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INTERACTION OF HALICHONDRIN B AND HOMOHALICHONDRIN B WITH BOVINE BRAIN TUBULIN

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Abstract—Halichondrin B is a polyether macrolide of marine origin which binds to tubulin and inhibits microtubule assembly *in vitro* and *in vivo*. As is the case with phomopsin A and dolastatin 10, halichondrin B is a non-competitive inhibitor of vinblastine binding to tubulin. Analogous to maytansine, which by contrast is a competitive inhibitor of vinblastine binding, halichondrin B has no effect on colchicine binding, which is greatly stabilized by phomopsin A and dolastatin 10, but not by maytansine. We have previously developed assays which allow sensitive discrimination among the interactions of various ligands with tubulin, and examined the effects of ligands on the reactivity of tubulin sulfhydryl groups and the exposure of hydrophobic areas on the surface of the tubulin molecule. To classify the nature of the interaction between halichondrin B and tubulin, in this study we examined the effects of halichondrin B and its closely related analogue, homohalichondrin B, by these assays. We found that: (1) halichondrin B and homohalichondrin B both inhibited formation of an intra-chain cross-link between two sulfhydryl groups in β-tubulin, as do phomopsin A, dolastatin 10, maytansine, and vinblastine; (2) halichondrin B resembles maytansine in that it had no effect on alkylation of tubulin sulfhydryl groups by iodoacetamide, unlike phomopsin A, dolastatin 10 and vinblastine, all of which inhibit alkylation; (3) halichondrin B differs from other anti-mitotic drugs in that it enhanced exposure of hydrophobic areas on tubulin; (4) homohalichondrin B, like maytansine and in contrast to phomopsin A, dolastatin 10 and vinblastine, had no effect on exposure of hydrophobic areas; and (5) homohalichondrin B, contrary to maytansine, inhibited alkylation of tubulin sulfhydryl groups in the presence of GTP and $MgCl_2$. In their interactions with the tubulin molecule, halichondrin B and homohalichondrin B appear to have unique conformational effects which differ from those of other drugs and also from the effects of each other as well.

Tubulin, the subunit protein of microtubules, is a target for a large number of anti-mitotic drugs which have been very useful probes of the structure and function of tubulin [1]. We previously developed three assays to examine the effect of a drug on tubulin. These assays are particularly useful when only small amounts of the drug are available. One of the assays measures the ability of a drug to inhibit the formation of two intra-chain cross-links in β-tubulin induced by the bifunctional compound *N,N'*-ethylenebis(iodoacetamide) (EBI)§ [2, 3]. The two cross-links, designated β^* and β^s, are, respectively, between Cys^{239} and Cys^{354} and between Cys^{12} and either Cys^{201} or Cys^{211} [4, 5]. The other two assays measure the effect of a drug on stabilizing tubulin against decay. In one case we measure the effect of a drug on the time-dependent increase in binding to tubulin of bis-5,5'-[8-(*N*-phenyl)aminonaphthalene-1-sulfonic acid] (BisANS), a fluorescent probe which interacts with hydrophobic areas on the surfaces of proteins [6, 7]. In the second case, we measure the effect on an analogous increase in the availability of sulfhydryl groups for alkylation by iodo[^{14}C]acetamide [8, 9].

These assays have permitted the classification of tubulin ligands into the following groups: (1) *Colchicine* and its analogues inhibit formation of β^* and enhance formation of β^s [3, 8, 10–13]. They also inhibit both alkylation with iodo[^{14}C]acetamide and the binding of BisANS. (2) *BisANS* itself, at its high affinity site, where it blocks microtubule assembly, has no effect on either cross-link formation or alkylation; the same is true for analogues of BisANS [14–16]. (3) *Vinblastine* and drugs which inhibit its binding to tubulin all inhibit β^s formation and enhance that of β^* [3]. The third category may be subdivided as follows: (3a) *Vinblastine* causes only partial inhibition of β^s formation and is a stronger inhibitor than colchicine of alkylation and of BisANS binding [3, 7, 8]. (3b) *Phomopsin A and dolastatin 10*, which are non-competitive inhibitors of vinblastine binding to tubulin, completely inhibit β^s formation and are stronger inhibitors than vinblastine of alkylation and BisANS binding [17–20]. (3c) *Maytansine* and *rhizoxin*, which are competitive inhibitors of vinblastine binding [21–23], completely block β^s formation but have little or no effect on either alkylation or BisANS binding [3, 9, 24]. (3d) *GTP*, which promotes, rather than inhibits,

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§ Abbreviations: EBI, *N,N'*-ethylenebis(iodoacetamide); EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; and BisANS, bis-5,5'-[8-(*N*-phenyl)-aminonaphthalene-1-sulfonic acid].

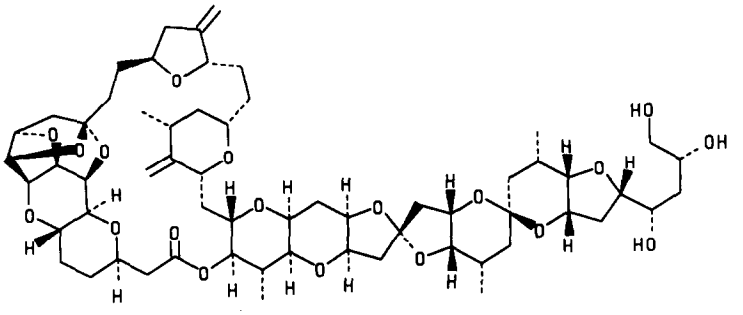

Fig. 1. Structure of halichondrin B.

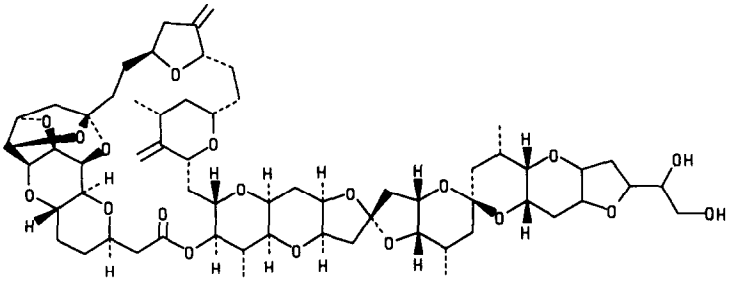

Fig. 2. Structure of homohalichondrin B.

microtubule assembly, completely inhibits β^s formation, but enhances that of β^*, and also inhibits alkylation [3].

Halichondrin B (Fig. 1) and homohalichondrin B (Fig. 2) are derived from marine sponges in the genera *Halichondra* and *Axinella*. Since they are common to more than one genus of sponge, it is possible that the drugs are actually made by bacteria which live on or in the sponges. With halichondrin B being a non-competitive inhibitor of vinblastine binding to tubulin [25] one might hypothesize that it would resemble phomopsin A and dolastatin 10 in its effects on tubulin. On the other hand, halichondrin B does not stabilize colchicine binding [25], whereas vinblastine, phomopsin A and dolastatin 10 do (maytansine and rhizoxin do not). Hence we decided to test the effects of halichondrin B and its slightly larger analogue, homohalichondrin B, on tubulin.

We found that halichondrin B resembles maytansine in that it completely inhibited β^s formation (while enhancing that of β^*). Halichondrin B also resembles maytansine in that it had no effect on the alkylation of tubulin by iodo[^{14}C]acetamide in the presence of GTP and $MgCl_2$. However, homohalichondrin B was different. Like halichondrin B, homohalichondrin B inhibited β^s formation and enhanced that of β^*. However, homohalichondrin B caused a small, but reproducible, inhibition of alkylation both in the presence and absence of GTP and $MgCl_2$. A further anomaly was observed when the effects of these compounds on BisANS binding were studied. Unlike most other tubulin ligands, the effects of halichondrin B and homohalichondrin B on BisANS binding did not parallel their effects on alkylation. Halichondrin B enhanced BisANS binding, whereas homohalichondrin B had no effect. Our results suggest that halichondrin B has a unique conformational effect on the tubulin molecule.

Possibly the decay of tubulin involves the moving apart of domains, causing exposure of hydrophobic sites and of sulfhydryl groups. Drugs which slow down decay may bridge between two domains thereby preventing them from moving apart. Drugs which do not affect decay may bind only to a single domain. Thus, homohalichondrin B, which is somewhat larger than halichondrin B, may be just large enough to begin to bridge between the domains and slow down exposure of sulfhydryl groups. These results were reported previously in preliminary form [26].

MATERIALS AND METHODS

Materials. Halichondrin B and homohalichondrin B were isolated from the Western Pacific (Palau) marine sponge *Axinella* sp. as recently reported [27]. All other materials were obtained or prepared as described elsewhere [28].

Tubulin preparation. Microtubules were purified from bovine cerebra and by cycling tubulin prepared therefrom by chromatography on phosphocellulose as previously described [29]. In experiments involving fluorimetry or tubulin alkylation, unless otherwise indicated, the buffer used was 0.1 2-(*N*-morpholino)-ethanesulfonic acid (Mes), pH 6.4, containing

1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA), 0.1 mM EDTA, 1 mM GTP and 0.5 mM $MgCl_2$. Cross-linking experiments were carried out in the same buffer lacking GTP and $MgCl_2$.

Cross-linking. For cross-linking experiments tubulin was purified on a composite phosphocellulose-Sephadex G-25 column, equilibrated in buffer lacking GTP and $MgCl_2$, as previously described [3]. The buffer for these experiments was 100 mM Mes, pH 6.4, containing 1 mM EGTA and 0.1 mM EDTA. Tubulin samples were incubated with EBI and then dialyzed, reduced and carboxymethylated as previously described [2, 3]. To measure the yields of both the β^s and β^* cross-links, samples were analyzed on 5.5% polyacrylamide gels in the system of Laemmli [30] as described previously [3]. If only the yield of β^s cross-link was being measured, electrophoretic analysis was performed using the modified system of Banerjee *et al.* [31]. In all cross-linking experiments, tubulin samples contained reduced and carboxymethylated conalbumin to serve as an internal standard to allow accurate measurement of cross-linked products [2]. Gels were stained with fast green and scanned at 640 nm using a Gilford 2400 spectrophotometer equipped with a linear transport and a microprocessor.

Alkylation. Tubulin samples were incubated for 1 hr at 37° with iodo[^{14}C]acetamide. Dilution to 5% in trichloroacetic acid precipitated the tubulin. The precipitates were collected by filtration and the radioactivity of the filters was determined as previously described [9].

Fluorescence. Samples of tubulin were incubated at 37°; from time to time aliquots were removed, mixed with BisANS, and placed in a SPF500C spectrofluorometer (SLM) set in the ratio mode. Excitation and emission were at 385 and 490 nm, respectively.

Other methods. The concentration of protein in a sample was determined using a modification by Schachterle and Pollack [32] of the method of Lowry *et al.* [33]. Bovine serum albumin was used as a standard.

RESULTS

When tubulin was incubated with EBI in the presence of these drugs, both halichondrin B and homohalichondrin B inhibited formation of the β^s cross-link and enhanced formation of the β^* cross-link (Table 1). As was noted previously [3], vinblastine gave only partial inhibition of β^s formation. To examine the effect more closely, the reaction with EBI was done in the presence of podophyllotoxin to inhibit β^* formation; a series of concentrations of halichondrin B and homohalichondrin B were tested. The results (Fig. 3) indicate that halichondrin B and homohalichondrin B half-maximally suppressed formation of the β^s cross-link at concentrations of approximately 3 and 6 μM, respectively.

Halichondrin B appeared to have very little effect on the alkylation of tubulin by iodo[^{14}C]acetamide (Table 2) either in the presence or absence of GTP and $MgCl_2$; the small suppressive effect was not significant. In contrast, homohalichondrin B had a statistically significant effect in the absence of GTP and $MgCl_2$ ($P < 0.05$ in Experiments 1 and 2). In Experiment 3, done in the presence of GTP and $MgCl_2$, in which each combination was tested in octuplicate aliquots, the suppressive effect of

Table 1. Effects of halichondrin B and homohalichondrin B on the intra-chain cross-linking of tubulin induced by EBI*

Drug added	Yield of β^s cross-link (%)	Yield of β^* cross-link (%)
Experiment 1†		
None	14, 17	25, 29
Halichondrin B, 60 μM	0	41
Homohalichondrin B, 60 μM	0	52
Vinblastine, 60 μM	8	55
GTP, 1 mM	1	26
Experiment 2‡		
None	16, 19	0
Halichondrin B, 60 μM	0	0
Homohalichondrin B, 60 μM	0	0
Vinblastine, 60 μM	7	0
GTP, 1 mM	0	0

* In both of these experiments, both the β^s and β^* band are assumed to be derived from the β_1 band; the β_1 band accounts for 75% of bovine cerebral tubulin [28]. The yields of β^s and β^* are expressed as a percentage of the total β_1 and calculated as described previously [3]. Control samples were processed in duplicate.

† In this experiment, tubulin (6.6 μM) was incubated with 0.91 mM EBI for 1 hr at 30°. Under these conditions both β^s and β^* cross-links are produced.

‡ In this experiment, conditions were as above, but all samples contained 50 μM podophyllotoxin to inhibit β^* formation.

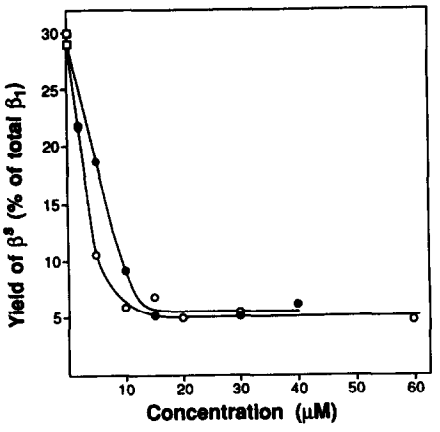

Fig. 3. Effects of halichondrin B and homohalichondrin B on the EBI-induced formation of the β^s cross-link. Aliquots (250 μL) of tubulin (0.66 mg/mL) containing reduced and carboxymethylated conalbumin (0.20 mg/mL) were incubated with 0.91 mM EBI for 1 hr at 30° in the absence (□) or presence of the indicated concentrations of either halichondrin B (○) or homohalichondrin B (●). All samples contained 50 μM podophyllotoxin to prevent formation of the β^* cross-link. After incubation, samples were processed as described in Materials and Methods and analyzed by polyacrylamide gel electrophoresis using the system of Banerjee *et al.* [31]. Samples incubated in the absence of ligand were done in duplicate; the average of the two determinations is shown.

homohalichondrin B, although amounting only to 11%, was also highly statistically significant ($P < 0.001$). Where the effect of vinblastine was tested, it was always markedly greater than that of either halichondrin B or homohalichondrin B.

We have previously used the binding of BisANS to tubulin as an indication of the exposure of hydrophobic areas that accompanies the decay of tubulin; decay is the name given to the time-dependent loss of the ability of tubulin to polymerize and bind to drugs such as colchicine [7]. In the experiment shown in Fig. 4, the effects of vinblastine, halichondrin B, and homohalichondrin B on BisANS binding are shown. Vinblastine gave its usual suppressive effect (64 ± 26% of control). In contrast, halichondrin B appeared to enhance BisANS binding; the average increase over control for each of the seven time points was 150 ± 24%; homohalichondrin B had a very small effect (109 ± 10% of control). The effect of vinblastine was significant ($P < 0.01$) as was that of halichondrin B ($P < 0.005$), but that of homohalichondrin B was only marginally significant ($P < 0.05$). In a similar experiment (not shown), vinblastine also suppressed BisANS binding (averaging 51 ± 19% of control over eight time points), while halichondrin B enhanced BisANS binding (164 ± 31% of control over four time points). Although in this experiment, the effect of vinblastine was significant ($P < 0.0005$) as was that of halichondrin B ($P < 0.025$), homohalichondrin B had no significant effect (102 ± 3% of control over eight time points).

DISCUSSION

Halichondrin B has been found previously to be

Table 2. Effects of halichondrin B and homohalichondrin B on the alkylation of tubulin by iodo[^{14}C]acetamide*

Addition	moles ^{14}C/100 kDa	% of Control
Experiment 1†‡		
None	6.09 ± 0.13	100 ± 2 (4)
Halichondrin B, 60 μM	6.11 ± 0.10	100 ± 3 (4)
Homohalichondrin B, 60 μM	5.51 ± 0.35§	90 ± 6 (4)
Vinblastine, 60 μM	4.76 ± 0.11	78 ± 2 (4)
Experiment 2‡‖		
None	7.16 ± 0.32	100 ± 4 (4)
Halichondrin B, 60 μM	6.86 ± 0.19	96 ± 5 (4)
Homohalichondrin B, 60 μM	6.69 ± 0.09§	94 ± 4 (4)
Vinblastine, 60 μM	5.69 ± 0.09	79 ± 4 (4)
Experiment 3‖		
None	2.75 ± 0.07	100 ± 3 (8)
Halichondrin B, 20 μM	2.65 ± 0.12	97 ± 5 (8)
Homohalichondrin B, 20 μM	2.46 ± 0.07¶	89 ± 3 (8)

* Aliquots (250 μL) of tubulin (0.66 mg/mL) were incubated with 1.36 mM iodo[^{14}C]acetamide for 1 hr at 37°. They were then precipitated and filtered and the radioactivity of the filters was determined as previously described [9]. The values given in the table represent means ± SD. The numbers in parentheses represent the number of aliquots used to make the measurement.

† In this experiment, the specific activity of the iodo[^{14}C]acetamide was 0.54 Ci/mol.

‡ In this experiment, the buffer lacked GTP and $MgCl_2$.

§ $P < 0.05$ vs control.

‖ In this experiment, the specific activity of the iodo[^{14}C]acetamide was 0.49 Ci/mol.

¶ $P < 0.001$ vs control.

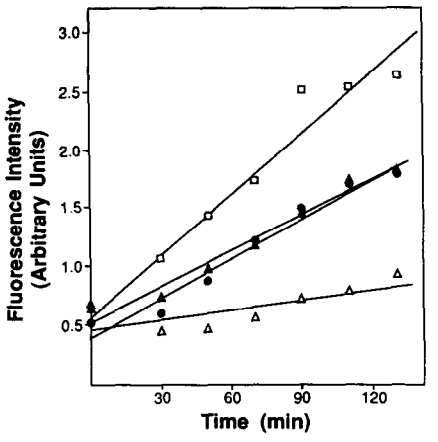

Fig. 4. Effects of halichondrin B, homohalichondrin B, and vinblastine on the binding of BisANS to tubulin. Samples of tubulin (0.2 mg/mL) were incubated at 37° in the absence (●) or presence of 20 μM concentrations of either halichondrin B (□), homohalichondrin B (▲) or vinblastine (△). At the indicated times, 1-mL aliquots of each sample were removed, made 10 μM in BisANS, and placed in fluorescence cuvettes; the fluorescence was determined as described in Materials and Methods.

a non-competitive inhibitor of vinblastine binding to tubulin [25]. Although the effect of homohalichondrin B on vinblastine binding has not been studied, its overall great similarity to halichondrin B suggests that it would bind to the same region on tubulin. So far, every compound that inhibits vinblastine binding to tubulin, either competitively or non-competitively, also inhibits the EBI-induced formation of the β^s cross-link, while enhancing that of β^*. Therefore, it is no surprise that both halichondrin B and homohalichondrin B also have this effect. Homohalichondrin B is about half as effective as halichondrin B, which is consistent with the finding of Bai *et al.* [25] that homohalichondrin B is slightly less than half as effective as halichondrin B at inhibiting microtubule assembly *in vitro*.

Previous evidence indicated that halichondrin B could have a unique interaction with tubulin. It was reported to resemble phomopsin A and dolastatin 10 in being a non-competitive inhibitor of vinblastine binding to tubulin, but to resemble maytansine in that it did not stabilize colchicine binding [25]. The results we have obtained illustrate that halichondrin B is indeed unique in its interaction with tubulin. Although it resembled phomopsin A, dolastatin 10, vinblastine and rhizoxin in its inhibition of β^s formation (and its enhancement of β^* formation) and it resembled maytansine in that it had no effect on the alkylation of tubulin by iodo[^{14}C]acetamide in the presence of GTP and $MgCl_2$, halichondrin B is unique in that it enhanced BisANS binding to tubulin. No other tubulin ligand has been reported to do this; maytansine and rhizoxin have no effect on BisANS binding, while vinblastine, phomopsin A, dolastatin 10, colchicine, and podophyllotoxin all inhibit BisANS binding [7, 18, 20, 24]. Furthermore, when the effect on alkylation is measured in the absence of GTP and $MgCl_2$, maytansine has a small but significant inhibitory effect [3], whereas halichondrin B did not (Table 2). In short, it appears that halichondrin B belongs in a unique subclass of tubulin ligands.

Interestingly, our results suggest that homohalichondrin B should also be classified as belonging to its own subclass of tubulin ligands, distinct from that to which halichondrin B belongs. Although homohalichondrin B resembles halichondrin B in that it inhibited β^s formation and enhanced that of β^*, homohalichondrin B had a small but significant inhibitory effect on the alkylation of tubulin by iodo[^{14}C]acetamide, both in the presence and absence of GTP and $MgCl_2$. No other tubulin ligand has this combination of properties. In the presence of GTP and $MgCl_2$, neither maytansine nor halichondrin B inhibit alkylation, while in their absence, only maytansine does [3]. Although vinblastine, phomopsin A, and dolastatin 10 also inhibit alkylation, they do so to significantly greater extents than does homohalichondrin B. To add to its apparent oddity, homohalichondrin B had little or no effect on BisANS binding. In that respect, it resembles maytansine and rhizoxin, which also have no effect, but it differs greatly from vinblastine, phomopsin A, and dolastatin 10, which strongly inhibit BisANS binding. In short, it appears that homohalichondrin B also deserves its own subclass among tubulin ligands. Table 3 summarizes some of the features of various tubulin ligands.

Another unique feature of halichondrin B and homohalichondrin B is that they are the only known tubulin ligands in which the effects on alkylation and on BisANS binding are uncoupled. In every case previously reported, the two effects are parallel: maytansine, in the presence of GTP and $MgCl_2$, has no effect on either alkylation or BisANS binding, while podophyllotoxin, colchicine, vinblastine, phomopsin A and dolastatin 10 inhibit both. Even their relative effects are parallel, with podophyllotoxin causing the least inhibition of both alkylation and BisANS binding, colchicine causing more inhibition, vinblastine still more, and phomopsin A and dolastatin 10 inhibiting both to the greatest extent. In contrast, halichondrin B enhanced BisANS binding and had no effect on alkylation, while homohalichondrin B inhibited alkylation and had either no effect on BisANS binding or else had a slight enhancing effect.

Although the increase in BisANS binding caused by halichondrin B is a unique effect, possible explanations are not difficult to find. Conceivably the decay of tubulin involves a time-dependent conformational change in which two domains on the molecule move apart exposing a region (the "decay region") which contains both sulfhydryl groups and hydrophobic areas. The exposure of the former increases alkylation by iodo[^{14}C]acetamide and the exposure of the latter increases BisANS binding. Perhaps the binding of halichondrin B to tubulin induces a slow conformational change which exposes another region containing only hydrophobic areas and no sulfhydryl groups. Alternatively, the binding

Table 3. Summary of the effects of the drugs which bind to tubulin in or near the vinblastine region, contrasted to the effects of colchicine and podophyllotoxin*

Drug	Effect on: β^* formation	β^s formation	BisANS binding	alkylation	binding of: VBL†	binding of: COL‡
Vinblastine	↑	↓	↓↓↓	↓↓↓	C§	↑
Maytansine	↑	↓↓	—	—	C	—
Rhizoxin	↑	↓↓	—	—	C	—
Phomopsin A	↑	↓↓	↓↓↓↓	↓↓↓	NC‖	↑↑
Dolastatin 10	↑	↓↓	↓↓↓↓	↓↓↓	NC	↑↑
Halichondrin B	↑	↓↓	↑	—	NC	—
Homohalichondrin B	↑	↓↓	—	↓	?	?
Colchicine	↓↓	↑	↓↓	↓↓	↑	C
Podophyllotoxin	↓↓	↑	↓	↓	?	C

* Symbol key: (↑) enhancement; (↓) inhibition; (—) no effect; and (?) not determined. The number of arrows is a qualitative indication of the magnitude of the effect, intended only for comparative purposes.

† VBL = either vinblastine or vincristine.

‡ COL = colchicine.

§ C = competitive inhibitor.

‖ NC = non-competitive inhibitor.

of halichondrin B could destabilize a region of the tubulin molecule, which would then unfold in a time-dependent fashion, concomitantly exposing hydrophobic areas. Either model would explain the increase in BisANS binding due to halichondrin B. A third possibility is that halichondrin B induces a conformational change which alters the environment around the hydrophobic areas that are exposed during decay so that the fluorescent quantum yield of BisANS increases without the binding of BisANS increasing.

Quite likely the effects of halichondrin B and homohalichondrin B on alkylation and BisANS binding are related. If tubulin decay represents the separation of two domains, exposing both hydrophobic areas and sulfhydryl groups, then one could postulate that a drug such as vinblastine, which inhibits both alkylation and BisANS binding, could be bridging between the two domains, slowing down their rate of separation. A drug such as maytansine would only bind to one of these domains, thereby having no effect on decay. Halichondrin B would do the same, acting like maytansine except for its additional effect discussed above leading to apparently greater BisANS binding. In contrast, homohalichondrin B, which is slightly larger than halichondrin B, would act very weakly as a bridge between these two domains. Hence, homohalichondrin B would inhibit exposure of sulfhydryl groups in the "decay region", thereby diminishing alkylation by iodo[^{14}C]acetamide, as we observed.

There are two ways we could account for the lack of effect of homohalichondrin B on BisANS binding. First, homohalichondrin B may simply not have the additional conformational effect which we have postulated above for halichondrin B. Alternatively, homohalichondrin B could indeed have that effect, but at the same time slowing down exposure of hydrophobic areas in the "decay region"; the two effects would compensate for each other, leading to no net change in the rate of BisANS binding. Another possibility is that the suppressive effect of homohalichondrin B on alkylation could be purely steric and not conformational. In other words, homohalichondrin B, being larger than halichondrin B, may extend over a sulfhydryl group on tubulin, thereby diminishing its accessibility to iodo[^{14}C]-acetamide. If this were true, then homohalichondrin B would not necessarily act as a bridge between subdomains, and the effects of homohalichondrin B and halichondrin B would not be related.

A situation parallel to the hypothesis advanced above may be illustrated by the effects of single ring analogues of colchicine on the alkylation of tubulin by iodo[^{14}C]acetamide [11]. Although colchicine inhibits alkylation, analogues of both the A- and C-rings of colchicine, separately or mixed, actually enhance alkylation. However, if the A- and C-rings are joined, as in either combretastatin A-4 or 5-(2′,3′,4′-trimethoxyphenyl)-2-methoxytropone, then they inhibit alkylation. The interpretation proposed for this observation was that colchicine inhibited alkylation by a conformational effect of some kind, perhaps by linking two subdomains of the tubulin molecule together. What we are arguing, essentially, is that homohalichondrin B, but not halichondrin B, can bridge between two subdomains of the tubulin molecule.

In the model of the vinblastine–maytansine–phomopsin A binding site proposed by Bai *et al.* [17], halichondrin B would probably lie in that portion of the site where maytansine and rhizoxin bind, but not overlapping the area where vinblastine binds and not overlapping at all with the peptide groove where phomopsin A and dolastatin 10 bind. The binding of homohalichondrin B would be similar

except that a very small part of the molecule might overlap into the peptide groove.

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