4$$\cdot$0.5H$_2$O: 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 X 10⁴ 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.

References


*Sample Availability:* Not Available