Notes

Pentamidine Congeners. 2. 2-Butene-Bridged Aromatic Diamidines and Diimidazolines as Potential Anti-*Pneumocystis carinii* Pneumonia Agents

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We have synthesized *cis* and *trans* geometric isomers **1–8** as semirigid congeners of pentamidine. Compounds **1–4** were more potent than pentamidine in treating *Pneumocystis carinii* pneumonia in immunosuppressed rats. These compounds also demonstrated no clinical toxicity or histopathologic abnormalities. Introduction of methoxy substituents meta to the amidine or imidazoline groups of the phenyl rings as in compounds **5–8** generally resulted in compounds with decreased anti-*P. carinii* activity and increased toxicity to the host. Compounds **1–4** were evaluated as DNA binders. These compounds showed greater affinity for poly(dA)·poly(dT) than for calf thymus DNA. The *cis* isomers, **1** and **2**, demonstrated greater affinity for DNA than their *trans* counterparts **3** and **4**. This difference in DNA binding affinity, however, did not reflect in a corresponding difference in the anti-*P. carinii* activity of these compounds.

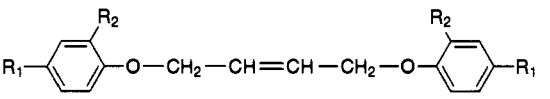
The increase in the number of patients afflicted with acquired immunodeficiency syndrome (AIDS) in the United states is paralleled by an increase in the incidence of *Pneumocystis carinii* pneumonia (PCP).[1,2] Pentamidine is one of the drugs used to treat PCP despite its numerous toxic adverse effects. The toxicity of pentamidine coupled with the fact that PCP is the leading cause of death of AIDS patients[3,4] suggests a need for new less toxic agents to prevent and/or treat PCP. Anti-*P. carinii* drug discovery is, however, limited to an empirical approach due to scanty knowledge available on *P. carinii* at the molecular level. Structure–activity relationship studies of pentamidine has led to the discovery of new analogues with improved therapeutic index.[5,6] Structurally, pentamidine is a highly flexible molecule; hence, it can assume a variety of interconvertible conformations. This conformational flexibility may account, at least in part, for the multiple pharmacological actions of the drug. We are interested in studying the effect of restricting the conformational freedom of pentamidine congeners on the anti-*P. carini* activity and host toxicity of such compounds. In this report we describe the syntheses, DNA binding affinity, anti-*P. carinii* activity, and host toxicity of eight semirigid aromatic diamidines and diimidazolines (Table 1, compounds **1–8**) as novel anti-PCP agents.

Results and Discussion

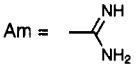
Compounds **1–8** were evaluated for their ability to reduce the severity of PCP in immunosuppressed rats as well as the extent of their toxicity to the host. The compounds were evaluated at a dose level of 10 mg/kg/day (or the highest soluble dose) by iv injection over 2 weeks. Anti-*P. carinii* activity was measured by histologic scoring of Grocott's methenamine silver (GMS)-stained lung sections and also by the number of cysts counted per gram of lung tissue expressed as a percentage of the number of cysts counted per gram of lung tissue of saline-treated control. Stained sections were coded, and each section was scored blindly by two examiners. Sections were read and scored according to the following system: 0.5, less than 10 cysts found per 2 sections examined; 1, scattered cysts with less than 10% of lung tissue involved; 2, scattered cysts with few focally intense areas of involvement and 10–25% of lung tissue involved; 3, scattered cysts with numerous focal areas of involvement and 25–50% of lung tissue involved; 4, cysts found throughout the tissue and numerous very intense focal areas of involvement with greater than 50% of lung tissue involved.

Toxicity to the host was evaluated at 10 mg/kg or as indicated in Table 2 by observing the health and general well-being of the animals daily. Sections of the spleen,

Table 1. Structure and Chemical Data of Pentamidine Congeners 1–8



compd	R_1[a]	R_2	double bond geometry	mp, °C	formula[b]
1	Am	H	cis	242	$C_{18}H_{20}N_4O_2 \cdot 2HCl \cdot 1.5H_2O$
2	Im	H	cis	270	$C_{22}H_{24}N_4O_2 \cdot 2HCl \cdot 0.25H_2O$
3	Am	H	trans	270	$C_{18}H_{20}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$
4	Im	H	trans	282	$C_{22}H_{24}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$
5	Am	OCH_3	cis	238	$C_{20}H_{24}N_4O_4 \cdot 2HCl \cdot 0.5H_2O$
6	Im	OCH_3	cis	263	$C_{24}H_{28}N_4O_4 \cdot 2HCl \cdot 0.5H_2O$
7	Am	OCH_3	trans	242	$C_{20}H_{24}N_4O_4 \cdot 1.8HCl$
8	Im	OCH_3	trans	264	$C_{24}H_{28}N_4O_4 \cdot 2HCl \cdot 0.5H_2O$

[a] Am =  Im = 

[b] Elemental analysis (C, H, N, Cl) within ±0.4% of the theoretical value.

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Table 2. Activity of Pentamidine Congeners against *Pneumocystis carinii* Pneumonia by Daily iv Injection

compound	dose (mg/kg/day)	toxicity[a]	mean histologic score (*n*)	no. of animals with the following histologic scores: 0.5	1	2	3	4	cysts/g of lung tissue[b] % of control ± SE (*n*)
saline			3.6 (10)	0	0	1	2	7	100.00 ± 9.83 (33)
pentamidine	10.0	2+	1.5 (9)[c]	1	4	3	1	0	5.31 ± 1.48 (30)[c]
1	10.0	0	0.9 (10)[c]	4	5	1	0	0	1.10 ± 0.84 (10)[c]
2	10.0	0	1.1 (10)[c]	2	6	2	0	0	1.22 ± 0.48 (10)[c]
3	10.0	0	0.9 (10)[c]	2	8	0	0	0	0.67 ± 0.17 (10)[c]
4	10.0	0	1.3 (10)[c]	3	3	4	0	0	1.22 ± 0.40 (10)[c]
5	5.0[d]	0	ND[e]						21.37 ± 8.13 (12)[c,f]
6	5.0[g]	2+	ND[e]						15.64 ± 7.40 (8)[c,h]
7	5.0[g]	2+	ND[e]						35.47 ± 5.52 (10)[c,i]
8	10.0	3+	ND[e]						1.50 ± 0.55 (11)[c]

[a] See text for explanation of toxicity. [b] $\times 10^6$. Saline control = 58.2×10^6 cysts/g of lung tissue. [c] $P < 0.001$ vs saline. [d] Administered at a lower dose due to insolubility. [e] ND, not done. [f] $P < 0.010$ vs pentamidine. [g] Dose lowered due to toxicity at 10.0 mg/kg. [h] $P < 0.05$ vs pentamidine. [i] $P < 0.001$ vs pentamidine.

liver, and kidney were also examined for pathologic changes by light microscopy. The following scoring criteria were used to evaluate the compounds for toxicity *in vivo*: 0 = no local, clinical, or histologic toxicity; 1+ = all animals survived with no observable distress, but some local toxicity at the site of injection; 2+ = most animals survived, some with severe distress and marked local toxicity, some clinical toxicity and histopathologic abnormalities were also observed; 3+ = an acute toxic effect occurred after a single dose and/or a sharp decrease in animals' health following multiple doses and resulted in a loss of less than 50% of the animals; 4+ = at least 50% of the animals died at the dose tested.

Tidwell[6] and Walzer[7] have shown that replacement of the amidine groups of aromatic diamidines with imidazoline groups give compounds with promising anti-PCP activity. In this series, however, amidine derivatives **1** and **3** appeared to be more potent than imidazoline derivatives **2** and **4**. This difference was especially pronounced in compounds with *trans* geometry. Compounds without methoxy substituents on the aromatic rings (i.e., **1**–**4**) showed lower mean histologic scores compared to pentamidine. The number of cysts counted per gram of lung tissue in rats treated with compounds **1**–**4** was also significantly lower than the number of cysts counted per gram of lung tissue in the pentamidine-treated animals. Thus compounds **1**–**4** were more potent than pentamidine in treating PCP in the rats. These compounds were also nontoxic compared to pentamidine. Compounds **5**–**8** have methoxy substituents meta to the amidine or imidazoline groups. Compound **5** was tested at a dose of 5 mg/kg/day due to solubility problems. At this dose level **5** was nontoxic; however, it was only moderately effective in treating PCP in the rats. Compounds **6** and **7** were evaluated at a dose of 5 mg/kg/day because they produced severe toxicity at a dose of 10 mg/kg/day. At the reduced dose of 5 mg/kg/day, compounds **6** and **7** were still significantly toxic. They were also less effective in treating PCP. Compound **8** was quite toxic albeit its effectiveness in treating PCP. Comparison of the activities of compounds **1**–**4** with those of **5**–**8** suggests that introduction of methoxy groups meta to the amidine or imidazoline moieties of the phenyl rings in these 2-butene-bridged analogues generally gives compounds with decreased anti-*P. carinii* activity and increased toxicity to the host.

Dose–response studies were performed with *trans*-1,4-bis(4-imidazolinophenoxy)-2-butene (**4**). Results of this study are shown in Table 3. At the highest dose level tested (10 mg/kg/day), **4** was found to be more potent and less toxic than pentamidine. When tested at a 2-fold reduction in dose (i.e., 5 mg/kg/day), **4** was as effective as 10 mg/kg/day of pentamidine in treating experimental PCP in rats. The anti-*P. carinii* activityof **4** was, however, significantly reduced when it was tested at a dose of 1 mg/kg/day.

Table 3. Dose–Response of *trans*-1,4-Bis(4-imidazolinophenoxy)-2-butene (**4**) against *Pneumocysts carinii* Pneumonia by Daily iv Injection

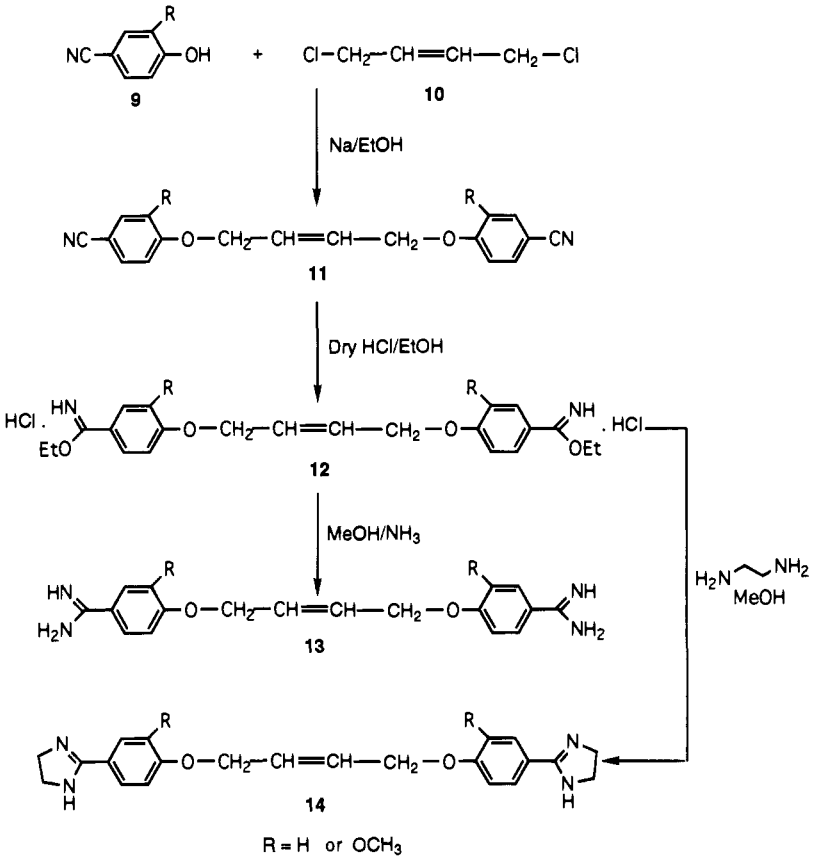
compound	dose (mg/kg/day)	toxicity[a]	cysts/g of lung tissue[b] % of control ± SE (*n*)
saline		0	100.00 ± 15.89 (26)
pentamidine	10.0	2+	4.95 ± 1.29 (25)[c]
4	10.0	0	1.38 ± 0.45 (10)[c]
	5.0	0	5.61 ± 2.10 (10)[c]
	1.0	0	39.60 ± 11.22 (10)[d,e]

[a] See text for explanation of toxicity. [b] $\times 10^6$. Saline control = 52.8×10^6 cysts/g of lung tissue. [c] $P < 0.001$ vs saline. [d] P, 0.5 vs saline. [e] P, 0.001 vs pentamidine.

Aromatic diamidines such as pentamidine have been reported to bind to DNA.[8-12] We were therefore interested in studying the DNA binding affinity of these compounds. Compounds **1**–**4** were found to bind calf thymus DNA with ΔT_ms ranging from 7.7 to 11.0 °C. These compounds also bind poly(dA)·poly(dT) in the range of 20.8-31.5°C. The *cis* isomers were better binders to both calf thymus DNA and poly(dA)·poly(dT) than the *trans* isomers. A clear-cut relationship between geometric isomerism, DNA binding affinity, and anti-*P. carinii* activity was, however, not apparent. The compounds showed greater binding affinity for poly(dA)·poly(dT) than for calf thymus DNA, suggesting that these compounds may bind to DNA in a manner similar to pentamidine. Footprinting[13] and molecular modeling[14] studies of pentamidine have demonstrated that the drug binds in the minor groove of DNA at AT-rich regions.

In summary these studies indicate that replacement of the pentyl bridge of pentamidine with a 2-butene bridge as in compounds **1**–**4** gives semirigid congeners of pentamidine that are more potent and less toxic than pentamidine in treating experimental PCP in rats. Replacement of the amidine moieties with imidazoline groups in these semirigid aromatic diamidines generally gives less active anti-PCP agents. Introduction of methoxy groups meta to the amidine or imidazoline moieties of the phenyl rings of these semirigid analogues of pentamidine as in compounds **5**–**8** generally results in decreased ant-*P. carinii* activity and increased toxicity to the host. Though the *cis* isomers were better

Scheme 1



binders to DNA than their *trans* counterparts, no significant differences between these geometric isomers in terms of anti-*P. carinii* activity or toxicity to the host were observed. Further studies are in progress using rigid rather than semirigid congeners of pentamidine to study the relationship between conformation and anti-*P. carinii* activity and/or host toxicity of aromatic diamidines. Results of these studies will be the subject of future communication.

Experimental Section

Synthesis of Compounds 1–8. The compounds were synthesized in an analogous manner to the synthesis of previously reported pentamidine analogues.[5] Briefly, the syntheses of **1–8** were initiated by reaction of the appropriately substituted 4-cyanophenol (**9**) with either *cis*-1,4-dichloro-2-butene or *trans*-1,4-dichloro-2-butene to give the appropriate dicyano derivative **11** (Scheme 1). Treatment of dicyano **11** in dry benzene/EtOH mixture with dry HCl gas afforded imidate hydrochloride **12**. Reaction of **12** with either $MeOH/NH_3$ or ethylenediamine gave the corresponding diamidine or diimidazoline derivative, respectively. The structures of the compounds were consistant with their spectral data.

Biological Evaluation. Evaluation of the compounds for anti-PCP activity and host toxicity in a rat model of the disease was carried out following standard procedures.[7,15,16] Pentamidine was used as positive control and saline as negative control.

DNA-Binding Properties. The procedure used for this study has been reported.[17] The DNA binding affinity of the compounds was measured at low ionic strength by determining the change in midpoint (ΔT_m) of the thermal denaturation curve of sonicated calf thymus DNA as well as poly(dA)·poly(dT) at a 1:10 drug to base ratio. The magnitude of ΔT_m is approximately proportional to the binding constant of the compound under these conditions.

Statistical Studies. Student's *t*-test was used to calculate the *p* values of each test group when compared to the saline-treated and pentamidine-treated groups. The statistical analysis was carried out using the STATS PLUS program (Human Systems Dynamics, Northridge, CA) on an Apple IIe personal computer.

Supplementary Material Available: Additional experimental details and tables of DNA binding data and analytical data (9 pages). Ordering information is given on any current masthead page.

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