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N,*N*′-Bis[4-(*N*-alkylamidino)phenyl]homopiperazines as Anti-*Pneumocystis carinii* Agents

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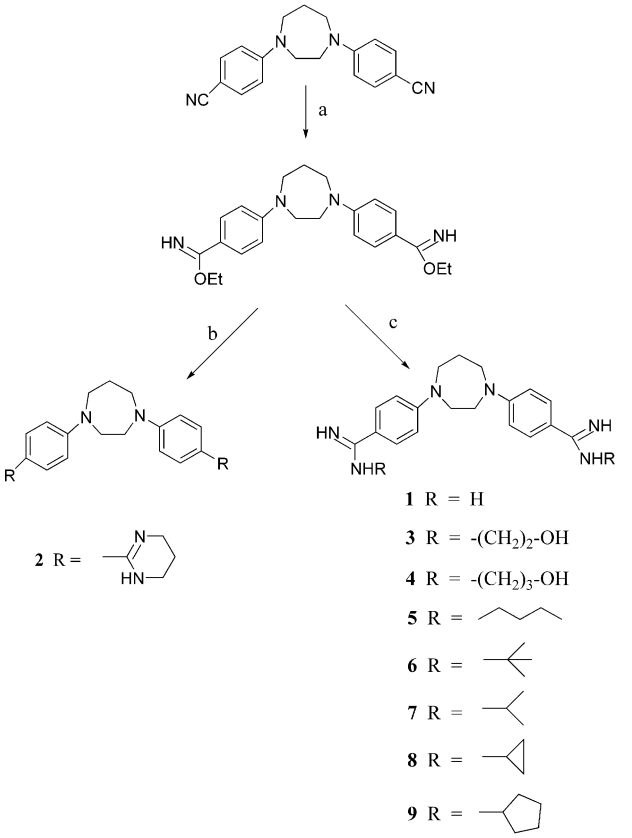
Abstract—The synthesis, anti-*Pneumocystis carinii* activity and DNA binding properties of eight new *N*,*N*′-bis[4-(*N*-alkylamidino)phenyl]homopiperazines are reported. Compounds **2** and **8** were the most potent and caused about 70% inhibition of *Pneumocystis carinii* growth in a cell culture model at 1 μM concentrations.

There is considerable interest in the development of novel aromatic bis-amidines, based on the prototype agent pentamidine, as potential agents for the treatment of *Pneumocystis carinii* pneumonia (PCP).[1] These compounds have been shown to exhibit a wide range of biological activities including antimicrobial, anticancer and antiviral effects.[1,2] The current hypothesis for the mechanism of action of aromatic bis-amidines is that they first bind to the minor groove of DNA at AT-rich sites and subsequently inhibit the normal functions of DNA-dependent enzymes (e.g., topoisomerases, nucleases) or they may cause direct inhibition of transcription.[3]

Pentamidine is the only agent in this chemical class that is currently used clinically to treat PCP. The major factor hampering its clinical usage is due to its vast array of serious adverse reactions especially in patients with AIDS. There is therefore a critical need for more efficacious and less toxic agents to treat AIDS-related PCP. We have recently reported on the promising anti-*P. carinii* activity of several novel conformationally restricted analogues of pentamidine including *N*,*N*′-bis(4-amidinophenyl)homopiperazine **1**.[1d] In an effort to enhance the anti-*P. carinii* activity of this compound, we have synthesized and evaluated a series of analogues of **1** in which various alkyl groups have been attached to the bis-amidinium nitrogens. The rationale for making



Scheme 1. Reagents used for the synthesis of compounds **2**–**9**: (a) HCl (g)/EtOH/$CHCl_3$; (b) 1,3-propyldiamine/EtOH; (c) R-NH_2/EtOH.

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this structural modification was based on the observation that substitution of various alkyl groups at the amidinium nitrogens of 2,5-bis(4-amidinophenyl)furan resulted in compounds with improved efficacy against PCP and reduced toxicity.[1c] The resultant analogues also showed significantly stronger binding to DNA, and this has been attributed to increased van der Waals interactions between the *N*-alkyl groups and the walls of the minor groove.[1c]

The synthesis of compound **1** was reported earlier[1d] whereas compounds **2**–**9** were synthesized using *N*,*N*′-bis(4-cyanophenyl)homopiperazine[1d] as the key intermediate, as outlined in Scheme 1. The classical Pinner reaction was employed to convert the bis-nitrile to the corresponding bis-imidate ester, which was then reacted with the appropriate alkylamine to form the desired products. All final compounds exhibited satisfactory ^{1}H NMR and elemental analysis data.[4]

The anti-*P. carinii* activity and DNA binding affinities of the synthesized compounds **2**–**9** are shown in Table 1. The usefulness of the *P. carinii* cell culture model as a rapid screen in the identification of promising anti-*P. carinii* agents has been discussed earlier.[1b,d] In this study, the untreated control was used as the negative control whereas pentamidine (1 μM) and trimethoprim/sulfamethoxazole (TMP/SMX 50/250 μg/mL) were used as the positive controls. The data for compound **1** and pentamidine conducted under experiment 1 were obtained from an earlier reported study[1d] and included here for comparison purposes.

Compounds **2**–**9** were evaluated for anti-*P. carinii* activity at 1 μM concentrations. Pentamidine, TMP/SMX and compound **1** were highly effective and caused total inhibition of *P. carinii* growth. However, none of the newly synthesized analogues of **1** were more potent than **1** or the positive controls. Among this series, compounds **2** and **8** were the most potent and caused about 70% inhibition of *P. carinii* growth. Compounds **5**, **6**, and **7** were less active with inhibition of *P. carinii* growth ranging from 35 to 42%. Compounds **3**, **4**, and **9** were inactive. With the exception of **6**, the other compounds exhibited strong binding affinity for both calf thymus DNA and Poly(dA-dT). A direct correlation between the DNA binding affinity and the anti-*P. carinii* activity was not evident for this series of compounds. It is interesting to note that the cyclopentyl analogue **9** has the strongest binding for DNA but was inactive when tested against *P. carinii* in culture. However, the cyclopentyl analogue of 2,5-bis(4-amidinophenyl)furan in the diaryl furan series was not only the strongest DNA binder but was also the most potent.[1c] This analogue was found to be about 100 times more effective than pentamidine in an animal model.[1c] These studies clearly indicate that the nature of the central linker between the benzamidine groups strongly influences the biological properties of aromatic bis-amidines. Furthermore, alkyl substituents on the amidino nitrogens may or may not

Table 1. Anti-*P. carinii* activity and DNA binding affinity of diaryl homopiperazines

Expt, Compd, Concn	Change in numbers of *P. carinii* trophozoites from day 1 through day 7 in culture ($\times 10^{-5}$)[a]	% of control growth	DNA binding (ΔT_m, °C)	
			Calf thymus DNA[b]	Poly(dA-dT)[c]
Expt 1[d], Control	24.10 ± 4.02			
Pentamidine 1.0 μM	−3.98 ± 1.64	< 0	11.1	20.6
Expt 2, Control	33.23 ± 3.20			
TMP/SMX 50/250 μg/mL	−0.31 ± 0.74	< 0		
Expt 1,[d] Compd **1**			15.0	23.1
1.0 μM	−3.32 ± 1.60	< 0		
Expt 2, Compd **2**			15.5	21.3
1.0 μM	10.73 ± 0.51	32		
Expt 2, Compd **3**			11.1	14.7
1.0 μM	33.62 ± 0.39	101		
Expt 2, Compd **4**			17.4	23.6
1.0 μM	34.79 ± 0.66	105		
Expt 2, Compd **5**			11.8	13.3
1.0 μM	19.19 ± 0.59	58		
Expt 2, Compd **6**			4.1	7.4
1.0 μM	21.57 ± 0.82	65		
Expt 2, Compd **7**			13.8	20.0
1.0 μM	20.20 ± 0.51	61		
Expt 2, Compd **8**			15.3	23.6
1.0 μM	10.92 ± 1.21	33		
Expt 2, Compd **9**			17.9	26.5
1.0 μM	34.32 ± 0.74	103		

[a]Values are reported as means ± SEMs. Values are calculated by subtracting the numbers of organisms on day 1 of culture from the numbers detected on day 7; thus, positive values denote growth, numbers near zero denote little change, and negative numbers denote decreasing organisms in the culture.

[b,c]Increase in thermal melting of calf thymus DNA and Poly(dA-dT), respectively.

[d]Data for pentamidine and compound **1** are taken from ref 1d in which the change in numbers of *P. carinii* trophozoites conducted in experiment 1 was from day 1 through day 5 in culture.

enhance the anti-*P. carinii* activity depending on the nature of the central linker.

Acknowledgements

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4. *N*,*N*′-Bis[4-(4,5,6-trihydro-1*H*-1,3-piperidin-2-yl)phenyl]homopiperazine (**2**): HCl salt, 78% yield, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 1.91–1.92 (m, 6H), 3.40–3.43 (m, 8H), 3.50 (m, 4H), 3.74 (s, 4H), 6.90 (d, J=9.6 Hz, 4H), 7.61 (d, J=9.6 Hz, 4H), 9.64 (bs, 4H).

Anal. calcd $C_{25}H_{32}N_6{\cdot}2HCl{\cdot}0.6H_2O$: C, 60.02; H, 7.09; N, 16.80; found: C, 60.42; H, 6.75; N, 16.40.

N,*N*′-Bis[4-(*N*-2-hydroxyethylamidino)phenyl]homopiperazine (**3**): HCl salt, 73% yield, mp 281–283 °C; ^{1}H NMR (DMSO-d_6) δ 1.93 (m, 2H), 3.44 (t, J=4.6 Hz, 4H), 3.51 (m, 4H), 3.61 (t, J=4.6 Hz, 4H), 3.74 (s, 4H), 5.16 (bs, 2H), 6.90 (d, J=8.7 Hz, 4H), 7.65 (d, J=8.0 Hz, 4H), 8.58 (s, 2H), 8.90 (s, 2H), 9.23 (s, 2H). Anal. calcd $C_{23}H_{32}N_6O_2{\cdot}2HCl{\cdot}0.5H_2O$: C, 54.54; H, 6.97; N, 16.59; found: C, 54.35; H, 6.80; N, 16.50.

N,*N*′-Bis[4-(*N*-3-hydroxypropylamidino)phenyl]homopiperazine (**4**): HCl salt, 59% yield, mp 284 °C; ^{1}H NMR (DMSO-d_6) δ 1.76 (m, 4H), 1.94 (m, 2H), 3.40 (m, 4H), 3.49 (m, 8H), 3.74 (s, 4H), 4.73 (bs, 2H), 6.91 (d, J=8.8 Hz, 4H), 7.63 (d, J=8.8 Hz, 4H), 8.57 (s, 2H), 9.04 (s, 2H), 9.30 (s, 2H). Anal. calcd $C_{25}H_{36}N_6O_2{\cdot}2HCl{\cdot}2H_2O$: C, 53.47; H, 7.54; N, 14.97; found: C, 53.65; H, 7.28; N, 14.94.

N,*N*′-Bis[4-(*N*-*n*-butylamidino)phenyl]homopiperazine (**5**): HCl salt, 81% yield, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 0.82–0.87 (m, 6H), 1.27–1.32 (m, 4H), 1.51–1.55 (m, 4H), 1.88 (m, 2H), 3.26–3.28 (m, 4H), 3.45 (s, 4H), 3.68 (s, 4H), 6.82 (d, J=8.7 Hz, 4H), 7.50 (d, J=8.7 Hz, 4H), 8.46 (s, 2H), 8.84 (s, 2H), 9.14 (s, 2H). Anal. calcd $C_{27}H_{40}N_6{\cdot}2HCl{\cdot}0.6H_2O$: C, 60.91; H, 8.18; N, 15.79; found: C, 60.88; H, 7.89; N, 15.78.

N,*N*′-Bis[4-(*N*-*tert*-butylamidino)phenyl]homopiperazine (**6**): HCl salt, 78% yield, mp 271–273 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (m, 18H), 1.98 (m, 2H), 3.43 (m, 4H), 3.67 (s, 4H), 6.76 (q, J=8.8 Hz, 2.8 Hz, 4H), 7.64 (d, J=8.8 Hz, 4H), 7.97 (s, 4H), 8.40 (bs, 2H). Anal. calcd $C_{27}H_{40}N_6{\cdot}2HCl{\cdot}H_2O$: C, 62.18; H, 8.12; N, 16.11; found: C, 62.15; H, 7.99; N, 16.24.

N,*N*′-Bis[4-(*N*-isopropylamidino)phenyl]homopiperazine (**7**): HCl salt, 71% yield, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (d, J=6.3 Hz, 12H), 1.93 (m, 2H), 3.49 (m, 4H), 3.73 (s, 4H), 4.02 (m, 2H), 6.90 (d, J=8.7 Hz, 4H), 7.60 (d, J=8.7 Hz, 4H), 8.66 (s, 2H), 9.09 (m, 4H). Anal. calcd $C_{25}H_{36}N_6{\cdot}2HCl{\cdot}H_2O$: C, 58.70; H, 7.88; N, 16.43; found: C, 58.92; H, 7.50; N, 16.63.

N,*N*′-Bis[4-(*N*-cyclopropylamidino)phenyl]homopiperazine (**8**): HCl salt, 69% yield, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 0.76 (m, 4H), 0.89 (m, 4H), 1.91 (m, 2H), 2.71 (m, 2H), 3.50 (s, 4H), 3.73 (s, 4H), 6.87 (d, J=8.8 Hz, 4H), 7.67 (d, J=8.8 Hz, 4H), 8.72 (bs, 2H), 9.31 (bs, 2H), 9.56 (bs, 2H). Anal. calcd $C_{25}H_{32}N_6{\cdot}2HCl{\cdot}0.8H_2O$: C, 59.59; H, 7.12; N, 16.68; found: C, 59.93; H, 7.05; N, 16.89.

N,*N*′-Bis[4-(*N*-cyclopentylamidino)phenyl]homopiperazine (**9**): HCl salt, 73% yield, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 1.46–1.64 (m, 12H), 1.94 (m, 6H), 3.46 (s, 4H), 3.68 (s, 4H), 4.04 (m, 2H), 6.90 (d, J=8.7 Hz, 4H), 7.78 (d, J=8.7 Hz, 4H), 8.60 (s, 2H), 9.04 (s, 2H), 9.16 (s, 2H). Anal. calcd $C_{29}H_{40}N_6{\cdot}2HCl{\cdot}0.6H_2O$: C, 62.60; H, 7.83; N, 15.10; found: C, 62.41; H, 7.89; N, 15.43.