ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1992, p. 779–782
0066-4804/92/040779-04$02.00/0

Vol. 36, No. 4

Antimicrotubule Benzimidazoles Inhibit In Vitro Growth of *Pneumocystis carinii*

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Received 4 November 1991/Accepted 22 January 1992

Nine antimicrotubule benzimidazole derivatives tested in a *Pneumocystis carinii* culture system with human embryonic lung fibroblast monolayers inhibited organism proliferation. The concentrations of drugs inhibitory in culture ranged from 10 to 0.1 μg/ml, with thiabendazole being the least effective (10 μg/ml) and parbendazole being the most effective (0.1 μg/ml). The parent compound, benzimidazole, was inactive at 10 μg/ml. Demonstration that this group of compounds has activity against *P. carinii* provides a new potential target that can be exploited, the microtubules. Also, the variability in the effectiveness of the compounds provides the basis for studies of structure-activity relationships, which were initiated in this study.

Pneumocystis carinii is a frequent cause of infection in immunocompromised individuals. Between 60 and 80% of all patients with AIDS suffer one or more episodes of pneumonia caused by *P. carinii*. Although prophylaxis with the combination of trimethoprim and sulfamethoxazole is effective in preventing pneumonia in children being treated for acute lymphocytic leukemia or in those with congenital immune deficiencies (10), severe adverse reactions preclude its use for prophylaxis in many patients with AIDS (12). Likewise, as therapeutic agents, both pentamidine and trimethoprim-sulfamethoxazole cause adverse reactions. Additional drugs are needed both for prophylaxis and for therapy of *P. carinii* pneumonia, particularly for those patients with AIDS. Both antiparasitic and antifungal drugs have been proposed as potential therapeutic agents. Although most antifungal agents have not proven useful (4) and some antiparasitic agents have been shown to have activity (19), most drug regimens have incorporated compounds which act as anti-folate pathway agents. Drugs which act against *P. carinii* by new and unique mechanisms could greatly enhance our ability to treat or prevent *P. carinii* pneumonia.

Benzimidazole derivatives are known to act against microtubules in other organisms, and it was hypothesized that they might show activity against the microtubules of *P. carinii*. Microtubules are important components of all eukaryotic cells. They form the mitotic spindle for chromosome segregation. In many cells, they function in motility by forming cilia and flagella, and they also serve as major cytoskeletal elements. Microtubules are formed by the polymerization of α- and β-tubulin protein subunits. Several drug groups inhibit microtubule polymerization; however, the benzimidazoles are the most important of these in terms of their clinical applications (15). At least 10 currently marketed benzimidazole derivatives have high inhibitory activities against helminths but low toxicities for mammalian cells. The related benzimidazoles benomyl and carbendazim are highly active antifungal agents used in agriculture. Recently, it has been reported that some protozoa (*Trichomonas vaginalis* and *Giardia lamblia*) also are highly susceptible to benzimidazoles, specifically the anthelminthic derivatives (9, 13). Results of the studies with a group of benzimidazoles that are active against fungi, helminths, and protozoa reported here demonstrate that they have potentially useful activity against *P. carinii* as well.

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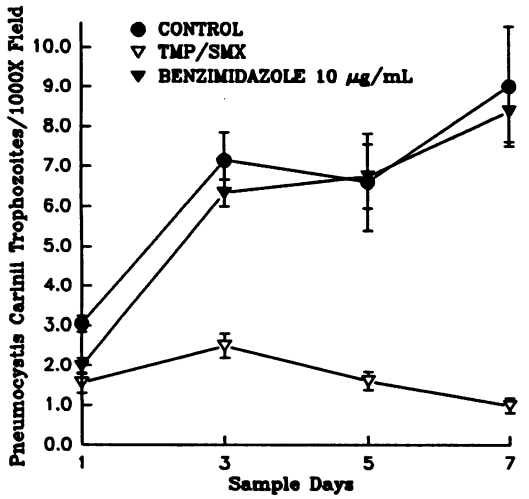
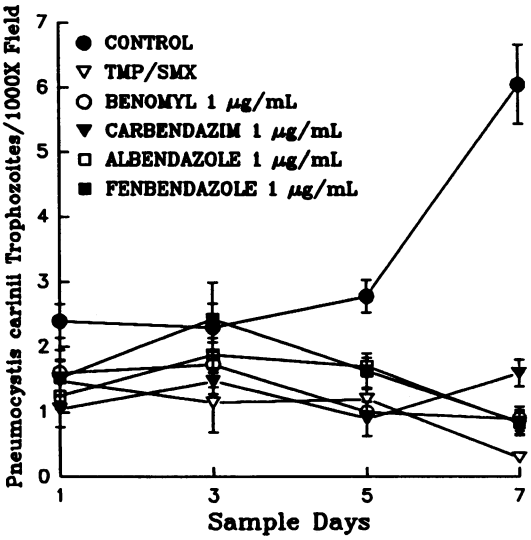
MATERIALS AND METHODS

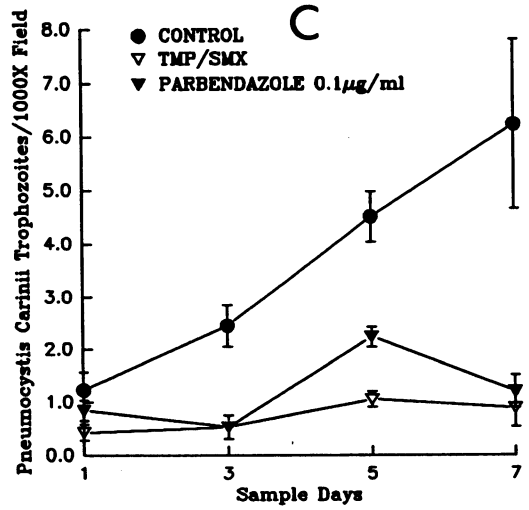
Ten benzimidazole derivatives were tested by using an in vitro system described previously (1). Briefly, drugs dissolved in dimethyl sulfoxide were diluted to concentrations of 10, 1.0, and 0.1 μg/ml in minimum essential medium used for the culture of human embryonic lung fibroblasts. The final maximum dimethyl sulfoxide concentration was 0.1%, a concentration of dimethyl sulfoxide that did not affect *P. carinii* proliferation when it was used alone and that gave *P. carinii* growth curves comparable to those of organisms in untreated control wells. Cell cultures in 24-well plates were inoculated with *P. carinii* trophozoites (final concentration, about 7×10^5 per ml) obtained from infected rat lungs (2). Each culture plate contained untreated and trimethoprim-sulfamethoxazole-treated (50/250 μg/ml) wells, in addition to the benzimidazole-treated wells. Plates were incubated at 35°C in a gas mixture of 5% O_2–10% CO_2–85% N_2 for up to 7 days. Plates were sampled on days 1, 3, 5, and 7 by removal of 10-μl amounts after agitation of the cultures. The samples were placed on slides in 1-cm^2 areas, fixed in methanol, and stained with Giemsa stain; and then they were examined microscopically as unknowns by two individuals. For each parameter there were four wells, making eight values for each parameter. Means and standard errors were used to construct growth curves.

RESULTS

All nine anthelminthic and antifungal benzimidazole derivatives tested were inhibitory to *P. carinii* at 10 μg/ml. In comparison, the parent compound, benzimidazole, had no effect at that concentration (Fig. 1A). The only derivative tested that was effective at 10 but not at 1.0 μg/ml was thiabendazole (Fig. 1A). All other antimicrotubule drugs tested were effective at 1.0 μg/ml (Fig. 1B). Only one of the drugs tested, parbendazole, was effective at 0.1 μg/ml (Fig. 1C).







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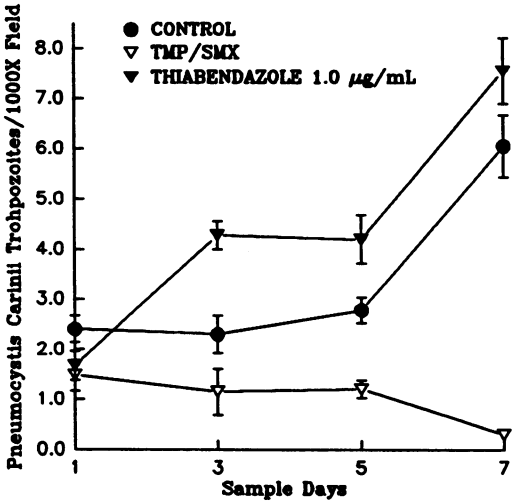

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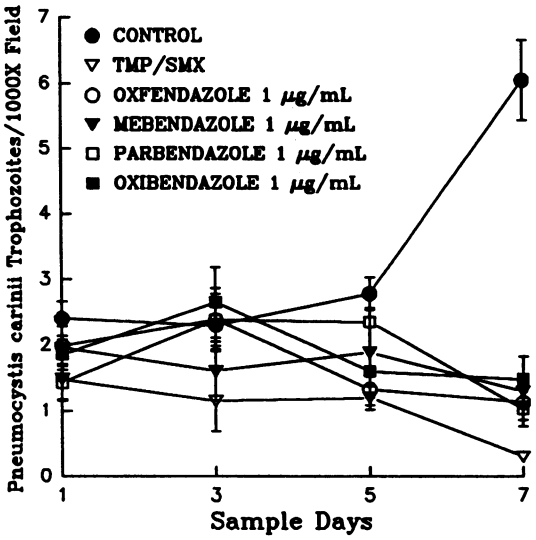


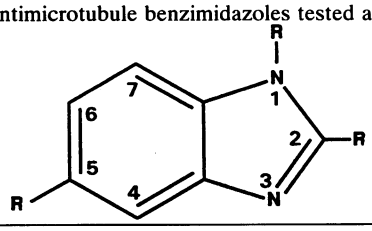
FIG. 1. (A to C) Growth curves are plots of means ± standard errors for *P. carinii* detected in Giemsa-stained samples taken on days 1, 3, 5, and 7 of culture. TMP/SMX, trimethoprim-sulfamethoxazole.

The variation in effectiveness of the benzimidazole derivatives tested led us to examine the structures for chemical correlates of effectiveness (Table 1). The two compounds with substituents other than $NHCOOCH_3$ (carbamate) at R_2 (i.e., benzimidazole and thiabendazole) were more than 10-fold less effective than all other derivatives tested. Among the remaining compounds, differences in potency were associated with the nature of the substituent on carbon 5, with a simple aliphatic substituent (i.e., parbendazole) being favored over sulfur- and oxygen-containing aliphatic substituents (albendazole, oxibendazole), aromatic substituents (fenbendazole, oxfendazole, mebendazole), or a simple hydrogen substituent (carbendazim).

DISCUSSION

The short-term culture method used here has predicted the efficacies of a variety of compounds for the treatment of *P. carinii* in animal models and in humans. These compounds

TABLE 1. Structures of antimicrotubule benzimidazoles tested against *P. carinii* in culture[a]



Derivative	R_1	R_2	R_5	Lowest effective concn (μg/ml)
Benzimidazole	-H	-H	-H	>10
Benomyl	$-CONH(CH_2)_3CH_3$	$-NHCOOCH_3$	-H	1
Thiabendazole	-H	-4-Thiazole	-H	10
Carbendazim	-H	$-NHCOOCH_3$	-H	1
Albendazole	-H	$-NHCOOCH_3$	$-SCH_2CH_2CH_3$	0.5
Fenbendazole	-H	$-NHCOOCH_3$	-S-Phenyl	1
Oxfendazole	-H	$-NHCOOCH_3$	-SO-Phenyl	1
Oxibendazole	-H	$-NHCOOCH_3$	$-OCH_2CH_2CH_3$	1
Mebendazole	-H	$-NHCOOCH_3$	-CO-Phenyl	1
Parbendazole	-H	$-NHCOOCH_3$	$-CH_2CH_2CH_2CH_3$	0.1

include trimetrexate (17), 9-deazainosine (3, 19), the 8-aminoquinolines WR6026 and WR238605 (5), and primaquine-clindamycin (18). The concentrations of these compounds that demonstrated activity in the in vitro culture system and that later proved to be effective in animal models were similar to the effective benzimidazole derivative concentrations reported here. For example, in the in vitro evaluation system, primaquine was shown to be effective at 0.45 μM (0.1 μg/ml), leading to the successful combination of clindamycin and primaquine for the treatment of *P. carinii* pneumonia (6, 20). Other 8-aminoquinolines tested in vitro had activities at 1.7 to 2.4 μM (approximately 1.0 μg/ml) and were shown to be very effective in the rat model of *P. carinii* pneumonia (5). Most of the benzimidazole derivatives evaluated were effective at about 3.3 μM, which is close to that range. Parbendazole was exceptional, being effective at 0.4 μM (0.1 μg/ml), while benzimidazole was not effective at 85 μM (10 μg/ml). Albendazole and mebendazole have been used to treat human tissue helminth infections as well as human intestinal helminth and protozoal infections. Assay methods have been reported (11, 16); and concentrations of the compounds in serum, plasma, and tissue have been determined. A very broad range of concentrations has been detected (7, 14). For example, after 15-mg/kg doses, albendazole was found at concentrations of 169 to 3,073 ng/ml, with mean peak levels of 788 ± 145 to 1,230 ± 58 ng/ml. Although therapeutic levels in liver tissue have been reported, therapeutic levels of neither drug in lung tissue have been reported. With the great variability in concentrations detected among the patients that have been tested, it is difficult to correlate effective drug concentrations with achievable levels in serum; however, it appears that inhibitory concentrations could be achieved.

The activities of the antimicrotubule benzimidazoles in this model suggest another group of potentially effective agents for the treatment of *P. carinii* pneumonia and provide an opportunity to correlate structures with efficacies to learn more about the mechanisms of action of these compounds. With information on the activities of these compounds in vitro, those that appear to be the most promising may be selected for use in trials in animal models. In addition, structural changes may be evaluated for their influence on the activities of the compounds in the culture method before progressing to evaluation in animals.

The effectiveness of parbendazole at the low concentration of 0.1 μg/ml should prompt early studies of this compound in animals. Finally, the discovery of compounds with activities against microtubules in other organisms provides a strong impetus for pursuing molecular studies of microtubules in *P. carinii*. Those studies are under way (8).

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service grants NO1-AI-72647 and UO1-AI-25859 from the National Institutes of Health.

We thank Janssen Biochimica/Biotech and SmithKline Beecham for providing compounds for testing.

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