0026-895X/87/030432-05/0$02.00/0

MOLECULAR PHARMACOLOGY, 32:432-436

The Effects of 6-Benzyl-1,3-benzodioxole Derivatives on the Alkylation of Tubulin

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Received January 23, 1987; Accepted June 19, 1987

SUMMARY

Derivatives of 6-benzyl-1,3-benzodioxole are known to bind to tubulin and inhibit tubulin polymerization. For a better understanding of the mechanism of action of the 6-benzyl-1,3-benzodioxole derivatives, we have examined their effect on the alkylation of tubulin sulfhydryls by iodo[^{14}C]acetamide and *N,N'*-ethylene(bis)iodoacetamide. We have found that the 6-benzyl-1,3-benzodioxole derivatives with an intact dioxole ring affect alkylation to an extent proportional to their ability to inhibit tubulin polymerization. Those derivatives with the strongest resemblance to podophyllotoxin have the weakest effects. However, derivatives with a disrupted dioxole ring show little or no ability to inhibit polymerization, but their effect on alkylation is directly related to the degree of resemblance they bear to the trimethoxy ring of podophyllotoxin. It thus appears that the relatively simple approach of using alkylating agents can generate a significant amount of information on the mechanism by which various drugs interact with tubulin.

Microtubules are long cylindrical organelles critically involved in cell division and other processes (1). They are a target for various anti-cancer drugs (2). Screening drugs for anti-microtubule activities is a long process involving expensive cell cultures and measuring the effect of the drugs on microtubule assembly *in vitro*, colchicine's binding to tubulin, and tubulin's intrinsic GTPase activity (3). We have previously reported that tubulin's sulfhydryl groups are highly specific reporters of the interaction of tubulin with ligands (4–7). We have found that most tubulin ligands have specific effects on the reaction of tubulin's sulfhydryl groups with iodo(^{14}C)acetamide (5, 6). In addition, we have observed that when tubulin is reacted with EBI, a bifunctional derivative of iodoacetamide, two covalent cross-links, designated as β^* and β^s, form in the β subunit of tubulin (4, 7). Formation of β^* is blocked by colchicine, podophyllotoxin, and nocodazole, which bind to the same or overlapping sites on tubulin (5, 8–10), whereas that of β^s is blocked by GTP, vinblastine, vincristine, and maytansine, compounds that bind to other sites on tubulin (7, 11, 12). These assays are simple; they not only indicate the fact that a drug may bind to tubulin, but they also give information about the site on tubulin where the drug binds.

Recently, a class of compounds, the derivatives of 6-benzyl-1,3-benzodioxole, has been described which inhibits mitosis and blocks tubulin polymerization *in vitro*; the 6-benzyl-1,3-benzodioxole derivatives block colchicine binding to tubulin, and their binding to tubulin is said to resemble that of podophyllotoxin (3). We tested 13 of the 6-benzyl-1,3-benzodioxole compounds on our systems and found that they behave very much like colchicine and podophyllotoxin, and that the anti-tubulin effect of each 6-benzyl-1,3-benzodioxole derivative could be correlated with the extent of its effect on cross-linking, provided its dioxole ring was intact. Derivatives without an intact dioxole ring had little or no ability to inhibit tubulin polymerization, but their effect on cross-linking could be correlated with the resemblance of their C-ring (see Fig. 1) with the E-ring of podophyllotoxin. These results suggest that the approach which we have used could be a rapid and inexpensive preliminary screening method for anti-mitotic drugs that interact with tubulin.

Materials and Methods

Materials. The 5-alkoxy-6-benzyl-1,3-benzodioxole derivatives were prepared, as previously described, by acid-catalyzed condensation of appropriately substituted benzylic alcohols with sesamol, followed by alkylation of the phenolic hydroxyl group of the resulting 5-hydroxy-6-benzyl-1,3-benzodioxole. The purity and structural identity of the synthetic products were confirmed by thin layer chromatography, elemental analysis, and by ^{1}H nuclear magnetic resonance spectral measurements (13, 14). Their structures are shown in Fig. 1 and are described in Table 1. Tubulin was purified from bovine brain cerebra by a cycle of assembly and disassembly, followed by chromatography according to the method of Fellous *et al.* (15). All other materials were obtained as previously described (16).

Supported by Grant CA 26376 from the National Institutes of Health (to R.F.L.).

ABBREVIATION: EBI, *N,N'*-ethylene(bis)iodoacetamide.

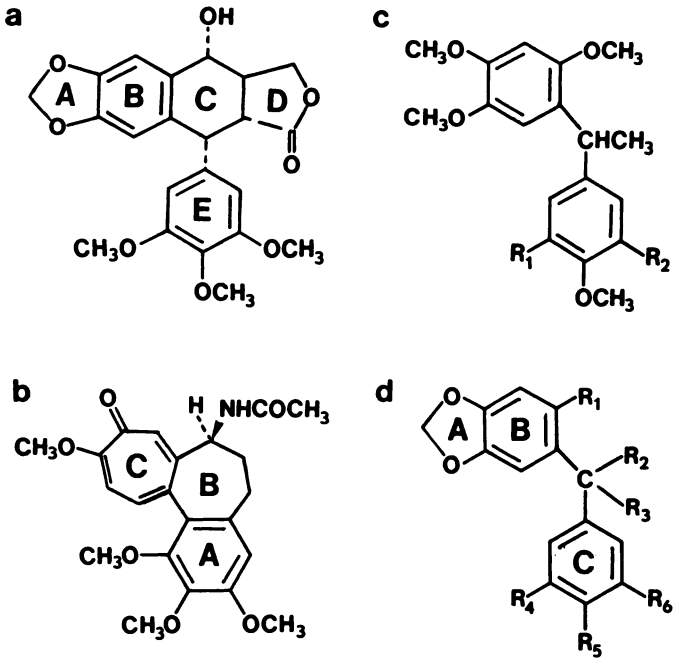

Fig. 1. Structures of analogues and derivatives of 6-benzyl-1,3-benzodioxole. The figure shows the following compounds: podophyllotoxin (a), colchicine (b), derivatives of 6-benzyl-1,3-dimethoxybenzene (c), and derivatives of 6-benzyl-1,3-benzodioxole (d). The R groups for the individual derivatives in c and d are described in Table 1.

Alkylation reactions. Tubulin was reacted with the 6-benzyl-1,3-benzodioxole derivatives in the presence of either iodo[^{14}C]acetamide or EBI. In the former case, the tubulin solutions were precipitated with trichloroacetic acid and filtered, and the radioactivity of the filters was measured as previously described (6). Tubulin that was reacted with EBI was reduced and carboxymethylated and subjected to electrophoresis on 5.5% polyacrylamide gels in the system of Laemmli (17), as previously described (4, 7). The yield of the β^* cross-link was calculated (7). Unless otherwise indicated, all experiments were carried out in the presence of the following buffer: 100 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.4, 1 mM ethyleneglycolbis-(β-aminoethyl ether)-*N*, *N'*-tetraacetic acid, 0.1 mM EDTA, 0.5 mM $MgCl_2$, and 1 mM GTP. Under these conditions, in the presence of EBI, only the β^* cross-link would form (7). Stock solutions of the 6-benzyl-1,3-benzodioxole derivatives were prepared in dimethyl sulfoxide. Unless otherwise indicated, the final concentrations of dimethyl sulfoxide in the experiments involving iodo[^{14}C]acetamide and EBI were 2% and 4%, respectively.

Results

The effects of the 6-benzyl-1,3-benzodioxole compounds on the reaction of tubulin with iodo[^{14}C]acetamide are shown in Table 2. As can be seen, most of them were not as effective as either podophyllotoxin or colchicine at suppressing the reaction with iodoacetamide. One of them, NSC 353649, had no effect at all, and NSC 357035, 3528 and NSC 352976 had relatively weak effects. In contrast, the effect of NSC 350102 was indistinguishable from that of colchicine or podophyllotoxin. When the effect of a series of NSC 350102 concentrations was tested (Fig. 2), it was found that 2–5 μM concentrations of NSC 350102 gave half-maximal suppression of alkylation, as was previously found for podophyllotoxin (5). In a separate experiment, the effect on alkylation of tubulin (0.66 mg/ml) of quadruplicate aliquots of 100 μM concentrations of each of two batches of NSC 321567 was tested; it was found that in the presence of the two batches, the extent of alkylation was 90 ± 3% and 85 ± 6% of that of an untreated control (data not shown). Because of the lesser solubility of NSC 321567 in dimethyl sulfoxide, this experiment was carried out in the presence of a dimethyl sulfoxide concentration of 10%. This makes it difficult to give a strict comparison of the effects of NSC 321567 and the other compounds, which were tested in the presence of a dimethyl sulfoxide concentration of 2%; nevertheless, it would appear that NSC 321567 is moderately effective at suppressing alkylation of tubulin by iodo[^{14}C]acetamide.

We tested the effects of the 6-benzyl-1,3-benzodioxole derivatives on the formation of the intra-chain cross-link, β^*, by EBI. From previous work, it is known that colchicine, podophyllotoxin, and nocodazole inhibit formation of this cross-link (5). As seen in Table 3, podophyllotoxin completely blocks β^* formation. The same is true for all of the 6-benzyl-1,3,-benzodioxole derivatives, except for NSC 321567. The most effective suppression is observed with NSC 321584, NSC 350102, NSC 352692, NSC 321567, and NSC 364720, whereas NSC 369686, NSC 352683, and NSC 357035 inhibit β^* formation by a small but significant amount. The other 6-benzyl-1,3-benzodioxole derivatives have intermediate effects. We examined the effects of a series of concentrations of an apparently highly effective suppressor (NSC 364720) and of a moderately effective one (NSC 269130) (Fig. 3). The results show that NSC 364720 and NSC 269130 gave half-maximal suppression of β^* formation in the concentration ranges of 1–2 μM and 5–10 μM, respectively.

TABLE 1
Identification of R groups in Fig. 1, c and d

Compound	R_1	R_2	R_3	R_4	R_5	R_6
Figure 1c						
NSC 369686	H	H				
3528	OCH_3	OCH_3				
Figure 1d						
NSC 269130	OCH_3	H	H	H	OCH_3	H
NSC 321584	OCH_2CH_3	H	H	H	OCH_3	H
NSC 350102	OCH_3	H	CH_3	H	OCH_3	H
NSC 321567	OCH_2CH_3	H	CH_3	H	OCH_3	H
NSC 364720	OCH_3	CH_3	CH_3	H	OCH_3	H
NSC 357035	OCH_2CH_3	H	CH_3	H	$OCH_2CH_2CH_3$	H
NSC 352692	$OCH_2CH{=}CH_2$	H	CH_3	H	OCH_3	H
NSC 352875	OCH_2CH_2OH	H	CH_3	H	OCH_3	H
NSC 353649	OCH_3	H	CH_3	H	OCH_3	OCH_3
NSC 352683	OCH_3	H	CH_3	OCH_3	OCH_3	OCH_3
NSC 352876	OCH_2CH_2OH	H	CH_3	OCH_3	OCH_3	OCH_3

TABLE 2

Effect of 6-benzyl-1,3-benzodioxole derivatives on the alkylation of tubulin by iodo[^{14}C]acetamide

Aliquots (250 μl) of tubulin (0.66 mg/ml) were incubated at 37°C for 1 hr with 1.36 mM iodo[^{14}C]acetamide (0.51 Ci/mol) in the presence of the indicated compounds. Incorporation of ^{14}C was measured as described in Materials and Methods. Incubations were done in quadruplicate. Standard deviations are shown.

Addition	Incorporation of ^{14}C	
	Mole label/mole protein	% of control
(μM)		
Experiment 1		
None	2.09 ± 0.05	100 ± 3
NSC 364720 (100)	1.77 ± 0.02	84 ± 2
NSC 350102 (100)	1.68 ± 0.07	80 ± 4
NSC 357035 (100)	1.95 ± 0.03	93 ± 3
NSC 353649 (100)	2.08 ± 0.09	100 ± 5
NSC 352875 (100)	1.81 ± 0.01	87 ± 2
NSC 269130 (100)	1.80 ± 0.04	86 ± 3
Colchicine (100)	1.57 ± 0.01	75 ± 2
Podophyllotoxin (50)	1.65 ± 0.06	79 ± 4
Experiment 2		
None	2.64 ± 0.08	100 ± 3
NSC 321584 (100)	1.84 ± 0.01	70 ± 2
NSC 352692 (100)	1.90 ± 0.03	72 ± 2
NSC 352876 (100)	2.15 ± 0.05	81 ± 3
NSC 352683 (100)	1.92 ± 0.26	73 ± 10
NSC 369686 (100)	1.95 ± 0.16	74 ± 6
3528 (100)	2.38 ± 0.08	90 ± 4
Colchicine (100)	1.78 ± 0.02	67 ± 2
Podophyllotoxin (50)	1.81 ± 0.02	69 ± 2

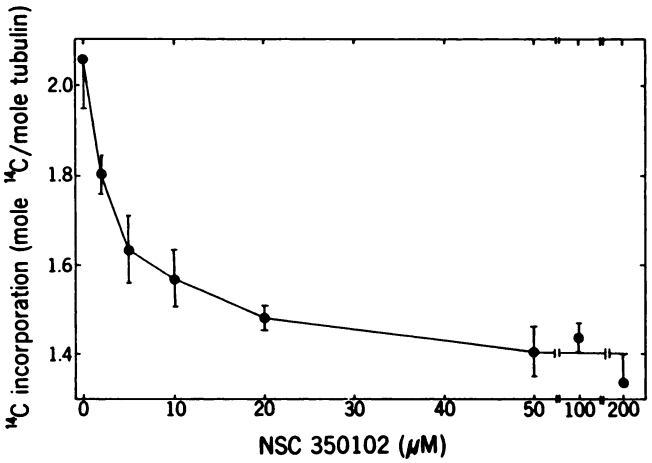

Fig. 2. Effects of a series of concentrations of NSC 350102 on the alkylation of tubulin by iodo[^{14}C]acetamide. Aliquots (250 μl) of tubulin (0.66 mg/ml) were incubated for 1 hr at 37°C in the presence of the indicated concentrations of NSC 350102 and in the presence of 1.36 mM iodo[^{14}C]acetamide (0.51 Ci/mol). At the end of the incubation, samples were precipitated by the addition of trichloroacetic acid, and incorporation of ^{14}C was determined. All incubations were done in quadruplicate. Standard deviations are shown.

TABLE 3

Effect of 6-benzyl-1,3-benzodioxole derivatives on the formation by EBI of the β* cross-link in β-tubulin

Aliquots (250 μl) of tubulin (0.66 mg/ml) in buffer lacking $MgCl_2$ were incubated for 1 hr at 30°C in the presence of 0.91 mM EBI, as well as the indicated drugs. At the end of 1 hr, the samples were dialyzed against buffer, reduced, and carboxymethylated and analyzed on 5.5% gels, as previously described (5). The yield of the β* cross-link was calculated as described (5). *Note*: We have previously shown (16) that, of the tubulin isotypes present in the two bands (β_1 and β_2) generated when reduced and carboxymethylated mammalian brain β-tubulin is subjected to electrophoresis in the system of Laemmli (17), only the isotypes in the β_1 band can form the β* cross-link. The β_2 isotype does not form this cross-link. Hence, the yield of the β cross-link is expressed as a percentage of the total β_1 in the sample.

Addition	Yield of β* (% of total β_1)
(μM)	
None	30.0, 32.5
Podophyllotoxin (50)	0
NSC 321584 (100)	3.2
NSC 269130 (100)	7.0
NSC 350102 (100)	3.1
NSC 321567* (100)	2.5
NSC 352692 (100)	4.4
NSC 364720 (100)	1.7
NSC 352875 (100)	6.4
NSC 357035 (100)	10.8
NSC 352876 (100)	7.9
NSC 353649 (100)	8.6
NSC 352683 (100)	22.3
NSC 369686 (100)	16.0
3528 (100)	6.7

Because of poor solubility, NSC 321567 was tested in the presence of a dimethyl sulfoxide concentration of 10%, as opposed to the 4% concentration in which the other derivatives were tested. The yield of β, obtained in a control sample containing 0.91 mM EBI and 10% dimethyl sulfoxide, was 49 ± 7%.

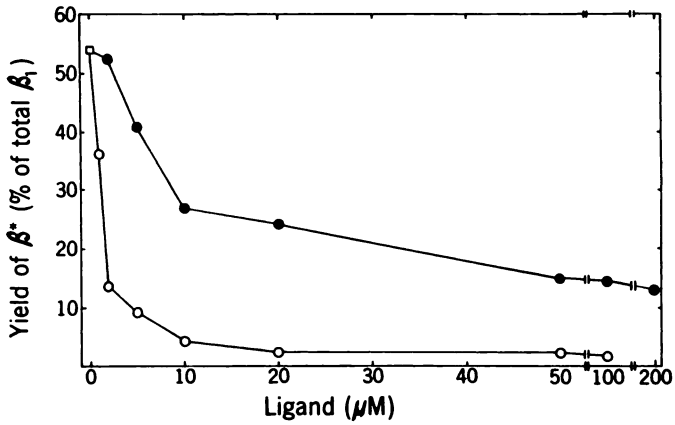

Fig. 3. Effect of a series of concentrations of NSC 364720 and NSC 269130 on the formation of the β* cross-link in β_1-tubulin by EBI. Aliquots (250 μl) of tubulin (0.66 mg/ml) were incubated for 1 hr at 30°C in the absence (□) or presence of the indicated concentrations of either NSC 364720 (○) or NSC 269130 (●), as well as 0.91 mM EBI. At the end of the incubation, samples were dialyzed, reduced, and carboxymethylated and analyzed on polyacrylamide gels. The yield of the β* cross-link was determined.

Discussion

Our previous work shows very clearly that the effect of drugs on the alkylation of tubulin falls into four very sharply defined categories: (*1*) drugs that inhibit alkylation with iodo[^{14}C] acetamide and block β* formation, namely, colchicine, podophyllotoxin, and nocodazole (5); all of these drugs are known to bind to one site or a set of overlapping sites on the tubulin molecule (8, 9); (*2*) drugs that inhibit alkylation with iodo[^{14}C] acetamide and block β* formation, namely, vinblastine and vincristine (5, 7); these drugs are known to bind to a site or sites on tubulin which are distinct from the colchicine-binding site (18); (*3*) drugs that do not affect alkylation with iodo[^{14}C] acetamide, but block β* formation, namely, maytansine (6, 7); and (*4*) drugs that have no effect on alkylation and have no effect on cross-link formation, namely, bis (8-anilinonaphthalene-1-sulfonate), which binds to a site on the tubulin molecule distinct from that of any other drug (19, 20). It is clear from the data presented in this paper that some of the 6-benzyl-1,3-benzodioxole derivatives belong in the first class, in that they inhibit alkylation by iodo[^{14}C]acetamide and inhibit β* forma-

tion. This is not surprising, in that they are known to block colchicine binding to tubulin, and their structures resemble that of podophyllotoxin (3). The maximal extents of the effects on these drugs on alkylation and β^* formation are slightly smaller than those of podophyllotoxin and considerably smaller than those of colchicine. We have previously proposed that the reason why podophyllotoxin's effects are smaller than colchicine's is that podophyllotoxin's binding is reversible and that of colchicine is not (5). It is reasonable to speculate that the 6-benzyl-1,3-benzodioxole derivatives bind reversibly to tubulin, and that this in part accounts for the smaller effects. Another possible reason for the lesser magnitude of their effects is that they are considerably smaller than podophyllotoxin, and they may cover a smaller area on the tubulin molecule.

Table 4 shows a comparison between the anti-tubulin effects of the 6-benzyl-1,3-benzodioxole derivatives and their effects on alkylation of tubulin by iodo[^{14}C]acetamide and on β^* formation. It is clear that there is close correlation of these effects among those derivatives that have an intact dioxole ring and a single methoxy group on the C-ring. The correlation is particularly impressive between anti-tubulin effect and the effect on β^* formation. The one with the strongest anti-tubulin effect, NSC 364720, whose ID_{50} is 5 μM, inhibits β^* formation by 95%. NSC 321584, NSC 321567, and NSC 350102, whose ID_{50} levels are in the 5–10 μM range, inhibit β^* formation by 90%. NSC 352875 and NSC 352692, whose ID_{50} levels are in the 10–20 μM range, inhibit β^* formation by 80–86%, and NSC 269130, with an ID_{50} of 20–25 μM, inhibits β^* formation by 78%. The correlation between anti-tubulin effect and the effect on alkylation by iodo[^{14}C]acetamide is not so striking, but in this particular group of derivatives, there is a loose correlation between these two effects. It is likely that the correlations in this group reflect the binding affinities of these derivatives for tubulin. The correlations are somewhat less apparent in those derivatives that have an intact dioxole ring, but have more than one methoxy group on the C-ring. The anti-tubulin activities of these derivatives, NSC 353649, NSC 352683, and NSC 352876, are very low, but they inhibit β^* formation by 29–75%, a lesser extent than any of the derivatives that have an intact dioxole ring and a single methoxy group on the C-ring. In contrast, their effects on alkylation by iodo[^{14}C]acetamide are very variable, ranging from no effect with NSC 353649 to an effect 84% as great as colchicine's with NSC 352683.

TABLE 4

Comparison of anti-tubulin activity of the 6-benzyl-1,3-benzodioxole derivatives with their effects on alkylation

Derivative	ID_{50} for tubulin polymerization[a]	Effect on ^{14}C incorporation (% of colchicine's effect)	Suppression of β^* formation (% of podophyllotoxin's effect)[b]
	μM		
Disrupted dioxole ring, 1 methoxy on C-ring			
NSC 369686	50–100	80	49
Disrupted dioxole ring, 3 methoxys on C-ring			
3528	Inactive	30	79
Intact dioxole ring, 1 methoxy on C-ring			
NSC 269130	20–25	56	78
NSC 321584	5–10	93	90
NSC 350102	5–10	79	90
NSC 321567	5–10	ND[c]	95
NSC 364720	5	62	95
NSC 352692	15–20	86	86
NSC 352875	10–15	54	80
Intact dioxole ring, 2 methoxys on C-ring			
NSC 353649	>100	2	72
Intact dioxole ring, 3 methoxys on C-ring			
NSC 352683	>100	84	29
NSC 352876	>100	57	75
Intact dioxole ring, 1 propoxy ring			
NSC 357035	ND	27	35

[a] From Ref. 3.
[b] Podophyllotoxin completely inhibited EBI-induced formation of the β^* cross-link; hence, podophyllotoxin is assumed to be 100% effective.
[c] ND = not determined.

The effect of the derivatives with a disrupted dioxole ring are surprising. NSC 369686 and 3528 have little or no anti-tubulin activity, but they inhibit alkylation by iodo[^{14}C]acetamide and β^* formation. 3528, which has three methoxy groups on the C-ring, inhibits β^* formation by 79%, as great as the effect of NSC 269130, which has strong anti-tubulin activity. NSC 369686, the other derivative with a disrupted dioxole ring and a single methoxy on the C-ring, inhibits β^* formation by 49%.

The results presented in this paper are consistent with the following models. In the derivatives of 6-benzyl-1,3-benzodioxole with an intact dioxole ring, the strongest interaction with tubulin is achieved with a single methoxy group on the C-ring. Addition of more methoxy groups to this ring weakens the binding of the compound to tubulin, as shown by a loss of anti-tubulin activity and a decrease in the ability to inhibit β^* formation and alkylation by iodo[^{14}C]acetamide. This is true even though the addition of more methoxy groups increases the resemblance to podophyllotoxin, which as three methoxy groups on this ring. It may be that the presence of the D-ring on the podophyllotoxin molecule could affect the angle at which its trimethoxy ring interacts with tubulin in such a way that the presence of the three methoxy groups makes for better binding. If the D-ring is lacking, as is the case with the 6-benzyl-1,3-dioxole derivatives, the angle at which the C-ring interacts with tubulin is such that the presence of more than one methoxy group on this ring hinders its binding to tubulin. Alternatively, perhaps tubulin can adopt two different conformations, depending on the absence or presence of the D-ring on the podophyllotoxin molecule. In the former case, the presence of three methoxy groups on the C-ring makes for worse binding; in the latter case it makes for better binding.

A different situation ensues when the dioxole ring is not intact. Here, the binding of the molecule to tubulin is strongly inhibited, as shown by the loss of anti-tubulin activity. Our results suggest that although the binding of the molecule as a whole is weakened, the binding of the C-ring to tubulin is facilitated, in that it is no longer as constrained as it is when the dioxole ring is intact. In this case, the binding of the C-ring to tubulin is increased when there are three methoxy groups on it, as opposed to just one. The presence of three methoxy groups on this ring makes it resemble the trimethoxy ring of either colchicine or podophyllotoxin. We have previously shown that 3,4,5-trimethoxybenzaldehyde, an analogue of this ring,

will bind weakly to tubulin and inhibit β* formation, and we have suggested that one of the β* sulfhydryls is located at the region of the tubulin molecule where this ring binds (10).

One contrast between the effects of 3,4,5-trimethoxybenzaldehyde and the 6-benzyl-1,3-benzodioxole derivatives is that the former increases alkylation by iodo[^{14}C]acetamide and the latter inhibits it. In our previous work, we presented evidence that colchicine's inhibition of alkylation by iodo[^{14}C]acetamide was, in part, a conformational effect that required that both the A- and the C-ring of colchicine be bound (10). The reason that the 6-benzyl-1,3-benzodioxole derivatives inhibit alkylation may be that both the dioxole ring and the C-ring bind to tubulin. It is possible that even the disrupted dioxole ring can still bind weakly to tubulin and, together with the C-ring, inhibit alkylation.

The assays we have used to test the 6-benzyl-1,3-benzodioxole derivatives are rapid and require very little protein. They appear to have some predictive value in determining the possible anti-tubulin activity of these derivatives. It is clear that they can generate false positives, in that compounds that have little or no anti-tubulin activity, and presumably, therefore, no anti-mitotic activity, can inhibit alkylation. Nevertheless, no false negatives were generated; derivatives that had a very small effect on alkylation had little or no anti-tubulin activity. Hence, the methods described here may be useful as a preliminary screening of compounds such as these. In addition, our results suggest that the detailed mechanism of the binding of these derivatives to tubulin and their effects on the tubulin molecule may be complex, and suggest that further experimentation may yield substantial information about the mechanism of tubulin-ligand interactions.

References

1. Dustin, P. *Microtubules*. Berlin, Springer-Verlag (1978).
2. Luduena, R. F. Biochemistry of tubulin, in *Microtubules* (K. Roberts and J. Hyams, eds.). Academic Press, London, 65–115 (1979).
3. Batra, J. K., L. Jurd, and E. Hamel. Structure-function studies with derivatives of 6-benzyl-1,3-benzodioxole, a new class of synthetic compounds which inhibit tubulin polymerization and mitosis. *Mol. Pharmacol.* **27**:94–102 (1985).
4. Luduena, R. F., and M. C. Roach. Interaction of tubulin with drugs and alkylating agents. 1. Alkylation of tubulin by iodo(^{14}C)acetamide and *N,N'*ethylenebis(iodoacetmide). *Biochemistry* **20**:4437–4444 (1981).
5. Luduena, R. F., and M. C. Roach. Interaction of tubulin with drugs and alkylating agents. 2. Effects of colchicine, podophyllotoxin, and vinblastine on the alkylation of tubulin. *Biochemistry* **20**:4444–4450 (1981).
6. Luduena, R. F., and M. C. Roach. Contrasting effects of maytansine and vinblastine on the alkylation of tubulin sulfhydryls. *Arch. Biochem. Biophys.* **210**:498–504 (1981).
7. Roach, M. C., and R. F. Luduena. Different effects of tubulin ligands on the intrachain cross-linking of β_1-tubulin. *J. Biol. Chem.* **259**:12063–12071 (1984).
8. Cortese, F., B. Bhattacharyya, and J. Wolff. Podophyllotoxin as a probe for the colchicine binding site of tubulin. *J. Biol. Chem.* **252**:1134–1140 (1977).
9. Hoebeke, J., G. van Nijen, and M. DeBrabander. Interaction of oncodazole (R 17934), a new anti-tumoral drug, with rat brain tubulin. *Biochem. Biophys. Res. Commun.* **69**:319–324 (1976).
10. Roach, M. C., S. Bane, and R. F. Luduena. The effects of colchicine analogues on the reaction of tubulin with iodo(^{14}C)acetamide and *N,N'*-ethylenebis(iodoacetamide). *J. Biol. Chem.* **260**:3015–3023 (1985).
11. Mandelbaum-Shavit, F., M. K. Wolpert-DePhilippes, and D. G. Johns. Binding of maytansine to rat brain tubulin. *Biochem. Biophys. Res. Commun.* **72**:47–54 (1976).
12. Bhattacharyya, B. and J. Wolff. Maytansine binding to the vinblastine sites of tubulin. *FEBS Lett.* **75**:159–162 (1977).
13. Jurd, L. Quinones and quinone-methides. 1. Cyclization and dimerization of crystalline ortho-quinone methides from phenol oxidation reactions. *Tetrahedron* **33**:163–168 (1977).
14. Jurd, L., R. L. Fye, and J. Morgan, Jr. New types of insect chemosterilants: benzyphenols and benzyl-1,3-benzodioxole derivatives as additives to housefly diet. *J. Agric. Food Chem.* **27**:1007–1016.
15. Fellous, A., J. Francon, A.-M. Lennon, and J. Nunez. Microtubule assembly *in vitro*. Purification of assembly-promoting factor. *Eur. J. Biochem.* **78**:167–174 (1977).
16. Luduena, R. F., M. C. Roach, P. P. Trcka, M. Little, P. Palanivelu, P. Binkley, and V. Prasad. β_2-Tubulin, a form of chordate brain tubulin with lesser reactivity towards an assembly-inhibiting sulfhydryl-directed cross-linking reagent. *Biochemistry* **21**:4787–4794 (1982).
17. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680–685 (1970).
18. Bryan, J. Definition of three classes of binding sites in isolated microtubule crystals. *Biochemistry* **11**:2611–2616 (1972).
19. Prasad, A. R. S., Luduena, R. F., and Horowitz, P. M. Bis-(8-anilinonaphthalene-1-sulfonate) as a probe for tubulin decay. *Biochemistry* **25**:739–742 (1986).
20. Luduena, R. F., Roach, M. C., and Horowitz, P. M. The effects of the anilinonaphthalenesulfonates on the alkylation of tubulin: correlation between the appearance of sulfhydryl groups and apolar binding sites. *Biochim. Biophys. Acta* **873**:143–146 (1986).

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