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8-Aminoquinolines Effective against *Pneumocystis carinii* In Vitro and In Vivo

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The activities of 25 8-aminoquinolines were compared in tests assessing the ability of the compounds to inhibit the growth of *Pneumocystis carinii* in culture. Six compounds were effective at or below 0.03 μM: CDRI 80/53, NSC19894, NSC305805, NSC305812, WR182234, and primaquine. Four others were effective at between 0.2 and 0.03 μM: NSC305835, WR225448, WR238605, and WR242511. Fourteen drugs were also tested in a standard model of *P. carinii* pneumonia in rats at daily doses of 2 mg/kg of body weight in drinking water. CDRI 80/53, NSC305805, NSC305835, and WR225448 were extremely effective in the animal model. The effectiveness of WR238605, WR242511, and primaquine in the rat model has been reported elsewhere (M. S. Bartlett, S. F. Queener, R. R. Tidwell, W. K. Milhouse, J. D. Berman, W. Y. Ellis, and J. W. Smith, Antimicrob. Agents Chemother. 35:277–282, 1991). The length of the alkyl chain separating the nitrogens in the substituent at position 8 of the quinoline ring was a strong determinant of anti-*P. carinii* activity.

Primaquine, an approved agent for the treatment of malaria, is effective against *Pneumocystis carinii* in humans when administered in combination with clindamycin and is currently used for the treatment of moderate infections or as salvage therapy (5, 9, 10, 14, 17, 18). The activity of this 8-aminoquinoline was detected initially by culture susceptibility testing and substantiated subsequently with the rat model (11). The effectiveness of primaquine prompted the study of other 8-aminoquinolines, and three members of this class, developed by Walter Reed Army Institute of Research (WRAIR), were found to be very active against *P. carinii* (4, 12).

The goal of the present study was to increase understanding of the structural elements producing antipneumocystis activity among 8-aminoquinolines. This information is crucial to the design of the ideal drug of this class, an 8-aminoquinoline with high potency and minimal toxicity. To accomplish this goal, we tested 8-aminoquinolines from the National Institutes of Health (NIH), WRAIR, and India (8) first in vitro and then in a rat model for *P. carinii* pneumonia. Active compounds were identified by tests in culture on the basis of the success of culture screening in identifying the activity of primaquine (5, 11). The current study confirms that culture screening is an effective way to identify 8-aminoquinolines active against *P. carinii* and that diverse structures within this family of compounds possess strong antipneumocystis activity.

MATERIALS AND METHODS

Culture. Compounds were evaluated by a short-term culture method with inocula from *P. carinii*-infected rat lung and cell cultures of human embryonic lung fibroblasts (HEL cells) as described previously (1, 12). In brief, tissue cultures were prepared with 24-well tissue culture plates in which HEL cells had been grown to confluency in minimum essential medium (MEM) containing 10% fetal calf serum. They were inoculated with *P. carinii* from fresh lung homogenates so that there were approximately 7×10^5 viable organisms per ml in each inoculated well. An inoculum was prepared by grinding *P. carinii*-infected rat lung in MEM, spinning the homogenate slowly ($250 \times g$) to settle large pieces of tissue, and counting numbers of organisms in 10-μl samples of supernatant by Giemsa staining and fluorescein diacetate-ethidium bromide viability staining. The viability observed with the fresh inoculum, which was used within 1 h of preparation, was in excess of 90%. On the basis of this count, the number of organisms per milliliter was adjusted by adding MEM to achieve the desired concentration. Although the infected rat lung used to prepare the inoculum contained cysts and precysts, as well as trophic forms, the predominant form in the actively infected animals was the trophic form. After preparation of the inoculum, cysts represented <10% of the viable organisms present. For the rare cysts that were observed, intracystic bodies were counted individually and included in the total count for the inoculum.

Each drug concentration to be tested was incorporated into the medium in four wells on each of four plates. Each plate also contained four wells without drug but inoculated with *P. carinii*; these wells served as positive growth controls. Experiments were discarded when the numbers of organisms in these wells failed to increase more than threefold over 7 days.

Plates were incubated in a gaseous mixture of 5% O_2–10% CO_2–85% N_2 at 35°C.

Evaluation of growth in culture. (i) Morphology. For determination of the numbers of organisms by morphology, cultures were sampled by washing cells with medium by use

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of Pasteur pipets and removing 10-μl samples of the supernatant to 1-cm^2 areas that had been etched on slides (1, 13). Slides were air dried, fixed in methanol, and stained with Giemsa stain. Two individuals counted numbers of trophic forms in 10 oil immersion fields on each slide, and mean values were plotted. Cysts and cells were also looked for in these preparations but were only rarely found. Excessive numbers of cells released into the supernatant is one index of drug toxicity for the monolayer; at the concentrations used in these experiments, no evidence of mammalian cell toxicity was seen.

(ii) ELISA. For the determination of antigens by an enzyme-linked immunosorbent assay (ELISA) (7), 300-μl samples from each of the four wells used for each condition were pooled in a microcentrifuge tube. Samples were processed and kept in phosphate-buffered saline (PBS) (pH 7.2) containing 0.02% sodium azide in a refrigerator until the study was complete. An ELISA was performed on all samples from a single experiment at the same time.

To each study tube, 100 μl of 1 M urea containing 1 mg of dithiothreitol per ml was added, the antigens were vortexed, and PBS was added to bring the volume in each tube to 1 ml. One hundred microliters of each antigen solution was transferred to each of 3 wells on a 96-well microtiter plate. The plates were incubated for 45 min at 35°C and washed three times with PBS containing 0.05% Tween 20 and 0.02% sodium azide by use of a Corning Immunowash ELISA plate washer. Nonspecific binding sites were blocked with 3% bovine serum albumin in PBS (pH 7.2) containing 0.02% sodium azide. The primary antibody was pooled convalescent-phase rat antisera diluted 1:500 with PBS; the secondary antibody was goat anti-rat immunoglobulin G conjugated with alkaline phosphatase. After a wash with buffer containing 100 mg $MgCl_2$ and 1 ml of diethanolamine per liter, substrate (1 mg of *p*-nitrophenyl phosphate per ml) was added. The plates were incubated at 35°C for 20 to 30 min and read on a Molecular Devices ELISA reader at an optical density at 405 nm (OD_{405}).

Convalescent-phase antibodies were obtained from rats that had been immunosuppressed, inoculated with *P. carinii* (3), and allowed to develop a moderate infection. They recovered and developed antibodies when immunosuppression was withdrawn. Rats were bled weekly and tested for an antibody response by use of Western blots (immunoblots). When a strong response at a 1:100 dilution of serum was noted, the rats were exsanguinated and the serum was pooled with other tested sera and frozen. The pooled sera were tested for cross-reactivity with different antigens by Western blotting.

Transtracheal inoculation of rats. The development of an infection in female rats was accomplished by inoculation as previously described (3). Rats from Harlan Sprague-Dawley colony 202 (Indianapolis, Ind.) are virus free and *P. carinii* free when shipped from the supplier, i.e., fail to develop a *P. carinii* infection when immunosuppressed and maintained in isolation.

Animals used to prepare an inoculum (inoculum animals) were maintained in isolation cages and protected from infectious agents other than *P. carinii*. Inoculum and sentinel animals are routinely monitored in our facility and are free of rodent coronavirus, Sendai virus, or *Mycoplasma pulmonis*. As supplied from Harlan, the rats are certified virus free. After 6 to 8 weeks, the lungs of inoculum rats were removed aseptically and divided into pieces that weighed approximately 200 mg; the pieces were placed in MEM containing 20% fetal calf serum (Sigma) and 7% dimethyl sulfoxide and then slowly taken to liquid nitrogen temperature. The samples were rapidly thawed for use.

The inoculum was prepared from frozen portions of infected rat lung by grinding a sample in saline and centrifuging, staining, and counting it as described for the preparation of the culture inoculum but adjusting the numbers of trophozoites so that there were at least 2×10^6 per ml. When examination of the Giemsa-stained preparation revealed contamination with bacteria or fungi, the inoculum was discarded.

Female rats weighing between 120 and 140 g were given dexamethasone at 0.36 mg/kg of body weight per day in drinking water for 4 days prior to transtracheal inoculation. The animals were anesthetized intramuscularly with 0.2 ml of a ketamine hydrochloride (80-mg/ml) solution also containing acepromazine and atropine (1.78 and 0.38 mg/ml, respectively). For inoculation of an animal, a small midline incision was made, the trachea was exposed by blunt dissection, and 0.2 ml of inoculum with 0.5 ml of air behind was injected directly into the trachea. The wound was closed with a clip.

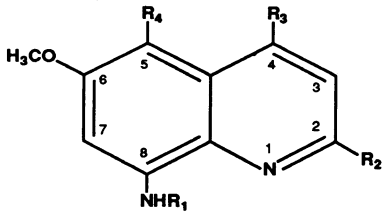
Therapy protocol. Drugs were administered to rats that had developed *P. carinii* infections after having been transtracheally inoculated as described previously (2). At 4 weeks postinoculation, rats were assigned by weight to treatment groups to achieve approximately equal mean weights for all groups. There were at least 10 rats in each group. Rats were housed in open cages and given water containing dexamethasone at 1.2 mg/liter and tetracycline at 0.5 g/liter. Animals received a normal diet containing 23% protein. For each drug study, a group of untreated animals served as a control to establish the ability of the inoculum to produce disease, and a group of trimethoprim-sulfamethoxazole-treated (50/250 mg/kg/day) animals served as a positive treatment control. Treatments with 8-aminoquinolines were given at 2.0 mg/kg/day in drinking water. Water was administered in brown bottles to protect the light-sensitive drugs.

Evaluation of therapy. At the end of 3 weeks of therapy, animals were anesthetized as described above and exsanguinated by cardiac puncture. Lungs were removed, and representative portions were used to make impression smears and reserved for the ELISA. Four impression smears, fixed in methanol, were used for staining with Giemsa and modified methenamine silver nitrate. Slides were blinded and examined microscopically by three readers, who scored them on the basis of the following system: >100 organisms per ×1,000 field, 5+; 11 to 100 per field, 4+; 1 to 10 per field, 3+; 2 to 9 in 10 fields, 2+; 1 in 10 or more fields, 1+; and no organisms in 50 fields, 0. This scale is approximately logarithmic; i.e., a sample scored as 4+ contains on average 10 times more organisms than a sample scored as 3+.

The ELISA was performed on samples from the same animals as an independent, second method of evaluation, by the technique described previously (7).

Scores from microscopic evaluations and OD_{405} values from the ELISA were entered on a Lotus worksheet. Mean scores and errors calculated from this program were used for comparisons; graphs were prepared by use of Cricket Graph. Statistical comparisons were made with the Kruskal-Wallis nonparametric analysis of variance test and the Mann-Whitney test for comparing two groups (InStat 2.0; GraphPad).

TABLE 1. Structures of 8-aminoquinolines studied



Compound	Designation	R_1	R_2	R_3	R_4
	Primaquine	$CH(CH_3)(CH_2)_3$-NH_2	H	H	H
1	WR182234	$CH(CH_3)(CH_2)_3$-NH_2	CH_3	H	H
2	NSC305805	$CH(CH_3)(CH_2)_3$-NH_2	H	CH_3	H
3	NSC19894	$CH(CH_3)(CH_2)_3$-NH_2	H	H	OCH_3
4	NSC305812	$CH(CH_3)(CH_2)_3$-NH_2	H	H	O-phenyl-p-Cl
5	NSC305841	$CH(CH_3)(CH_2)_3$-NH_2	CH_3	H	O-phenyl-p-F
6	WR183489	$CH(CH_3)(CH_2)_3$-NH_2	H	H	S-phenyl-p-Cl
7	WR242511	$CH(CH_3)(CH_2)_3$-NH_2	H	CH_3	$O(CH_2)_5CH_3$
8	WR225448	$CH(CH_3)(CH_2)_3$-NH_2	H	CH_3	O-phenyl-m-CF_3
9	WR238605	$CH(CH_3)(CH_2)_3$-NH_2	OCH_3	CH_3	O-phenyl-m-CF_3
10	NSC305833	$(CH_2)_6$-$NHCH(CH_3)_2$	H	CH_3	H
11	NSC305842	$(CH_2)_7$-$NHCH(CH_3)_2$	H	CH_3	H
12	NSC408744	$(CH_2)_2$-$N(CH_2CH_3)_2$	H	CH_3	H
13	NSC305840	$(CH_2)_5$-$N(CH_2CH_3)_2$	H	CH_3	H
14	NSC305839	$(CH_2)_7$-$N(CH_2CH_3)_2$	H	CH_3	H
15	NSC305835	$(CH_2)_4CH(CH_3)$-NH_2	H	CH_3	H
16	NSC305838	$(CH_2)_6$-$NHCH(CH_3)CH_2CH_3$	H	CH_3	H
17	NSC13713	$(CH_2)_3$-$N(CH_2CH_3)_2$	H	H	OCH_3
18	NSC19895	$CH(CH_3)(CH_2)_3$-$N(CH_3)_2$	H	H	OCH_3
19	NSC13314	$CH(CH_3)(CH_2)_3$-$NHCH(CH_3)_2$	H	H	OCH_3
20	NSC305845	$(CH_2)_6$-$N(CH_2CH_3)_2$	H	CH_3	F
21	NSC305843	$(CH_2)_6$-$N(CH_2CH_3)_2$	H	CH_3	OCH_3
22	NSC406300	$(CH_2)_5$-$NHCH(CH_3)_2$	H	H	O-phenyl-p-OCH_3
23	NSC290954	CH_2-phenyl-p-CH_2NH_2	H	H	O-phenyl-p-OCH_3
24	CDRI 80/53	$CH(CH_3)(CH_2)_3$-NH-3-acetyl-4,5-dihydro-2-furan	H	H	H

RESULTS

Twenty-five 8-aminoquinolines were available for study, 6 from WRAIR, 18 from NIH, and 1 from India. The structures of these compounds are shown in Table 1. All 25 compounds were tested for the inhibition of *P. carinii* growth in culture, at concentrations ranging from 0.01 to 10 μg/ml, and the cultures were evaluated both by direct counting of organisms and by the ELISA. Primaquine, WR238605 (compound 9), and WR242311 (compound 7) had previously been reported active in culture at 0.1 μg/ml, or approximately 0.2 μM, as determined by direct counting of Giemsa-stained samples as the method of evaluation (4). In the current study, the Indian compound (compound 24), three NIH compounds (compounds 2, 3, and 4), and two WRAIR compounds (compound 1 and primaquine) were shown to be effective in culture at less than 0.03 μM, as determined by the ELISA as well as a microscopic evaluation (Table 2). Four other compounds, 7, 8, 9, and 15, were effective at concentrations ranging from 0.2 to 0.03 μM.

Growth curves for representative compounds illustrated the range of inhibition of *P. carinii* by 8-aminoquinolines. Compound 2 showed activity similar to that of the positive control, primaquine (Fig. 1). Compound 8 showed intermediate activity, whereas compound 6 allowed growth virtually identical to that in the untreated control, until day 7, when the treated culture contained significantly fewer organisms than the control.

Compounds that were active in culture at 1.0 μg/ml or less were selected as the highest-priority compounds for testing in animals, but some compounds with lower activity in culture were also included to allow evaluation of the correlation of culture results with results in animal studies. All test compounds were administered at 2 mg/kg/day in drinking water, a dose and route of administration that had previously been demonstrated to be effective for WR6026, WR238605, and WR242511 (4). Evaluations were based upon counts of organisms in Giemsa-stained or silver-stained preparations and upon reactions in the ELISA with homogenized lung samples (Table 3). In the first experiment, compounds 2, 8, and 15 were extremely effective, eliminating detectable infection in all but one animal in each group; ELISA scores were not different from those seen with trimethoprim-sulfamethoxazole therapy (Table 3). Significant activity was also seen with compounds 1 and 24. Compound 6 did not appear effective, and animals tested with this compound were not different from untreated controls. In the second experiment with the animal model, compounds 4, 10, and 20 were active, but the activity of the last two compounds was modest (Table 3). Compound 2 was retested as the positive control in this experiment and was again very effective at 2 mg/kg/day. In other experiments not shown, this compound also has been effective at 1 and 0.5 mg/kg/day. Two compounds were tested in the third experiment (Table 3), but only compound 18 showed activity.

TABLE 2. Activity of 8-aminoquinolines tested in culture

Compound	Activity of drug at the indicated concn (μg/ml) as a percentage of control:								Calculated lowest effective dose (μM)[c]
	ELISA value[a]				Count[b]				
	0.01	0.1	1	10	0.01	0.1	1	10	
Primaquine	40	−14	−27		73	27	6	1	0.022
2	65	−11	−115		83	37	13		0.020
3	33	−4	−28	−35	62	22	7	1	0.021
4	68	52	−98		65	53	15		0.026
24	25	3			52	57	6	4	0.027
1	77	75	42		57	56	6	1	0.028
9	87	57			60	45			0.17
8	95	31	−20		78	72	13	2	0.18
7	63	−8			96	36	9	5	0.18
15	67	71	15		93	65	12		0.2
21		64	112			81	107		0.22
19		56	21	−29		60	53	7	0.24
22		108	43	−28		106	40	2	1.8
5		65	−9			103	16		2.0
6	129	113	64		66	89	11	1	2.2
14		106	80			103	64		2.3
20		99	36			75	35		2.3
16		75	89			103	50		2.4
18		100	54	−25		67	48	13	2.5
10		106	22			62	31		2.5
17			94	63			77	90	>17
23		75	79			119	99		>2
13		143	111			96	103		>1.9
11		100	87			157	49		>2.3
12		112	119			94	92		>3.0

[a] Computed with the following formula: 100 × [(day-7 experimental ELISA OD_{405} − day-0 ELISA OD_{405})/(day-7 control ELISA OD_{405} − day-0 ELISA OD_{405})]. With this scale, growth equivalent to control growth has a value of 100, cultures that grow less rapidly than the control have values of between 0 and 100, and cultures in which the numbers of organisms fell over 7 days have a negative value.

[b] Computed with the following formula: 100 × (average number of organisms seen per ×1,000 field in experimental wells on day 7/average number of organisms seen per ×1,000 field in control wells on day 7).

[c] Estimated to be the lowest concentration yielding values <85% of control values in both the ELISA and direct counts and converted to micromolar values to allow more direct comparisons of compounds.

DISCUSSION

The *P. carinii* culture system was used as a prescreen for 8-aminoquinolines. Of the 25 compounds tested, 10 were shown to be active at 0.2 μM or less: 1, 2, 3, 4, 7, 8, 9, 15, 24, and primaquine. All 8-aminoquinolines evaluated were analogs of primaquine and retained the 6-methoxy group. The effects of the 8-aminoalkylamino side chain and quinoline ring substitution on culture activity were examined in an attempt to identify structure-activity correlations. Of the 10 compounds that were active in culture at less than 0.2 μM, 8 (1, 2, 3, 4, 7, 8, 9, and primaquine) contained a 4-amino-1-methylbutyl-substituted 8-amino group. One key element seemed to be the length of the alkyl chain linking the two nitrogen atoms in R_1; four carbons were optimal for activity. Compounds such as 12 and 17, which contained fewer carbons in the chain, were devoid of activity. Extending the chain to more than four carbons did not have as dramatic an effect but may have diminished activity in culture, as is illustrated by the activities of compounds 13, 14, and 16 versus that of compound 2. Only one compound with an extended 8-aminoalkylamino side chain (WR6026) has shown high activity in culture (4). Compound WR6026 does not contain a chiral center in the 8-aminoalkylamino side chain, demonstrating that that feature is not required for activity.

The effect of ring substituents on the culture activity of primaquine analogs was also examined. Primaquine substituted with 2-methyl (compound 1), 4-methyl (compound 2), 4-methyl-5-substituted phenoxy (compound 8), and 4-methyl-5-hexoxy (compound 7) groups retained culture activity. The presence of a 5-methoxy group on a structure otherwise like primaquine (compound 3) had less of an effect on activity in culture than did the substitution of larger 5-alkoxy groups (compounds 4 and 5).

Seven of the most potent 8-aminoquinolines in these culture studies had not previously been tested against *P. carinii* pneumonia in animals. In the current study, of the six compounds most active in culture, two (compounds 2 and 24) were also exceedingly effective alone at a daily dose of 2 mg/kg for the therapy of rat *P. carinii* pneumonia, one (compound 1) showed modest activity, one (compound 4) showed weak activity, and two (compound 4 and primaquine) were inactive alone (4). Of the four compounds showing activity in culture at between 0.03 and 0.2 μM, 7, 8, 9, and 15, all were extremely effective alone at 2 mg/kg in the animal model (4). No compound devoid of activity in culture showed activity in the animal model. In previous studies in our laboratory, WR238605 (compound 9) and WR242511 (compound 7) were very effective therapy for rat *P. carinii* pneumonia when used alone at a dose of 2 mg/kg, but primaquine was active at this dose only when given with clindamycin (4, 11, 12). The lack of activity of primaquine alone in the animal model was suggested to relate to the very short half-life of primaquine in rats (12). Compound 3 was also inactive in the animal model at 2 mg/kg/day, despite showing high activity in culture. This compound is the only one in our series bearing both a methoxy group at highly

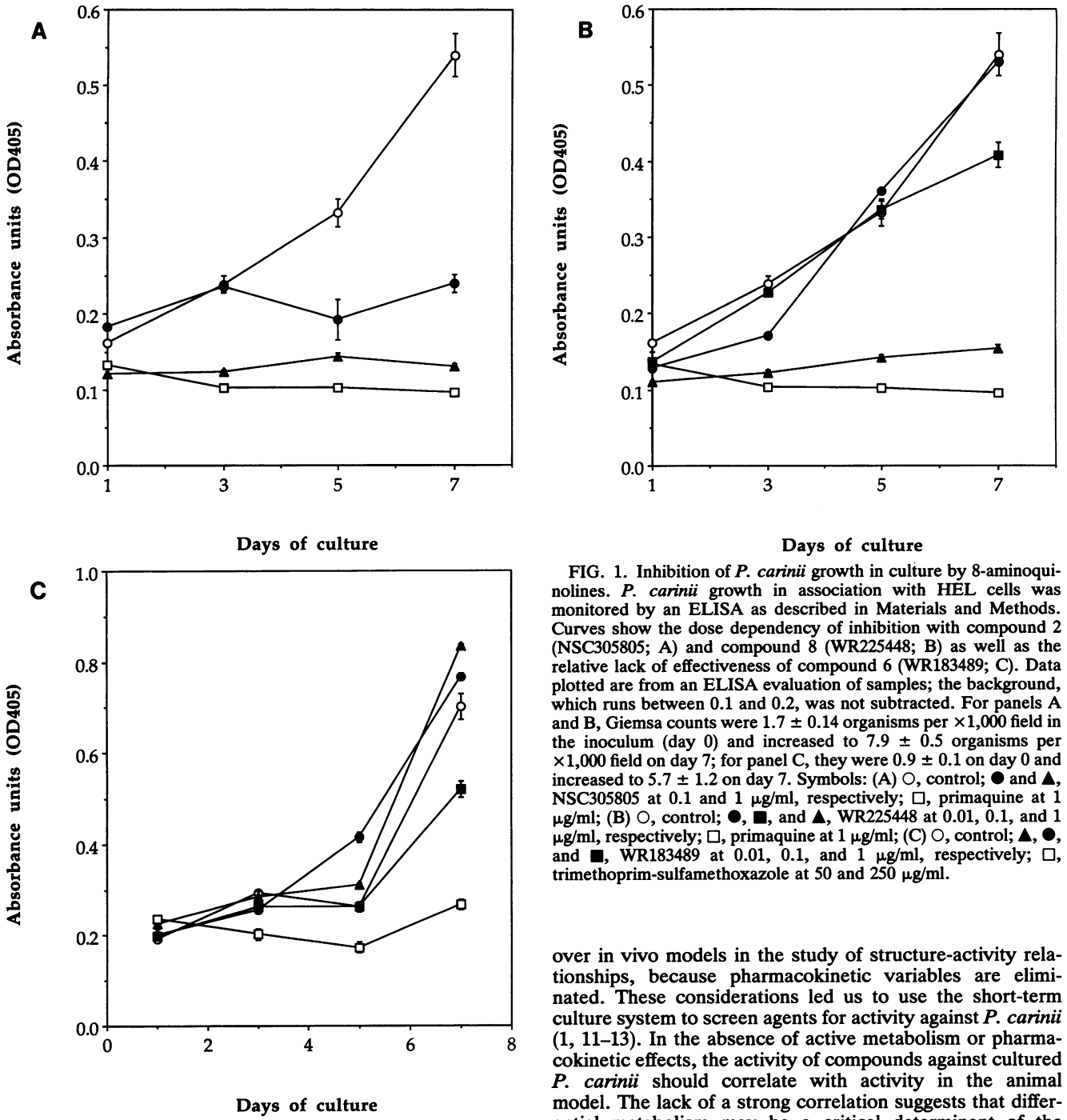


FIG. 1. Inhibition of *P. carinii* growth in culture by 8-aminoquinolines. *P. carinii* growth in association with HEL cells was monitored by an ELISA as described in Materials and Methods. Curves show the dose dependency of inhibition with compound 2 (NSC305805; A) and compound 8 (WR225448; B) as well as the relative lack of effectiveness of compound 6 (WR183489; C). Data plotted are from an ELISA evaluation of samples; the background, which runs between 0.1 and 0.2, was not subtracted. For panels A and B, Giemsa counts were 1.7 ± 0.14 organisms per ×1,000 field in the inoculum (day 0) and increased to 7.9 ± 0.5 organisms per ×1,000 field on day 7; for panel C, they were 0.9 ± 0.1 on day 0 and increased to 5.7 ± 1.2 on day 7. Symbols: (A) ○, control; ● and ▲, NSC305805 at 0.1 and 1 μg/ml, respectively; □, primaquine at 1 μg/ml; (B) ○, control; ●, ■, and ▲, WR225448 at 0.01, 0.1, and 1 μg/ml, respectively; □, primaquine at 1 μg/ml; (C) ○, control; ▲, ●, and ■, WR183489 at 0.01, 0.1, and 1 μg/ml, respectively; □, trimethoprim-sulfamethoxazole at 50 and 250 μg/ml.

reactive position 5 on the ring and a free amino group in the side chain at position 8. These structural elements may predispose the molecule to metabolic conversion, but the metabolism of this compound in rats has not been studied.

Culture models have traditionally been used for screening large numbers of compounds to select the most active agents for further testing and to avoid performing lengthy and expensive animal tests on compounds unlikely to have intrinsic activity. Culture systems have another advantage over in vivo models in the study of structure-activity relationships, because pharmacokinetic variables are eliminated. These considerations led us to use the short-term culture system to screen agents for activity against *P. carinii* (1, 11–13). In the absence of active metabolism or pharmacokinetic effects, the activity of compounds against cultured *P. carinii* should correlate with activity in the animal model. The lack of a strong correlation suggests that differential metabolism may be a critical determinant of the activity of 8-aminoquinolines when they are used for *P. carinii* pneumonia in animals, just as is the case for the use of these agents for protozoal diseases in animals and humans (15, 16).

Compounds 9 and 24 are being tested in humans for malaria because of their high potency against *Plasmodium* spp. (6, 8). Both compounds also rank among the most potent agents tested in both culture and animal models for *P. carinii*. These agents could afford the means to determine whether single-drug therapy with a very potent 8-aminoquinoline might replace the combination of clindamycin-primaquine for *P. carinii* pneumonia in human patients. Com-

TABLE 3. Activity of 8-aminoquinolines tested in the rat model of *P. carinii* pneumonia

Expt	Compound[a]	Giemsa stain infectivity score[b]	Total no. infected/ total no. tested[c]	Silver stain infectivity score[b]	Total no. infected/ total no. tested[c]	ELISA OD_{405}[b]
1	2	0[d]	0/9	0.1 ± 0.1[d]	1/9	0.10 ± 0.01[d]
	8	0[d]	0/8	0.1 ± 0.1[e]	1/8	0.10 ± 0.01[e]
	15	0.1 ± 0.1[d]	1/9	0.1 ± 0.1[d]	1/9	0.10 ± 0.01[d]
	24	0.6 ± 0.4[d]	2/7	1.2 ± 0.3[e]	6/7	0.16 ± 0.02[e]
	1	1.4 ± 0.7[f]	4/8	2.1 ± 0.4[f]	8/8	0.22 ± 0.06[f]
	6	4.1 ± 0.3	9/9	3.6 ± 0.2	9/9	0.42 ± 0.04
	TMP-SMX	0.1 ± 0.1[d]	1/9	0.3 ± 0.1[d]	4/9	0.10 ± 0.01[d]
	None (untreated)	4.3 ± 0.2	12/12	3.6 ± 0.2	12/12	0.34 ± 0.03
2	2	0.3 ± 0.3[e]	1/5	0.5 ± 0.3[e]	2/5	0.16 ± 0.01[e]
	10	1.1 ± 0.5[f]	4/7	1.3 ± 0.2[d]	7/7	0.20 ± 0.03[d]
	20	2.3 ± 0.5[f]	7/8	2.1 ± 0.4[e]	7/8	0.31 ± 0.04[e]
	4	3.4 ± 0.3[f]	9/9	3.4 ± 0.2	9/9	0.48 ± 0.07[f]
	3	4.0 ± 0.2	7/7	3.6 ± 0.2	7/7	0.65 ± 0.08
	5	4.2 ± 0.2	6/6	3.8 ± 0.2	6/6	0.57 ± 0.04
	17	4.5 ± 0.5	7/7	4.0 ± 0.1	7/7	0.78 ± 0.03
	None (untreated)	4.4 ± 0.2	12/12	4.0 ± 0.1	12/12	0.68 ± 0.07
3	18	3.3 ± 0.5[f]	7/8	3.1 ± 0.3[f]	8/8	0.35 ± 0.04[f]
	19	4.3 ± 0.1	3/3	3.7 ± 0.3	3/3	0.53 ± 0.03
	TMP-SMX	0.2 ± 0.1[d]	4/11	0.8 ± 0.2[d]	9/11	0.15 ± 0.01[d]
	None (untreated)	4.6 ± 0.1	7/7	3.9 ± 0.1	7/7	0.57 ± 0.04

[a] TMP-SMX, trimethoprim-sulfamethoxazole. Numbered compounds were used at 2 mg/kg/day; TMP-SMX was used at 50/250 mg/kg/day.
[b] Reported as means ± SEMs. *P* values were determined by the Mann-Whitney test.
[c] Number of rats in which organisms could be detected at the end of the study/number of rats evaluated at the end of the study.
[d] $P < 0.0001$.
[e] $P < 0.001$.
[f] $P < 0.01$.

pound 9 is in preclinical testing and is expected to enter phase-I trials for *P. carinii* pneumonia soon. Equally active compounds, such as 2 and 15, also have the potential for development, but these compounds cannot be made available as quickly because preclinical toxicity tests have not been reported.

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