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SYNTHESIS AND ANTI-*PNEUMOCYSTIS CARINII* ACTIVITY OF PIPERIDINE-LINKED AROMATIC DIIMIDAZOLINES

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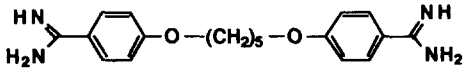
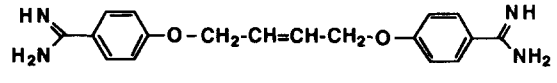
Abstract: A series of novel piperidine-linked aromatic diimidazolines (**3-7**) have been synthesized as conformationally restricted congeners of the anti-*Pneumocystis carinii* drug, Pentamidine. These compounds significantly inhibited the growth of *Pneumocystis carinii* in culture at 1 μg/mL. Copyright © 1996 Elsevier Science Ltd

Pneumocystis carinii pneumonia (PCP) is an opportunistic infection seen in many immunocompromised individuals, most notably in patients with acquired immunodeficiency syndrome (AIDS). About 50,000 cases of PCP have been estimated annually in the United States and it is the leading cause of mortality in AIDS patients.[1] Pentamidine **1** is one of the drugs of choice used extensively for the treatment of PCP. However, the drug is associated with serious toxicities, and this has prompted the search for new and safer agents to combat this fatal infection in AIDS patients. Although a number of pentamidine analogues have been developed,[2-5] there is still an ongoing need for more potent and less toxic congeners of pentamidine. Since pentamidine is a highly flexible molecule, it can exist in different conformational forms, and this may account for the multiple pharmacological actions of the drug. We are interested in studying the effect of restricting the conformational flexibility of pentamidine congeners on anti-*P. carinii* activity of these compounds. We recently reported the synthesis and biological activity of a series of *cis*- and *trans*-butamidine analogues **2** as semi-rigid congeners of pentamidine.[2,3] Several of these compounds were found to be more potent and less toxic than pentamidine in treating PCP in immunosuppressed rats. Earlier studies by other workers also showed that replacement of the amidine groups of pentamidine and related analogues with imidazoline moieties resulted in compounds with increased anti-PCP activity and reduced toxicity.[4] Based on these observations and to further investigate the optimal conformation of pentamidine analogues required for anti-*P. carinii* activity, we now report the synthesis and in vitro anti-*P. carinii* activity of a series of piperidine-linked aromatic diimidazolines, **3-7** (Fig. 1), as conformationally constrained analogues of pentamidine.



1



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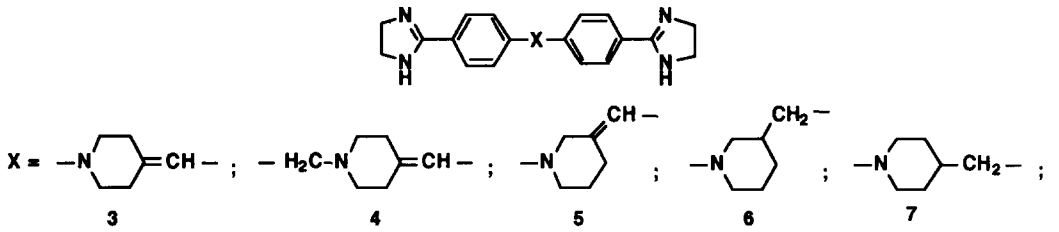
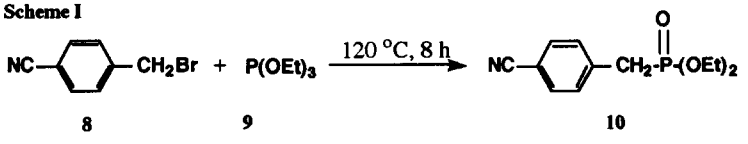


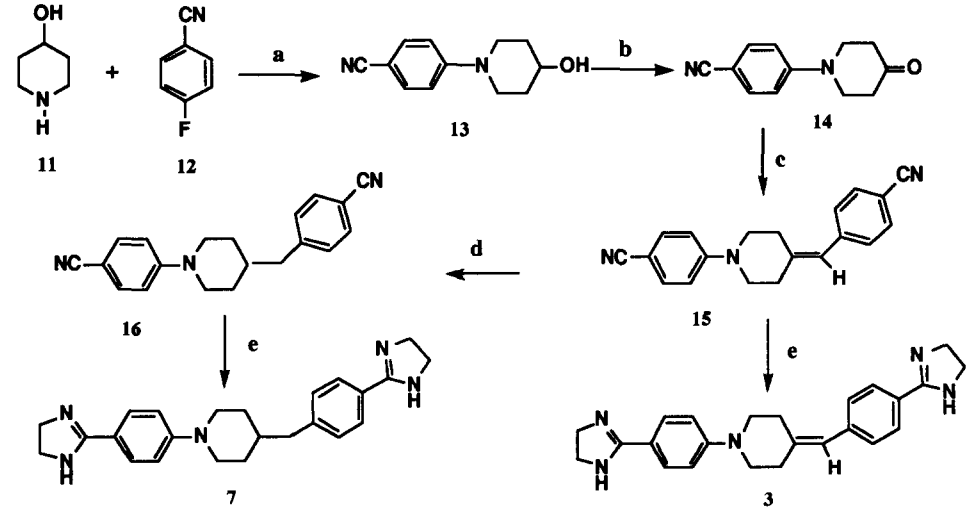
Fig. 1. Structures of Piperidine-linked aromatic diimidazolines.

Chemistry: The synthesis of **3** and **7** were accomplished according to the procedures shown in Schemes I and II. Similar procedures were used for the synthesis of compounds **4**, **5**, and **6**, using the appropriate starting materials. In Scheme I, 4-cyanobenzyl bromide **8** and triethyl phosphite **9** were heated to 120 °C for 8 h to give 4-cyanobenzyl phosphonate[6] **10** as a colorless liquid in 97% yield.

Scheme I



Scheme II



(a) DMSO, K_2CO_3, 120 °C, 6.5 h; (b) TFA, pyridine, DCC, DMSO/CH_2Cl_2 (1:2); (c) **10**, 60% NaH, THF; (d) 5% Pd/C, CH_3OH, H_2 gas, 2 h; (e) THF/EtOH, HCl gas; $NH_2CH_2CH_2NH_2$/EtOH reflux 4.5 h.

In scheme II, an aromatic nucleophilic displacement reaction between 4-hydroxypiperidine **11** and 4-fluorobenzonitrile **12** in dry DMSO with K_2CO_3 as acid scavenger afforded N-(4-cyanophenyl)-4-hydroxy piperidine **13** in 94% yield. Oxidation of the hydroxyl group of **13** with dicyclohexylcarbodiimide[7] gave N-(4-cyanophenyl)-4-piperidone **14** in 79% yield. A Wittig reaction[6] between **14** and phosphonate ester **10** with dry THF as solvent and NaH as proton abstractor resulted in dicyano product **15** in 70% yield. Treatment of **15** with dry HCl gas in dry THF/EtOH (9:1) gave an imidate intermediate. IR analysis of the intermediate indicated the absence of nitrile groups suggesting completion of the reaction. The imidate was refluxed with ethylene diamine[4] to give **3** in 33% yield. Reduction of the dicyano derivative **15** with 5% Pd/C in a Parr hydrogenator afforded **16** in 98% yield. Compound **16** was converted to **7** in 31% yield according to the procedure described for **3**. Following similar procedures, compound **4** was synthesized using 4-cyanobenzyl bromide and compounds **5** and **6** were synthesized using 3-hydroxypiperidine as the starting materials. All five compounds (**3-7**) exhibited satisfactory ^{1}H-NMR and elemental analysis data[8].

Biological results and discussion: Compounds **3-7** were tested in a short-term culture assay system with human embryonic lung cells with *P. carinii* from infected rat lung as described previously.[9-11] This cell culture model has proven to be an effective assay system in the identification of promising anti-*P. carinii* agents for further testing in animals. Compounds that significantly inhibit *P. carinii* cell growth at 1 μg/mL or less in the assay system have typically been selected for in vivo animal testing. In this study, the range of inhibition of *P. carinii* cell growth by compounds **3-7** are shown in Fig. 2. Pentamidine and the untreated control were used as the positive and negative controls respectively. Compounds **3** and **4** showed inhibitory activity similar to that of pentamidine. Additionally, compound **3** with a "fixed" conformation was over 3.6 times more active (at day 7) than its dihydro analogue **7** which is capable of assuming multiple conformations. Compounds **5** and **6** showed intermediate activity whereas, compound **7** was the least active agent of this series. Compound **5** was isolated and tested as the E isomer. Its stereochemical configuration was confirmed by NOE experiments[8]. Compound **6**

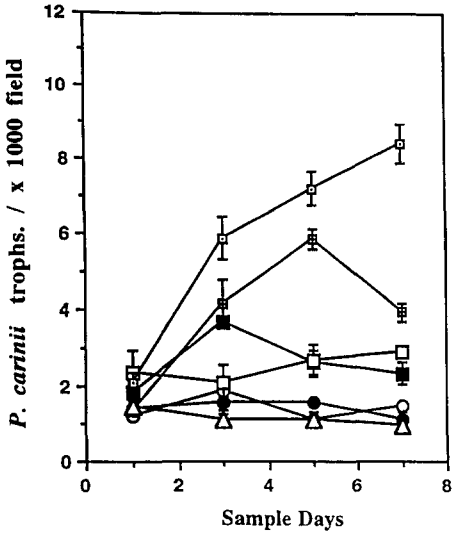


Fig. 2. Inhibition of *P. carinii* growth in culture by piperidine-linked aromatic diimidazolines tested at 1 μg/mL. *P. carinii* trophozoites were counted after Giemsa staining. The number of *P. carinii* trophozoites per x 1,000 field may be converted to the number of *P. carinii* trophozoites per milliliter of culture supernatant by multiplying by 3.9 x 10^5 (dilution and magnification factor). Each measurement is the mean for four wells read in duplicate, with the standard error of the mean.
Symbols: ▫ control; △ pentamidine;
● 3; ○ 4; ■ 5; □ 6; ▪ 7.

was not separated into its stereoisomers and was tested as a racemic mixture. The conformations of some of these compounds (e.g. **3** and **5**) are more rigidly defined than the previously reported butamidine analogues.[2,3] However, a direct comparison in anti-*P. carinii* activity between the two series of compounds cannot be made since the butamidine analogues were evaluated in vivo, [2,3] and not in the in vitro culture model. The imidazoline moieties in these compounds exist predominantly in the ionized form at physiological pH. There is considerable evidence to suggest that specific hydrogen-bonding interactions between the protonated imidazoline or amidine moieties of pentamidine analogues and the pathogenic genome are important for the anti-PCP actions.[1,3,12] The piperidine nitrogen in these compounds remains largely unionized at physiological pH and their presence did not adversely affect the anti-*P. carinii* activity. Similar observations were reported for pentamidine analogues in which the ether oxygens were replaced with nitrogen atoms.[4] Based on these results, it is clear that compounds **3** and **4** merit further investigation and that the conformations of these compounds do influence their anti-*P. carinii* activity. We are actively pursuing the synthesis of additional analogues related to these compounds for evaluation as anti-*P. carinii* agents.

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References and Notes

1. Queener, S. F. *J. Med. Chem.* **1995**, *38*, 4739.
2. Donkor, I. O.; Jones, S. K.; Tidwell, R. R. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1137.
3. Donkor, I. O.; Tidwell, R. R.; Jones, S. K. *J. Med. Chem.* **1994**, *37*, 4554.
4. Tidwell, R. R.; Jones, S. K.; Geratz, J. D.; Ohemeng, K. A.; Cory, M.; Hall, J. E. *J. Med. Chem.* **1990**, *33*, 1252.
5. Tidwell, R. R.; Jones, S. K.; Naiman, N. A.; Berger, L. C.; Brake, W. B.; Dykstra, C. C.; Hall, J. E. *Antimicrob. Agents Chemother.* **1993**, *37*, 1713.
6. Tominaga, Y.; Pratap, R.; Castle, R. N.; Lee, M. L. *J. Hetrocyclic Chem.* **1982**, *19*, 871.
7. Taylor, E. C.; Skotnicki, J. S.; Fletcher, S. R. *J. Org. Chem.* **1985**, *50*, 1005.
8. **3**: HCl salt; ^{1}H NMR (DMSO-d_6) δ 2.55 (t, 2 H), 3.48-3.58 (m, 6 H), 3.86 (d, 4 H), 3.94 (d, 4 H), 6.48 (s, 1 H), 6.97 (d, 1 H), 7.04 (d, 1 H), 7.42 (d, 1 H), 7.47 (d, 1 H), 7.86 (d, 1 H), 7.89 (d, 1 H), 7.99 (d, 1 H), 8.03 (d, 1 H), 10.29 (d, 2 H), 10.85 (d, 2 H); *Anal.* Calcd for $C_{24}H_{27}N_5$: C, 74.77; H, 7.06; N, 18.17. Found: C, 75.00; H, 7.15; N, 18.16. **4**: HCl salt; ^{1}H NMR (DMSO-d_6) δ 2.48-3.01 (m, 8 H), 3.96 (s, 4 H), 3.99 (s, 4 H), 4.43 (s, 2 H), 6.53 (s, 1 H), 7.50 (d, 2 H), 7.94 (d, 2 H), 8.04 (d, 2 H), 8.15 (d, 2 H), 10.82 (s, 2 H), 11.00 (s, 2 H); *Anal.* Calcd for $C_{25}H_{29}N_5$: C, 75.15; H, 7.32; N, 17.53. Found: C, 74.89; H, 7.44; N, 17.34. **5**: HCl salt; ^{1}H NMR (DMSO-d_6) δ 1.73 (p, 2 H), 2.60 (t, 2 H), 3.58 (t, 2 H), 3.89 (s, 4 H), 3.97 (s, 4 H), 4.20 (s, 2 H), 6.67 (s, 1 H), 7.10 (d, 2 H), 7.50 (d, 2 H), 7.87 (d, 2 H), 8.00 (d, 2 H), 10.17 (s, 2 H), 10.71 (s, 2 H); *Anal.* Calcd for $C_{24}H_{27}N_5$: C, 74.77; H, 7.06; N, 18.17. Found: C, 74.78; H, 7.17; N, 17.92. NOE experiments showed that **5** assumes an E configuration. The free base of **5** (in CD_3OD) was used in these experiments. Irradiation of the alkene proton (δ 6.53, s) of **5** caused enhancements of the nearest methylene protons (δ 3.98, s) adjacent to the nitrogen of the piperidine ring. Similarly, irradiation of the methylene protons caused an enhancement of the alkene proton. **6**: HCl salt; ^{1}H NMR (DMSO-d_6) δ 1.19-1.60 (m, 4 H), 1.80 (m, 1 H), 2.58-2.85 (m, 6 H), 3.81 (s, 4 H), 3.93 (s, 4 H), 6.89 (d, 2 H), 7.48 (d, 2 H), 7.79 (d, 2 H), 8.04 (d, 2 H), 10.37 (s, 2 H), 10.89 (s, 2 H); *Anal.* Calcd for $C_{24}H_{29}N_5$: C, 74.38; H, 7.54; N, 18.07. Found: C, 74.19; H, 7.47; N, 17.94. **7**: HCl salt; ^{1}H NMR (DMSO-d_6) δ 1.24 (q, 2 H), 1.65 (q, 2 H), 1.91 (m, 1 H), 2.69 (d, 2 H), 2.89 (t, 2 H), 3.93 (s, 4 H), 4.01 (s, 4 H), 4.22 (t, 2 H), 7.07 (d, 2 H), 7.50 (d, 2 H), 7.90 (d, 2 H), 8.04 (d, 2 H), 10.26 (s, 2 H), 10.80 (s, 2 H); *Anal.* Calcd for $C_{24}H_{29}N_5$: C, 74.38; H, 7.54; N, 18.07. Found: C, 74.16; H, 7.65; N, 17.92.
9. Queener, S. F.; Bartlett, M. S.; Nasr, M.; Smith, J. W. *Antimicrob. Agents Chemother.* **1993**, *37*, 2166.
10. Chio L-C.; Bolyard, L. A.; Nasr, M.; Queener, S. F. *Antimicrob. Agents Chemother.* **1996**, *40*, 727.
11. Bartlett, M. S.; Queener, S. F.; Shaw, M. M.; Richardson, J. D.; Smith, J. W. *Antimicrob. Agents Chemother.* **1994**, *38*, 1859.
12. Nunn, C. M.; Neidle, S. *J. Med. Chem.* **1995**, *38*, 2317.

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