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Identification of 2-Aminobenzimidazole Dimers as Antibacterial Agents

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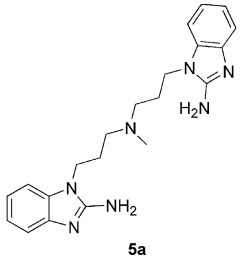
Abstract—The preparation and evaluation of 2-aminobenzimidazole dimers as antibacterial agents is described. Biological screening of the dimers indicated that compounds with multiple chloro substituents possessed optimal antibacterial activity.

The rapid emergence of multidrug resistant pathogenic bacteria has become a serious health threat worldwide.[1] It has been postulated that the development of resistance to known antibiotics could be overcome by identifying new drug targets via genomics, improving existing antibiotics and by identifying new antibacterial agents with novel structures and modes of action.[2–4] As part of a program aimed at identifying new antibacterial agents, 2-aminobenzimidazole dimer **5a** was identified as having weak bacterial growth inhibition activity (Fig. 1). A survey of the existing literature indicated that there were no reports, which described the use of 2-aminobenzimidazole dimers as antibacterial agents. Therefore, we initiated a study to further explore the antibacterial activity of this class of compounds.

Synthesis of 2-aminobenzimidazole dimers **5a–i** was accomplished by SN_{Ar} displacement of fluorine in **1a–i** with amine **2** to provide nitroanilines **3a–i**. The nitroanilines **3a–i** were reduced using Raney–Nickel to provide the corresponding phenylenediamines **4a–i**, which were cyclized using CNBr to provide the desired 2-aminobenzimidazole dimers **5a–i** in moderate yield (Scheme 1, 20–30% over three steps).[5] All dimers were purified by reverse phase preparative HPLC (>95% purity) prior to biological testing.



5a

Figure 1. 2-Aminobenzimidazole dimer.

Dimers **5a–i** were evaluated for their ability to inhibit bacterial growth against both Gram positive and Gram negative organisms (Table 1).[6–8] Initial screening revealed that dimers **5b** and **5c** that possess a nonpolar chloro and trifluoromethyl group, respectively, at the 5-position of the benzimidazole nucleus, showed a slight improvement in antibacterial activity (entries 2–3) as compared to the unsubstituted dimer **5a**. However, the antibacterial activity disappeared when the substituent at C-5 was an electron withdrawing cyano (**5e**) or ester (**5f**) group or a bromine (**5d**) atom (entries 4–6). Similarly, dimers **5g** and **5h** that possess an electron donating substituent at C-6 were devoid of antibacterial activity (entries 7–8). A dramatic improvement in antibacterial activity was observed for dimer **5i**, which has chloro substituents at the 5- and 6-position of the benzimidazole nucleus (entry 9).

Due to the enhanced biological activity of dichloro substituted dimer **5i**, it was decided to maintain this substitution pattern for further synthetic and biological studies. To explore the importance of the tertiary nitrogen atom in the alkyl spacer, dimers **8** and **9** were prepared as shown in Scheme 2. Screening of dimers **8** and **9** indicated that they did not inhibit bacterial growth at 100 μM. To ascertain the significance of the alkyl spacer

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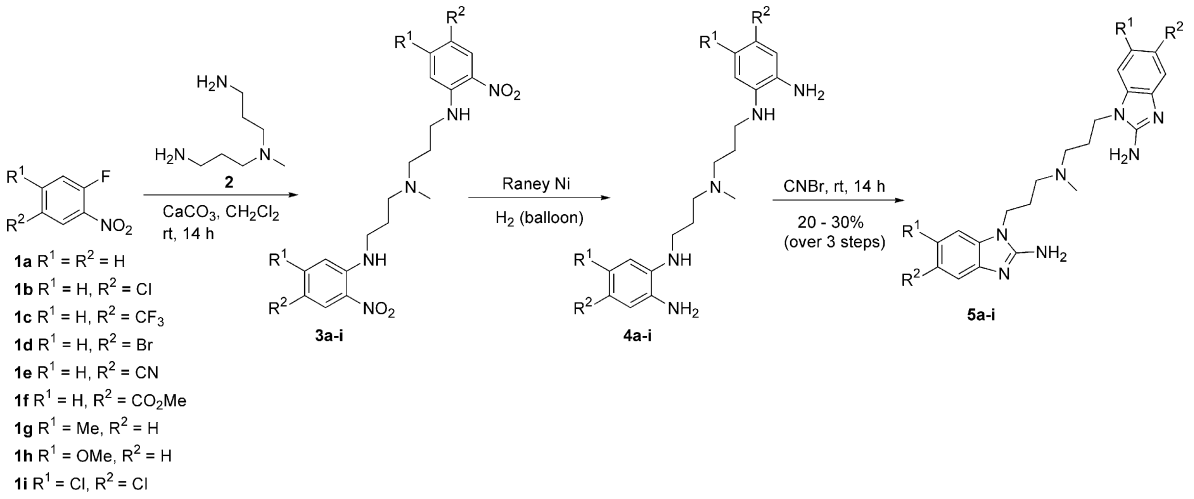


Scheme 1. Preparation of 2-aminobenzimidazole dimers.

length, dimer **11** was also prepared (Scheme 2).[5] Biological screening indicated that dimer **11** also possessed moderate to good antibacterial activity against both, Gram positive and Gram negative bacteria (entry 12). To examine the optimal position of the alkyl spacer, N2 substituted dimers **13** and **14** were prepared starting from 2,5,6-trichlorobenzimidazole[9] (Scheme 2). Biological screening indicated that the N2 substituted dimers had good antibacterial activity, comparable to the N1 substituted dimers **5i** and **11** (entries 13 and 14).

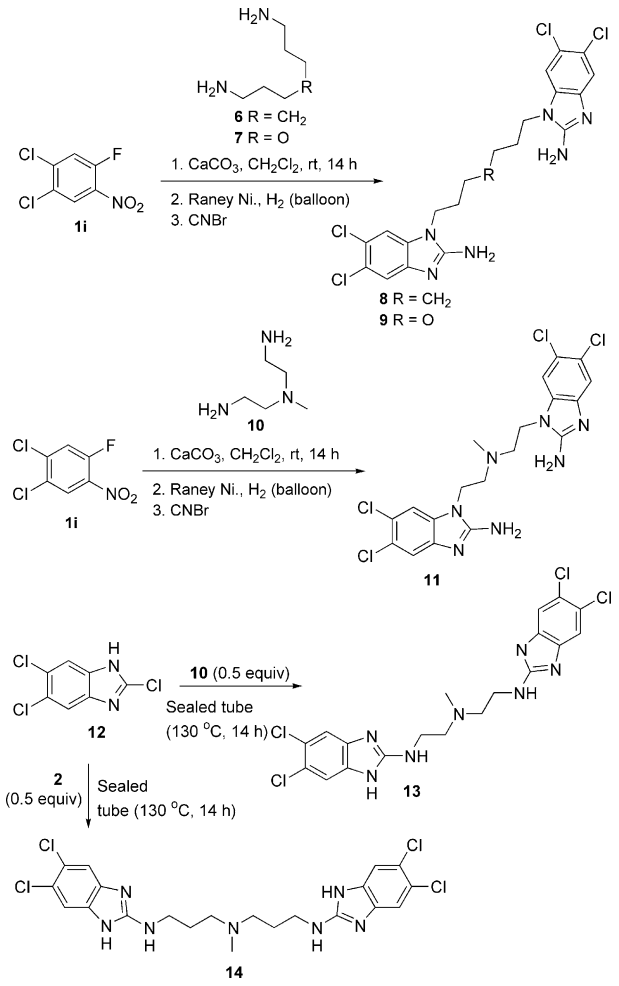
Lastly, importance of the 2-aminobenzimidazole dimer pharmacophore was probed by evaluating 2-aminobenzimidazoles **15** and **16** as well as benzimidazole dimer **17** for antibacterial activity (Fig. 2). Screening results indicated that 2-aminobenzimidazoles **15** and **16** were not biologically active. Benzimidazole dimer **17** missing the 2-amino group, showed only weak antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus* (entry 15).

Analysis of the biological data shows that the 2-amino group (primary or secondary) and the dimeric structure are crucial for antibacterial activity. Depending on the organism, removal of the 2-amino group (**17**) results in a 4- to 16-fold reduction of biological activity while removal of the dimeric structure (**15** and **16**) abolishes activity completely. Replacement of the tertiary amine in the tether with an oxygen (**9**) or methylene (**8**) abolishes biological activity. This result suggests that a positively charged nitrogen (at physiological pH) in the tether is important for antibacterial activity. Activity is retained when the alkyl spacer chain is moved from the N1 ring nitrogen to the 2-amino group on the benzimidazole nucleus (**13** and **14**). The length of the spacer between the aromatic rings also appears to have some effect on the biological activity. In general, dimers with a three carbon spacer (**5i** and **14**) between the aromatic ring and the basic amine possess better activity compared to similar dimers (**11** and **13**) with a two carbon spacer. Substitution at the 5-position of the benzimidazole

Table 1. In vitro antibacterial activity of 2-aminobenzimidazole dimers

Entry	Compd[a]	MIC[b,c] (μM) *E. coli*	MIC (μM) *S. aureus*	MIC (μM) *S. pyogenes*	MIC (μM) *E. faecalis*
1	**5a** (R^1 = H, R^2 = H)	50–100	50–100	NT[d]	NT
2	**5b** (R^1 = H, R^2 = Cl)	25–50	25–50	25–50	12–25
3	**5c** (R^1 = H, R^2 = CF_3)	12–25	25–50	12–25	25–50
4	**5d** (R^1 = H, R^2 = Br)	> 100	> 100	NT	NT
5	**5e** (R^1 = H, R^2 = CN)	> 100	> 100	NT	NT
6	**5f** (R^1 = H, R^2 = CO_2Me)	> 100	> 100	NT	NT
7	**5g** (R^1 = Me, R^2 = H)	> 100	> 100	NT	NT
8	**5h** (R^1 = OMe, R^2 = H)	> 100	> 100	NT	NT
9	**5i** (R^1 = Cl, R^2 = Cl)	3–6	6–12	3–6	3–6
10	**8** (R^1 = Cl, R^2 = Cl)	> 100	> 100	NT	NT
11	**9** (R^1 = Cl, R^2 = Cl)	> 100	> 100	NT	NT
12	**11**	12–25	6–12	3–6	1.5–3
13	**13**	6–12	12–25	6–12	1.5–3
14	**14**	6–12	3–6	3–6	3–6
15	**17**	50–100	25–50	NT	NT

[a]All dimers were purified by preparative HPLC and tested as their trifluoroacetate or acetate salts.
[b]Minimum inhibitory concentration of compound that inhibited visible growth.
[c]As a control, ciprofloxacin gave MIC values of 0.04–0.07 μM (*E. coli*), 0.3–0.6 μM (*S. aureus*), 1.2–2.5 μM (*S. pyogenes*) and 1.2–2.5 μM (*E. faecalis*) in our assay.
[d]Not tested.



Scheme 2. Preparation of dichlorosubstituted 2-aminobenzimidazole dimers.

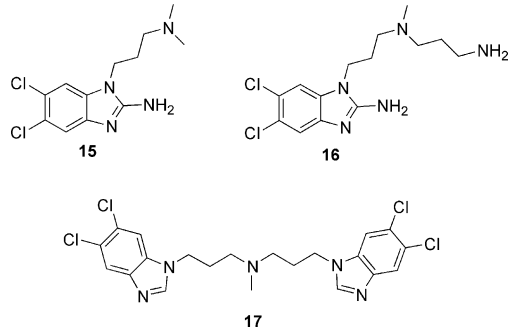


Figure 2. Other benzimidozole structures evaluated for antibacterial activity.

nucleus with nonpolar groups such as chloro and trifluoromethyl is tolerated and results in a modest increase in MIC activity (**5b–c**). The same beneficial effect was not seen when the substituent at C-5 was an electron withdrawing cyano or ester group or a bromine atom. Similarly, substitution at C-6 with a halogen such as chlorine is beneficial (**5i**) while substitution with an electron donating substituents such as methyl or methoxy (**5g–h**) abolishes activity. Best results were seen when both C-5 and C-6 were substituted with a chlorine atom (**5i**). The improved biological activity of the more lipophilic, dichloro substituted benzimidazole dimers could be attributed to their ability to penetrate bacterial cell membranes more efficiently and reach their cellular targets.[10] Interestingly, the dichlorosubstituted dimers **11** and **13** appeared to be especially effective against *Enterococcal* bacterial strains with MIC values comparable to those obtained with ciprofloxacin (Table 1).

In summary, the preparation and evaluation of two types of 2-aminobenzimidazoles dimers (N1 and N2 substituted) as antibacterial agents is described. The 2-aminobenzimidazole dimers represent a novel scaffold that possesses antibacterial activity and as such may aid in the discovery of new antibacterial agents.

References and Notes

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2. Walsh, C. *Nature* **2000**, *406*, 775.
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5. Preparation for dimer **5i**: 3,3′-diamino-*N*-methyldipropylamine **2** (2 mmol, 0.28 g) was added to a mixture of 1,2-dichloro-3-fluoro-4-nitrobenzene **1i** (0.26 mL, 1 mmol) and $CaCO_3$ (0.3 g, 4 mmol) in CH_2Cl_2 (2 mL). After stirring for 12 h at rt, the reaction was filtered through Celite and the filter bed was washed with additional CH_2Cl_2. The filtrate was concentrated under reduced pressure to provide **3i** as a yellow solid that was used without any further purification. Crude **3i** (1 mmol) obtained above was hydrogenated using Raney–Nickel (catalytic) and H_2 gas (balloon) in EtOH (10 mL) for 12 h at rt. The reaction was filtered through Celite and the filter bed was washed with additional EtOH. The filtrate was concentrated under reduced pressure to provide phenylenediamine **4i** as a dark oil that was used without further purification. Crude phenylenediamine **4i** (1 mmol) was dissolved in EtOH (5 mL) and treated with CNBr (3 mmol, 0.31 g). The reaction was stirred for 12 h at rt after which it was basified using 4M NaOH (pH >12). The aqueous layer was extracted with EtOAc (2×) and the combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. Crude dimer **5i** was purified by reverse-phase preparative HPLC using a Gilson 215 system with a Waters PrepPak (25×100 mm) C18 column, eluting with a linear gradient of 20–40% mobile phase B for 30 min with a flow rate of 5 mL/min (A = 0.1% TFA in water, B = 0.1% TFA in acetonitrile). **5i**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.03 (s, 4H), 7.97 (s, 2H), 7.68 (s, 2H), 4.18 (t, 4H, $J = 6.9$), 3.19 (m, 4H), 2.78 (s, 3H), 2.09 (m, 4H). LC/MS: LC 2.44 min, m/z 515. **11**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.99 (s, 4H), 7.81 (s, 2H), 7.58 (s, 2H), 4.32 (m, 4H), 3.19 (m, 4H), 2.78 (s, 3H). LC/MS: LC 2.75 min, m/z 488.
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7. Barry, A. L. *The Antimicrobic Susceptibility Test: Principles and Practice*; Lea and Febiger: Philadelphia, 1976.
8. The MIC assays were carried out in a 150-μL volume in duplicate in 96-well clear flat-bottom plates. The bacterial suspension from an overnight culture growth in the appropriate medium was added to a solution of test compound in 0.5% DMSO in water. Final bacterial inoculum was approximate 10^2–10^3 CFU/well. The percentage growth of the

bacteria in the test wells relative to that observed for a control well containing no compound was determined by measuring absorbance at 595 nm (A_{595}) after 20–24 h at 37 °C. The MIC was determined as a range of concentrations where complete inhibition of growth was observed at the higher concentration and the bacterial cells were viable at the lower concentration. The bacterial strains used for the assays include *E. coli* ATCC 25922, *S. aureus* ATCC 13709, *E. faecalis* ATCC 29212, *S. pyogenes* ATCC 49399.

9. For a preparation of 2,5,6-trichlorobenzimidazole, see: Hinkley, J. M.; Porcari, A. R.; Walker, J. A., II; Swayze, E. E.; Townsend, L. B. *Synth. Comm.* **1998**, *28*, 1703. Experimental for dimer **13**: A mixture of 2,4,5-trichlorobenzimidazole 12 (0.15 g, 0.68 mmol), 2,2′-diamino-*N*-methyl-diethylamine (0.029 mL, 0.23 mmol), triethylamine (0.031 mL, 0.23 mmol) in EtOH (0.5 mL) was heated in a sealed tube at 130 °C for 6 h. The reaction was then concentrated and purified by reverse-phase preparative HPLC to provide **13**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.27 (s, 4H), 6.88 (m, 2H), 3.4 (m, 4H), 2.63 (t, 4H, $J = 6.3$), 2.30 (s, 3H). LC/MS: LC 2.93 min, m/z 488. 14: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.29 (s, 4H), 7.15 (m, 2H), 3.34 (m, 4H), 2.77 (t, 4H, $J = 7.0$), 2.46 (s, 3H), 1.85 (m, 4H). LC/MS: LC 214 min, m/z 515.

10. All 2-aminobenzimidazole dimers prepared did not inhibit bacterial transcription/translation (T/T) in a cell free assay at 100 μM. This result indicated that the dimers do not interfere with bacterial T/T and therefore exert their antibacterial activity by a different mode of action. For a description of the cell free T/T assay, see: Lesley, S. A.; Brow, M. A. D.; Burgess, R. R. *J. Biol. Chem.* **1991**, *266*, 2632.