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The interaction of benzimidazole carbamates with mammalian microtobule protein

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A range of benzimidazole compounds are now important as clinically- and agriculturally-useful drugs. Benomyl (methyl-1[butylcarbamoyl]benzimidazol-2-yl carbamate) and thiabendazole are fungicides; albendazole, cambendazole, fenbendazole, mebendazole, oxfendazole, oxibendazole, parbendazole and thiabendazole are anthelmintics; oncodazole was first investigated as an antineoplastic agent [1]. The similarities between the structures of these drugs suggests a common mechanism of action. Benomyl and methylbenzimidazol-2-yl carbamate (carbendazim) interfere with mitosis in fungi, plants and mammalian cells [2, 3]. Mebendazole causes degenerative changes in the intestinal cells of parasitic nematodes, an effect attributed to interaction with cytoplasmic microtubules [4]. Oncodazole interacts with microtubules *in vivo* [5] and *in vitro*, inhibiting assembly by binding to the colchicine-sensitive site of tubulin [6].

It has been proposed that the action of benomyl, and possibly other compounds, depends on the conversion within the cell to methylbenzimidazol-2-yl carbamate, which may be the effective agent [7]. Others, such as thiabendazole, cannot act in this way, but may still bind to the same site of sensitive microtubule systems [8].

In this paper, we compare the sensitivity of mammalian microtubules to a range of benzimidazoles with their efficacy *in vivo* and discuss structure–function relationships and the possibilities of variation in drug sensitivity between groups of organisms.

Experimental. Microtubule protein was purified from sheep brain by two cycles of assembly–disassembly as previously described [9], and was stored at −130°. Before use, the protein was depolymerized at 0° and clarified by centrifugation (130,000 *g*, 30 min, 4°). Tubulin dimer was separated from associated proteins by further assembly at 37° in the presence of 10% (v/v) dimethyl sulphoxide [10].

Assembly of microtubules (2 mg/ml) was monitored by turbidimetry at 400 nm in a Gilford 250 recording spectrophotometer; reaction was initiated by warming to 37° [11]. The increase in absorbance reflects the extent of microtubular aggregation [15]. Stock solutions of drugs were prepared in dimethyl-formamide, and were diluted 50-fold into the reaction; controls contained 2% (v/v) dimethylformamide.

Colchicine binding assays were performed as decribed in [12].

[^{3}H]Colchicine was obtained from the Radiochemical Centre, Amersham, Bucks, U.K., and colchicine from the Sigma (London) Chemical Co., Poole, Dorset, U.K. Other chemicals, of the purest grade available, were from standard suppliers.

Results and discussion. The time-course of the inhibition of brain microtubule assembly by parbendazole is shown in Fig. 1. Both the rate and the extent of polymerization are inhibited. This type of inhibition resembles that seen with colchicine [11] and is dissimilar from that with griseofulvin [11, 13]. Qualitatively similar effects are seen with several benzimidazoles. These results are summarized in Table 1. It is clear that inhibitory activity requires substitution at R_1 and R_2; the parent benzimidazole is ineffective. A range of hydrophobic R_1 substituents will suffice; nevertheless, the replacement of —CH_2— in parbendazole by —S— (albendazole) or of the sulphur of fenbendazole by sulphoxide (oxfendazole) considerably decreases the potency in this system. Methylbenzimidazol-2-yl carbamate, oxfendazole and thiabendazole were only weakly effective and their limited solubility precluded

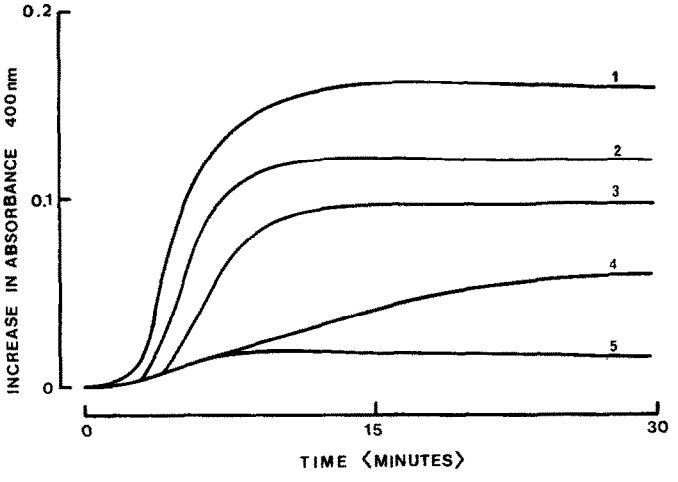
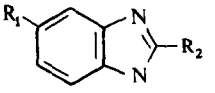
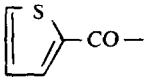
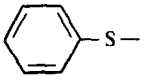
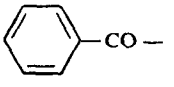
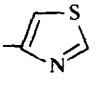
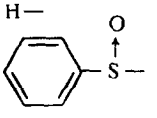
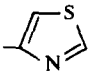


Fig. 1. Time-course of assembly of sheep brain microtubule protein in the presence of varying concentrations of parbendazole. All cuvettes contained assembly buffer (0.1 M-1,4-piperazinediethanesulphonic acid, 1 mM-$MgSO_4$, 2 mM-EGTA, 1 mM-GTP, pH 6.9) plus 2%-dimethylformamide and the following concentrations of parbendazole: Curve 1, none; Curve 2, 10^{-6} M; Curve 3, 2×10^{-6} M; Curve 4, 5×10^{-6} M; Curve 5, 2×10^{-5} M. Other experimental procedures were as described in the text.

Table 1. Relative effectiveness of benzimidazole carbamates and related compounds on assembly of sheep brain microtubules *in vitro* (IC_{50}) and on infections of *Nematospiroides dubis* (ED_{90}) in mice *in vivo*



Compound	R_1*	R_2*	IC_{50}‡	ED_{90}§
Parbendazole	$CH_3(CH_2)_3$—	—$NHCOOCH_3$	3	15
Oncodazole		—$NHCOOCH_3$	5	—
Fenbendazole		—$NHCOOCH_3$	6	90
Oxibendazole	$CH_3(CH_2)_2O$—	—$NHCOOCH_3$	7	<8
Mebendazole		—$NHCOOCH_3$	8	370
Albendazole	$CH_3(CH_2)_2S$—	—$NHCOOCH_3$	20	40
Cambendazole	$(CH_3)_2CHOCONH$—		68	130
Methylbenzimidazol-2-yl carbamate	H—	—$NHCOOCH_3$	>150†	—
Oxfendazole		—$NHCOOCH_3$	>250†	30
Thiabendazole	H—		>1000†	800
Benzimidazole	H—	—H	>5000†	—

* Substituents on the general structure shown above.
† Limit of solubility.
‡ Concentration of drug ($\times 10^6 M^{-1}$) causing 50 per cent inhibition of assembly.
§ P.p.m. of drug administered in diet (personal communication of Dr. J. Gee, Pfizer Ltd., Sandwich, Kent, U.K.).

accurate IC_{50} measurement. The effectiveness of oncodazole is marginally less marked than in a previous report [6].

There is some correlation between antimicrotubular activity and anthelmintic efficacy for parbendazole, fenbendazole, oxibendazole, albendazole, cambendazole and thiabendazole but none for mebendazole and oxfendazole (Table 1). Oxfendazole may be a special case since the sulphone it contains may well be reduced readily *in vivo*. It is clear, however, that there is no overall absolute correlation.

Incubation of whole microtubule protein with 50 μM-[^{3}H]colchicine led to the formation of the 1:1 colchicine–tubulin complex as previously described [12]. The inclusion of mebendazole in similar incubations decreased the extent of colchicine binding (Table 2). This was more marked at a lower colchicine concentration, suggesting that mebendazole, like oncodazole [6], competes with colchicine for the tubulin binding site. These experiments were repeated with purified tubulin dimer, with essentially similar results (data not shown). It is therefore unlikely that the associated proteins play any major role in these interactions.

Growth of the fungus *Aspergillus nidulans* is inhibited by both methylbenzimidazol-2-yl carbamate [7] and thiabendazole [8]. Extracts of mycelia bind both drugs in competition with colchicine and with each other, consistent with the participation of tubulins [7, 8]. In our experiments, the mammalian tubulin clearly binds methylbenzimidazol-2-yl carbamate to a very much smaller extent (see also [7]) and thiabendazole barely at all. Furthermore, the spectrum of effectiveness of benzimidazoles as anthelmintics does not correlate absolutely with their antimicrotubular activity. Although some of the discrepancies could be attributed to pharmacodynamic or metabolic factors, they might also be a consequence of difference between helminth and mammalian tubulins.

Fungal and mammalian tubulins co-polymerize [14], indicating a high degree of homology between them. Our observations suggest, however, that distinct differences may be present, probably around the colchicine binding site, and that these may constitute appropriate targets for specific chemotherapy not only of helminths but also of other eukaryotes.

Table 2. Competition between mebendazole and [^{3}H]colchicine for binding to sheep brain microtubule protein

[Colchicine](μM)	[Mebendazole](μM)	Colchicine : tubulin Molar ratio	Per cent of control
5	0	0.431	100
5	50	0.240	56
50	0	0.980	100
50	50	0.795	81
50	100	0.639	65

Microtubule protein was incubated with mebendazole (or buffer for controls) for 60 min at 37°, and then for a further 90 min with [^{3}H]colchicine. Other procedures were as in the text.

Note added in proof. Since this manuscript was prepared, Friedman and Platzer (*Biochim. biophys. Acta* **544**, 605 1978) have described similar results with calf brain tubulin.

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Oxidation of *p*-, *m*- and *o*-tyramine by type A and type B monoamine oxidase

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Mitochondrial monoamine oxidase [amine: oxygen oxidoreductase (deaminating, flavin-containing); EC 1.4.3.4] (MAO) is believed to exist in many animal tissues in two functional forms called type A and type B [1–3], based primarily on its sensitivity to clorgyline [1]. Recently, many monoamines have been characterized as substrates for type A and type B MAO; 5-hydroxytryptamine [4], 5-methoxytryptamine [5] norepinephrine and epinephrine [4] are specific for type A MAO, while β-phenylethylamine [2, 6], phenylethanolamine [7, 8] at low substrate concentrations, and benzylamine [4] are specific for type B MAO. Some substrates such as kynuramine [9], *p*-tyramine, tryptamine [1], dopamine [4], *p*-octopamine [8, 10] and *p*-synephrine [11] are oxidized by either type of MAO. Recently, *m*-tyramine was found in the brains of various species of mammals [12–15]. *o*-Tyramine was also identified in rat and human urine [16–18]. These findings have led us to characterize *m*- and *o*-tyramine as substrates for type A and type B MAO. The results on *p*-tyramine are also presented for comparison.

A crude mitochondrial fraction was isolated from whole