ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1994, p. 2572–2576
0066-4804/94/$04.00+0

Vol. 38, No. 11

Guanylhydrazones in Therapy of *Pneumocystis carinii* Pneumonia in Immunosuppressed Rats

PETER D. WALZER,[1,2]* JILANNA FOY,[2] JENNIFER RUNCK,[2] PAUL STEELE,[3] MICHAEL WHITE,[4] ROBERT S. KLEIN,[5] BRIAN A. OTTER,[5] AND RICHARD J. SUNDBERG[6]

Research Service, VA Medical Center, Cincinnati, Ohio 45220[1]; Division of Infectious Diseases, Department of Internal Medicine,[2] Department of Pathology and Laboratory Medicine,[3] and Division of Biostatistics, Department of Environmental Health,[4] University of Cincinnati College of Medicine, Cincinnati, Ohio 45267; Montefiore Medical Center, Bronx, New York 10461[5]; and Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901[6]

Received 12 May 1994/Returned for modification 15 August 1994/Accepted 2 September 1994

Guanylhydrazones are cationic heteroaromatic drugs similar to the diamidines which are effective in the treatment of African trypanosomiasis and pneumocystosis. On the basis of their antitrypanosomal activity, different guanylhydrazones were selected for evaluation in a rat model of *Pneumocystis carinii* pneumonia. The most active compounds were the 2-(4′-formylphenyl)-1-methylimidazo-[1,2-a]pyridinium guanylhydrazones which, at a dose of 2 mg/kg/day, were about as effective as trimethoprim-sulfamethoxazole at a dose of 50 mg of trimethoprim per kg/day plus 250 mg of sulfamethoxazole per kg/day. The anti-*P. carinii* activity of these guanylhydrazone derivatives was found with parenteral but not with oral administration. The 1,3-arylene diketone bis(guanylhydrazones) were generally ineffective, although a triacetyl derivative showed some anti-*P. carinii* activity. Nitroimidazole guanylhydrazone derivatives were also ineffective. Attempts to improve the therapeutic efficacy of the different guanylhydrazones were limited by problems of toxicity. We conclude that some guanylhydrazone derivatives are potent anti-*P. carinii* drugs and that further studies should be pursued to develop safer compounds and investigate structure-activity relationships.

Drugs currently licensed for the treatment of *Pneumocystis carinii* pneumonia in human immunodeficiency virus-infected patients are characterized by problems of efficacy and/or a high frequency of adverse reactions (18). Cationic aromatic compounds such as the diamidines, which were originally developed as antitrypanosomal drugs, are known to have potent anti-*P. carinii* properties (30, 32). The major limitation to the wider use of these agents is their toxicity, as evidenced by the fact that only one member of this class of compounds, pentamidine isethionate, has been licensed for use in this country (1, 19). Research efforts to develop better diamidine derivatives have proceeded along two general lines. Most of the work has been devoted to synthesizing new compounds and examining their structure-activity relationships (6, 15, 26, 27). Alternatively, our laboratory has explored existing classes of cationic drugs and found anti-*P. carinii* activity in the following: diamidines, carbanilides, aminoquinaldines, and phenanthridiniums (30, 32).

Guanylhydrazones represent another class of quaternary heteroaromatic drugs which have shown trypanocidal activity. In a previous study, we found that methylglyoxal-bis(guanylhydrazone) (MGBG) had limited efficacy in an immunosuppressed-rat model of pneumocystosis (32). Over the past decade, several hundred guanylhydrazone derivatives have been synthesized and their structure-activity relationships have been studied in a mouse model of African trypanosomiasis (24, 28, 29). In the present study, we used this information to select and compare the activities of guanylhydrazone compounds in a standard immunosuppressed-rat model of *P. carinii* pneumonia.

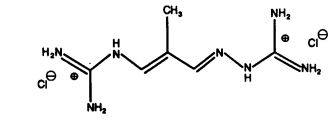
* Corresponding author. Mailing address: Research Service (151), VA Medical Center, 3200 Vine Street, Cincinnati, OH 45220. Phone: (513) 559-5061. Fax: (513) 559-5646.

MATERIALS AND METHODS

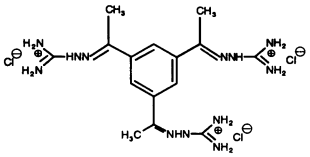
Drugs. The compounds used in this study and their structures are shown in Fig. 1. MGBG was purchased from Sigma Chemical Co., St. Louis, Mo. The following 1,3-arylene diketone bis(guanylhydrazone) derivatives were synthesized by R.S.K. and B.A.O. in accordance with procedures described by Ulrich and Cerami: 1,3-diacetylbenzene-bis(guanylhydrazone) hydrochloride (compound 4 in their series), 1,3,5-triacetylbenzene-tris(guanylhydrazone) hydrochloride (compound 12), and 1,3-diacetylpyridine-bis(guanylhydrazone) hydrochloride (compound 17) (28, 29). Several guanylhydrazones of 2-(4′-formylphenyl)-1-methylimidazo-[1,2-a]pyridinium salts were synthesized by R.J.S. as previously reported (24). These agents included compound 1a-53, a dibromide salt; compounds 1a-62 and JC-I-127, which are identical except that the former is the dibromide salt and the latter is the dichloride salt; and compounds li-39 and JC-I-134, which are identical except that li-39 has a 7-methyl group and is the dibromide salt and JC-I-134 has a 6-methyl group and is the dichloride salt. Two other agents synthesized by R.S.K. and B.A.O. included 2-(4-carbostyryl)-5-nitro-1-vinylimidazoleguanylhydrazone, which was prepared by using the method of Ross and Jamieson (21), and 2-(4-carbostyryl)-5-nitro-1-vinylimidazolethiosemicarbazone, which was newly synthesized in their laboratory.

The dose, route, and frequency of administration of the drugs used in these experiments were based mainly on experience obtained by using these compounds in the mouse model of African trypanosomiasis. Modifications were then made on the basis of efficacy and tolerance by the rats.

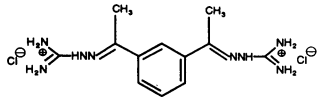
Animal model. The experimental protocol has been described in detail in our previous reports (16, 30–32). Briefly, adult male Sprague-Dawley rats (Harlan Industries, Madison, Wis., or Sasco, Inc., St. Louis, Mo.) with naturally acquired latent *P. carinii* infection were immunosuppressed with corticosteroids (methylprednisolone acetate [4 mg] injected subcu-



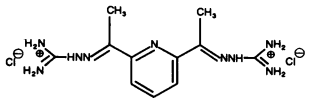
Methylglyoxal-bis(guanylhydrazone) hydrochloride (MGBG)



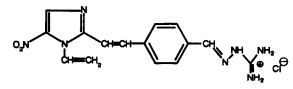
1,3,5-Triacetylbenzene-tris(guanylhydrazone) hydrochloride (#12)



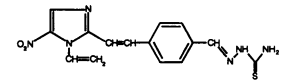
1,3-Diacetylbenzene-bis(guanylhydrazone) hydrochloride (#4)



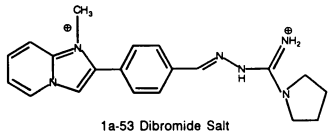
2,6-Diacetylpyridine-bis(guanylhydrazone) hydrochloride (#17)



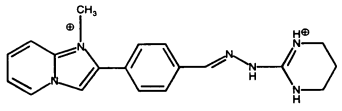
2-(4-Carbostyryl)-5-nitro-1-vinylimidazoleguanylhydrazone hydrochloride



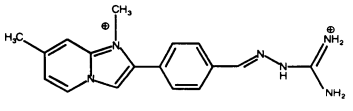
2-(4-Carbostyryl)-5-nitro-1-vinylimidazolethiosemicarbazone



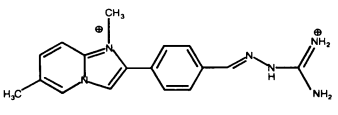
1a-53 Dibromide Salt



1a-62 Dibromide Salt
Jc-I-127 Dichloride Salt



1i-39 Dibromide Salt



Jc-I-134 Dichloride Salt

taneously [s.c.] once weekly) to induce pneumocystosis. After about 6 weeks, when the pneumonia reached moderate intensity, the rats were randomly divided into treatment and control groups of 10 to 20 animals each. Drugs to be studied were given by parenterally or by oral gavage in single or divided doses for 3 weeks, during which time the animals remained on the immunosuppressive regimen. At the end of this time, the rats were sacrificed by an overdose of carbon dioxide or halothane anesthesia.

Evaluation of drug activity. Analysis of the activity of the anti-*P. carinii* drugs, which was performed by using procedures described in our earlier reports (16, 30–32), was based on the severity of pneumocystosis in the lungs rather than on animal survival because the rats occasionally died from causes such as drug toxicity or infection with other organisms. Ordinarily, animals must have received at least 10 days of treatment to be included in the data analysis because it usually takes this long to observe an effect against *P. carinii*; however, with highly potent but toxic drugs (e.g., some of the guanylhydrazone compounds used here), this period is reduced to 7 days. To determine the magnitude of *P. carinii* pneumonia, the right lung was weighed, homogenized, and stained with cresyl echt violet to count the number of organism cysts per lung; in selected instances, the homogenate was stained with Diff-Quik, a variant of the Wright-Giemsa stain, to count all of the developmental stages of *P. carinii*. The lower limit of detection is 1.12×10^5 organisms per lung. The left lung was used for histologic studies, which were also performed on a selective basis; staining was performed with hematoxylin and eosin and methenamine silver, and pneumocystosis intensity was graded on a scale of 0 to 4+ based on the degree of alveolar involvement. All specimens were analyzed in a blinded manner.

Drug efficacy was determined by comparing the *P. carinii* infection in the treatment groups with that in the control steroid (C/S) group. In our previous studies, several different drugs and dose regimens were usually analyzed in a given experiment and the results were presented in a figure (16, 30–32). Since the data frequently did not follow a normal pattern of distribution, we used nonparametric statistical techniques, such as the Kruskall-Wallis test for analysis of variance and the Wilcoxin rank sum test with the Bonferroni correction for comparison of individual groups. The anti-*P. carinii* activity of drugs was also classified by calculating the reduction in the median cyst or nucleus counts in the treatment groups compared with controls.

The data presented here summarize our experience with guanylhydrazone derivatives in the treatment of experimental pneumocystosis over a period of several years. These compounds were evaluated in experiments along with other, unrelated candidate anti-*P. carinii* drugs; thus, we were interested in only a small portion of any given study. We felt that the most efficient way of presenting this information would be to express the mean ± the standard deviation of each group in tabular form. In preliminary experiments, we compared data analyses of different treatment regimens by use of geometric means ± standard deviation and the *t* test with analysis by use of medians and the Wilcoxin rank sum test. Anti-*P. carinii* drugs used included pentamidine, inhibitors of dihydrofolate reductase used with sulfamethoxazole or dapsone, atovaquone, and clindamycin used with primaquine. Excellent correlation between the two methods of data analysis was found. Therefore,

FIG. 1. Structural formulas of the guanylhydrazone derivatives tested in this study.

TABLE 1. Treatment of *P. carinii* pneumonia

Study	Drug group	Dose regimen[a]	No. of rats	Mean $\log_{10}$ cyst count ± SD	*P* value[b]
1–3	MGBG	25–75 mg/kg/day s.c., i.m., or i.p. 3–7 days/wk	60	8.59 ± 0.50	NS
	C/S		68	8.69 ± 0.57	
4	Compound 4	10→5 mg/kg 5 days/wk s.c.	11	8.36 ± 0.53	NS
	Compound 12	10→5→2.5 mg/kg 5 days/wk s.c.	9	7.20 ± 1.0	NS
	Compound 17	10→5 mg/kg 5 days/wk s.c.	11	8.08 ± 0.85	NS
	C/S		20	7.38 ± 1.51	
5	Compound 4	5 mg/kg 3× wkly s.c.	20	8.38 ± 0.80	NS
	Compound 12	5 mg/kg 3× wkly s.c.	14	7.55 ± 0.99	0.0001
	Compound 17	5–>3 mg/kg 3× wkly s.c.	13	8.39 ± 0.46	NS
	C/S		17	8.73 ± 0.57	
6	1a-53	3 mg/kg/day s.c.	13	8.45 ± 0.59	NS
	1a-62	3 mg/kg/day s.c.	9	7.44 ± 0.96	0.002
	1i-39	3 mg/kg/day s.c.	11	7.71 ± 0.86	0.006
	C/S		11	8.59 ± 0.56	
7	1a-62	3→1.5 mg/kg/day s.c.	19	6.53 ± 0.87	<0.0001
	1i-39	3→1.5 mg/kg/day s.c.	17	7.04 ± 0.30	<0.0001
	C/S		18	8.52 ± 1.18	
8	JC-I-127	10 mg/kg/day p.o.	19	7.94 ± 0.52	NS
	JC-I-127	30 mg/kg/day p.o.	18	7.76 ± 0.51	NS
	JC-I-134	10 mg/kg/day p.o.	19	7.78 ± 0.59	NS
	JC-I-134	30 mg/kg/day p.o.	17	7.59 ± 0.39	NS
	C/S		22	7.89 ± 0.79	
9	1a-62	0.5 mg/kg/day s.c.	15	6.24 ± 0.89	<0.0001
	1a-62	2 mg/kg/day s.c.	15	5.44 ± 0.51	<0.0001
	JC-I-127	0.5 mg/kg/day s.c.	15	5.97 ± 0.80	<0.0001
	JC-I-127	2 mg/kg/day s.c.	9	5.37 ± 0.75	<0.0001
	1i-39	0.5 mg/kg/day s.c.	16	7.15 ± 0.94	NS
	1i-39	2 mg/kg/day s.c.	13	6.17 ± 0.84	<0.0001
	JC-I-134	0.5 mg/kg/day s.c.	15	7.00 ± 0.79	NS
	JC-I-134	2 mg/kg/day s.c.	15	6.24 ± 0.43	<0.0001
	TMP-SMX	50 mg of TMP per kg/day, 250 mg of SMX per kg/day p.o.	13	5.13 ± 0.30	<0.0001
	C/S		19	7.88 ± 1.10	
10	*N*-vinyl-metro-guan[c]	12.5 mg/kg 5 days/wk, 6.25 mg/kg 2 days/wk i.m.	15	8.00 ± 0.88	NS
	N-vinyl-metro-guan	100→25 mg/kg 5 days/wk, 12.5 mg/kg 2 days/wk i.m.	9	8.11 ± 0.76	NS
	N-vinyl-metro-thio[d]	12.5 mg/kg 5 days/wk, 6.25 mg/kg 2 days/wk i.m.	17	8.13 ± 0.66	NS
	N-vinyl-metro-thio	100 mg/kg 5 days/wk, 50 mg/kg 2 days/wk i.m.	15	8.30 ± 0.72	NS
	C/S		16	7.76 ± 0.81	

[a] i.m., intramuscularly; i.p., intraperitoneally; p.o., orally.
[b] To determine values, each group was compared with the C/S group in the same experiment. $P < 0.05$ was considered significant. NS, not significant. →, dose reduction (e.g., 10→5 mg signifies 10 mg reduced to 5 mg).
[c] *N*-Vinyl-metro-guan, *N*-vinyl-metro-guanylhydrazone.
[d] *N*-Vinyl-metro-thio, *N*-vinyl-metro-thiosemicarbazone.

in this report we have used geometric means as the principal method of presenting the results; medians have been included on a selective basis to indicate that this close correlation extends to the analysis of guanylhydrazones.

RESULTS

Analysis by *P. carinii* cyst counts. MGBG served as the prototype compound, and the data from three experiments (studies 1 to 3), which were published previously (32), have been combined to summarize our experience with this agent (Table 1). As judged by *P. carinii* cyst counts in the MGBG and C/S groups, this experience was disappointing. MGBG caused severe reactions at injection sites, and thus a variety of dose regimens were explored. Although we found some differences among these regimens, we concluded that further testing of this drug was not justified.

In study 4, which investigated the bis(guanylhydrazone) derivatives synthesized by Ulrich and Cerami (28, 29), the drugs were administered at a dose of 10 mg/kg/day s.c.; however, this had to be reduced because of early deaths of the rats and other signs of severe systemic toxicity (Table 1). Compound 12 appeared to have the greatest anti-*P. carinii* activity, although the cyst count reduction did not reach statistical significance. In study 5, compound 12, used at dose of 5 mg/kg/day 3 days/week s.c. lowered the mean cyst count over 15-fold, from 8.73 per lung in the C/S group to 7.55 per lung, which was highly significant. Similar data were obtained by analysis of median cyst counts (Table 2). Even at the reduced doses, all three guanylhydrazone derivatives caused adverse reactions in the animals.

The first experiment (study 6) evaluating the imidazo[1,2-a]pyridinium guanylhydrazones synthesized by R.J.S. (24) compared compounds 1a-53, 1a-62, and 1i-39 at a dose of 3

TABLE 2. Evaluation of treatment of *P. carinii* pneumonia by different methods of analysis

Study	Drug group	Cyst count per lung		Median histologic score
		Mean ($\log_{10}$)	Median	
5	Compound 12	7.55	6.43×10^6	2+
	C/S	8.73	8.02×10^8	4+
9	1a-62	5.44	1.12×10^5	0.5+
	JC-1-127	5.37	1.12×10^5	0+
	li-39	6.17	1.56×10^6	1+
	Jc-134	6.24	1.56×10^6	0.5+
	C/S	7.88	1.43×10^8	3.5+

mg/kg/day s.c. (Table 1). Anti-*P. carinii* activity was found in 1a-62 and 1i-39 but not in 1a-53; however, all compounds caused early deaths and other evidence of systemic toxicity. In study 7, the initial dose was 3 mg/kg/day, which was then reduced to 1.5 mg/kg/day for improved tolerance and rat survival (Table 1). 1a-62 and li-39 lowered the mean cyst count from $\log_{10}$ 8.52 per lung in the C/S group to $\log_{10}$ 6.53 per lung (98-fold) and 7.04 per lung (30-fold), respectively. Study 8 analyzed JC-I-127, the dichloride salt analog of 1a-62, and JC-I-134, the dichloride salt analog of 1i-39 (Table 1). These compounds had been prepared in the hope that a more soluble chloride formulation would be better absorbed after oral administration than the dibromide salt. Although both preparations were well tolerated by the rats, they showed no anti-*P. carinii* activity. In study 9, we compared both formulations of these compounds by parenteral administration (Table 1). 1a-62 and its analog JC-I-127 exhibited comparable, dose-related activities against *P. carinii* and were well tolerated; at 2 mg/kg/day, these drugs lowered the mean cyst count from 7.88 per lung in the C/S group to 5.44 per lung (275-fold) and 5.37 per lung (323-fold), respectively; similar data were obtained when the data were analyzed by median counts (Table 2). These results compared favorably to the cyst count of 5.13 per lung achieved with the standard positive control drug, trimethoprim (TMP)-sulfamethoxazole (SMX) administered at 50 mg of TMP per kg/day and 250 mg of SMX per kg/day (Table 1). 1i-39 and its analog JC-I-134 also showed dose-related activity, but the magnitude of cyst reduction (45- to 50-fold) was not quite as great as that obtained with 1a-62 and JC-I-134.

2-(4-Carbostyryl)-5-nitro-1-vinylimidazole-guanylhydrazone and 2-(4-carbostyryl)-5-nitro-1-vinylimidazole-thiosemicarbazone were evaluated in a final experiment (Table 1). The drugs were administered in two doses but did not exhibit any anti-*P. carinii* activity. The doses of these agents also had to be reduced because of adverse effects.

Analysis by lung histology. In general, the results of histologic examination correlated well with *P. carinii* quantitation (Table 2). The most active drugs, 1a-62, li-39, and their JC counterparts, reduced the median histologic score from 3.5 to 4+ in the C/S group to 0 to 1+. On hematoxylin and eosin staining, heavily infected lungs exhibited minimal inflammatory changes and the typical vacuolated alveolar exudate; when stained with methenamine silver, clusters of organisms were seen.

DISCUSSION

Antitrypanosomal and anti-*P. carinii* activities have been shown to be properties of quaternary aromatic compounds and structurally unrelated drugs (5, 23, 30, 32). We undertook the present study to determine whether guanylhydrazone derivatives, which are effective in the treatment of trypanosomiasis in mice, have activity in our rat model of pneumocystosis. The results presented here summarize our experience with these compounds over several years. We also compared data analysis by geometric means and the *t*-test with analysis done by using medians and the Wilcoxin rank sum test and found that the two methods gave very similar information.

The bis(guanylhydrazones) of which MGBG is the prototype, have been investigated as antitrypanosomal and antitumor agents (4, 7). Since these drugs are relatively inexpensive and easy to synthesize, it was thought that they might be particularly suitable for use in poor African countries plagued by trypanosomiasis in cattle (29). Of the bis(guanylhydrazones) tested in a mouse model of African trypanosomiasis (28, 29) and in our rat model of pneumocystosis, compound 12, the triacetylbenzene derivative, was the most active.

The imidazo[1,2-a]pyridinium guanylhydrazones used in the present study were selected from a large number of compounds on the basis of favorable efficacy and toxicity in the therapy of *Trypanosoma rhodesiense* infection in mice (24). These drugs exhibited much greater anti-*P. carinii* activity than the other guanylhydrazone derivatives and are among the most potent cationic heteroaromatic agents we have ever tested. Compound 1a-62 (or JC-I-127, the dichloride salt) was the most active drug; when used at a dose of 2 mg/kg/day, this agent lowered the cyst count to levels comparable to those achieved with high dose TMP-SMX. 1i-39 was also very active, but 1a-53 was inactive. The latter result was somewhat surprising in light of the fact that 1a-53 appeared to be the most promising antitrypanosomal compound in mice (24).

Neither of the 5-nitro-1-vinylimidazole derivatives (21) examined here showed any anti-*P. carinii* activity. Our experience with nitroimidazole compounds with or without addition of guanylhydrazones in the treatment of pneumocystosis has generally been disappointing (30).

The principal structural characteristic shared by the guanylhydrazones, diamidines, and related compounds is the cationic nature of the heteroaromatic ring, which appears to play an important role in their antimicrobial properties (24). Current investigative interest in the mechanism of action of these drugs has focused on binding to the minor groove of DNA and inhibition of topoisomerase (8, 10, 17, 20, 22), although other pathways have also been studied (2, 3, 25). Studies of these drugs with trypanosomes have shown good correlation in the ability of these agents to bind to DNA, induce petite mutants in *Saccharomyces cerevisiae*, and exert trypanocidal activity in the mouse model (8, 12, 24). This approach has also been applied to *P. carinii* and other microbes, but further studies are needed to determine its value for prediction of in vivo activity (9, 11, 25, 27).

The major impediments to the clinical development of guanylhydrazones and other cationic compounds have been their toxicity and general need for parenteral administration. The successful use of aerosol pentamidine (4) suggests that other methods of administration should be explored. Among the more novel approaches which might be considered are topical administration (14) and conjugation to monoclonal antibodies directed towards the specific drug target (13).

In summary, this study has shown that some guanylhydrazone derivatives selected for activity in a mouse model of African trypanosomiasis are highly effective in the treatment of pneumocystosis in immunosuppressed rats. This information, combined with results of recent in vitro studies, should be

helpful in developing new anti-*P. carinii* drugs with improved potency and reduced toxicity.

ACKNOWLEDGMENTS

This study was supported by the Medical Research Services of the Department of Veterans Affairs and by Public Health Service contracts AI-72646 and AI-25139 and grants HL-46653, AI-31702, and AI-32899 from the National Institutes of Health.

REFERENCES

1. **Apted, F. I. C.** 1980. Present status of chemotherapy and chemoprophylaxis of human trypanosomiasis in the Eastern hemisphere. Pharmacol. Ther. **11**:391–413.
2. **Bacchi, C. J., P. P. McCann, H. C. Nathan, S. H. Hutner, and A. Sjoersdma.** 1983. Antagonism of polyamine metabolism—a critical factor in chemotherapy of African trypanosomiasis. Adv. Polyamine Res. **4**:331–341.
3. **Bitonti, A. J., J. A. Dumont, and P. P. McCann.** 1986. Characterization of *Trypanosoma brucei* S-adenosl-L-methionine decarboxylase and its inhibition by Berenil, pentamidine, and methylglyoxal bis(guanylhydrazone). Biochem. J. **237**:685–689.
4. **Chang, K.-P., R. F. Steiger, C. Dave, and Y.-C. Cheng.** 1978. Effects of methylglyoxalbis(guanylhydrazone) or trypanosomatid flagellates: inhibition of growth and nucleoside incorporation in *Trypanosoma brucei*. J. Protozool. **25**:145–149.
5. **Clarkson, A. B., Jr., M. Saric, and R. W. Grady.** 1990. Deferoxamine and eflornithine (DL-α-difluoromethylornithine) in a rat model of *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **34**:1833–1835.
6. **Cory, M., R. R. Tidwell, and T. A. Fairley.** 1992. Structure and DNA binding activity of analogs of 1,5-di(4-amidinophenoxy) pentane (pentamidine). J. Med. Chem. **35**:431–438.
7. **Dave, C., S. N. Pathak, and C. W. Porter.** 1978. Studies on the mechanism of cytotoxicity of methylglyloxal bis(guanylhydrazone) in cultured leukemia L1210 cells. Adv. Polyamine Res. **1**:153–171.
8. **Douc-Rasy, S., A. Kayser, J.-F. Riou, and G. Riou.** 1986. ATP-independent type II topoisomerase from trypanosomes. Proc. Natl. Acad. Sci. USA **83**:7152–7156.
9. **Dykstra, C. C., and R. R. Tidwell.** 1991. Inhibition of topoisomerases from *Pneumocystis carinii* by aromatic dicationic molecules. J. Protozool. **6**:78S–81S.
10. **Fairley, T., R. R. Tidwell, I. Donkor, N. Naiman, K. Ohemeng, R. Lombardy, J. Bentley, and M. Cory.** 1993. Structure, DNA minor groove binding, and base pair specificity of alkyl- and aryl-linked bis(amidinobenzimadazoles) and bis(amidinoindoles). J. Med. Chem. **36**:1746–1753.
11. **Feigon, J., W. A. Denny, W. Leupin, and D. R. Kearn.** 1989. Interactions of antitumor drugs with natural DNA: ^{1}H NMR study of binding mode and kinetics. J. Med. Chem. **27**:450–465.
12. **Ferguson, L. R., and R. J. Sundberg.** 1991. Petite mutagenesis in *Saccharomyces cerevisiae* by a series of bis-cationic trypanocidal drugs. Antimicrob. Agents Chemother. **15**:2318–2321.
13. **Goldenberg, D. M., R. M. Sharkey, S. Udem, R. Vagg, G. M. Levine, P. Conte, L. C. Swayne, H. J. Hansen, D. Cunniff, J. Anton, M. J. Linke, A. G. Smulian, and P. D. Walzer.** 1994. *Pneumocystis carinii* pneumonia in AIDS patients: a new method of diagnosis by radioimmunodetection (immunoscintigraphy). J. Nuclear Med. **35**:1028–1034.
14. **Jennings, F. W., J. M. Atouguia, and M. Murray.** 1993. Topical melarsoprol for trypanosomiasis. Lancet **341**:1341–1342.
15. **Jones, S. K., J. E. Hall, M. A. Allen, S. D. Morrison, K. A. Ohemeng, V. V. Reddy, J. D. Geratz, and R. R. Tidwell.** 1990. Novel pentamidine analogs in the treatment of experiment *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **34**:1026–1030.
16. **Kim, C. K., J. M. Foy, M. T. Cushion, D. Stanforth, M. J. Linke, H. L. Hendrix, and P. D. Walzer.** 1987. Comparison of histologic and quantitative techniques in evaluation of therapy for experimental *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **31**:197–201.
17. **Luck, G., K. E. Reinert, B. Baguley, and C. Zimmer.** 1987. Interaction of the nonintercalative antitumour drugs SN-6999 and SN-18071 with DNA: influence of ligand structure on the binding specificity. J. Biomol. Struct. Dyn. **14**:1079–1094.
18. **Masur, H.** 1992. Prevention and treatment of *Pneumocystis* pneumonia. N. Engl. J. Med. **327**:1853–1860.
19. **Pearson, R. D., and E. L. Hewlett.** 1985. Pentamidine for the treatment of Pneumocystis carinii pneumonia and other protozoal diseases. Ann. Intern. Med. **103**:782–786.
20. **Pjura, P. E., K. Grzeskowiak, and R. E. Dickerson.** 1987. Binding of Hoechst 33258 to the minor groove of *B*-DNA. J. Mol. Biol. **177**:257–271.
21. **Ross, W. J., and W. B. Jamieson.** 1975. Antiparasitic nitroimidazoles. 8. Derivatives of 2-(4-formylstyryl)-5-nitro-1-vinylimidazole. J. Med. Chem. **18**:158–161.
22. **Shapiro, T. A., and P. T. England.** 1990. Selective cleavage of kinetoplast DNA minicircles promoted by antitrypanosomal drugs. Proc. Natl. Acad. Sci. USA **87**:950–954.
23. **Smith, J. W., M. S. Bartlett, S. F. Queener, M. M. Durkin, M. A. Jay, M. T. Hull, R. S. Klein, and J. J. Marr.** 1987. *Pneumocystis carinii* pneumonia therapy with 9-deazainosine in rats. Diagn. Microbiol. Infect. Dis. **7**:113–118.
24. **Sundberg, R. J., D. L. Dahlhausen, G. Manikumar, B. Mavunkel, A. Biswas, V. Srinivasan, H. A. Musallan, W. A. Reid, Jr., and A. L. Ager.** 1990. Cationic antiprotozoal drugs. Trypanocidal activity of 2-(4′-formylphenyl)imidazo[1,2α]pyridinium guanylhydrazones and related derivatives of quaternary heteroatomic compounds. J. Med. Chem. **33**:298–307.
25. **Tidwell, R. R., and C. A. Bell.** 1994. Pentamidine and related compounds in the treatment of *Pneumocystis carinii* infection, p. 561–583. *In* P. D. Walzer (ed.), *Pneumocystis carinii* pneumonia. Marcel Dekker, Inc. New York.
26. **Tidwell, R. R., S. K. Jones, J. D. Geratz, K. A. Ohemeng, M. Cory, and J. E. Hall.** 1990. Analogues of 1,5-bis(4-amidinophenoxy)pentane (pentamidine) in the treatment of experimental *Pneumocystis carinii* pneumonia. J. Med. Chem. **33**:1252–1257.
27. **Tidwell, R. R., S. K. Jones, N. A. Naiman, L. C. Berger, W. B. Brake, C. C. Dykstra, and J. E. Hall.** 1993. Activity of cationically substituted bis-benzimidazoles against experimental *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **37**:1713–1716.
28. **Ulrich, P., and A. Cerami.** 1984. Trypanocidal 1,3 arylene diketone bis(guanylhydrazone)s. Structure-activity relationships among substituted and heterocyclic analogues. J. Med. Chem. **27**:35–40.
29. **Ulrich, P. C., R. W. Grady, and A. Cerami.** 1982. The trypanocidal activity of various aromatic bisguanylhydrazones in vivo. Drug Dev. Res. **2**:219–228.
30. **Walzer, P. D., J. Foy, P. Steele, and M. White.** 1992. Treatment of experimental pneumocystosis: review of 7 years of experience and development of a new system for classifying antimicrobial drugs. Antimicrob. Agents Chemother. **36**:1943–1950.
31. **Walzer, P. D., J. Foy, P. Steele, and M. White.** 1993. Synergistic combinations of Ro 11-8958 and other dihydrofolate reductase inhibitors with sulfamethoxazole and dapsone for therapy of experimental pneumocystosis. Antimicrob. Agents Chemother. **37**:1436–1443.
32. **Walzer, P. D., C. K. Kim, J. M. Foy, M. J. Linke, and M. T. Cushion.** 1988. Cationic antitrypanosomal and other antimicrobial agents in the treatment of experimental *Pneumocystis carinii* pneumonia. Antimicrob. Agents Chemother. **32**:896–905.