SUPPLEMENTARY MATERIAL FOR:

(-)-RHAZINILAM AND THE DIPHENYLPYRIDAZINONE NSC 613241: TWO COMPOUNDS INDUCING THE FORMATION OF MORPHOLOGICALLY SIMILAR TUBULIN SPIRALS BUT BINDING APPARENTLY TO TWO DISTINCT SITES ON TUBULIN

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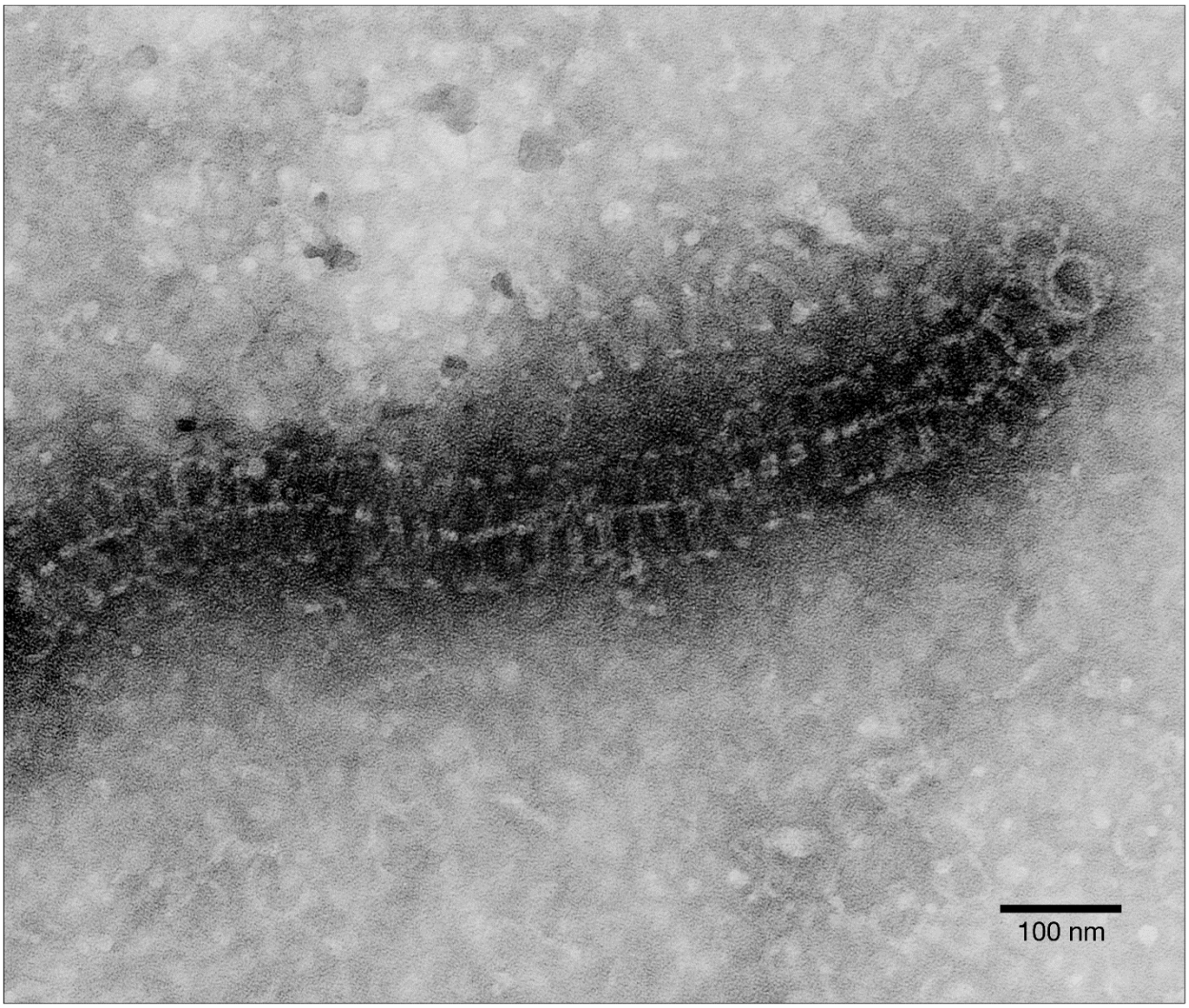
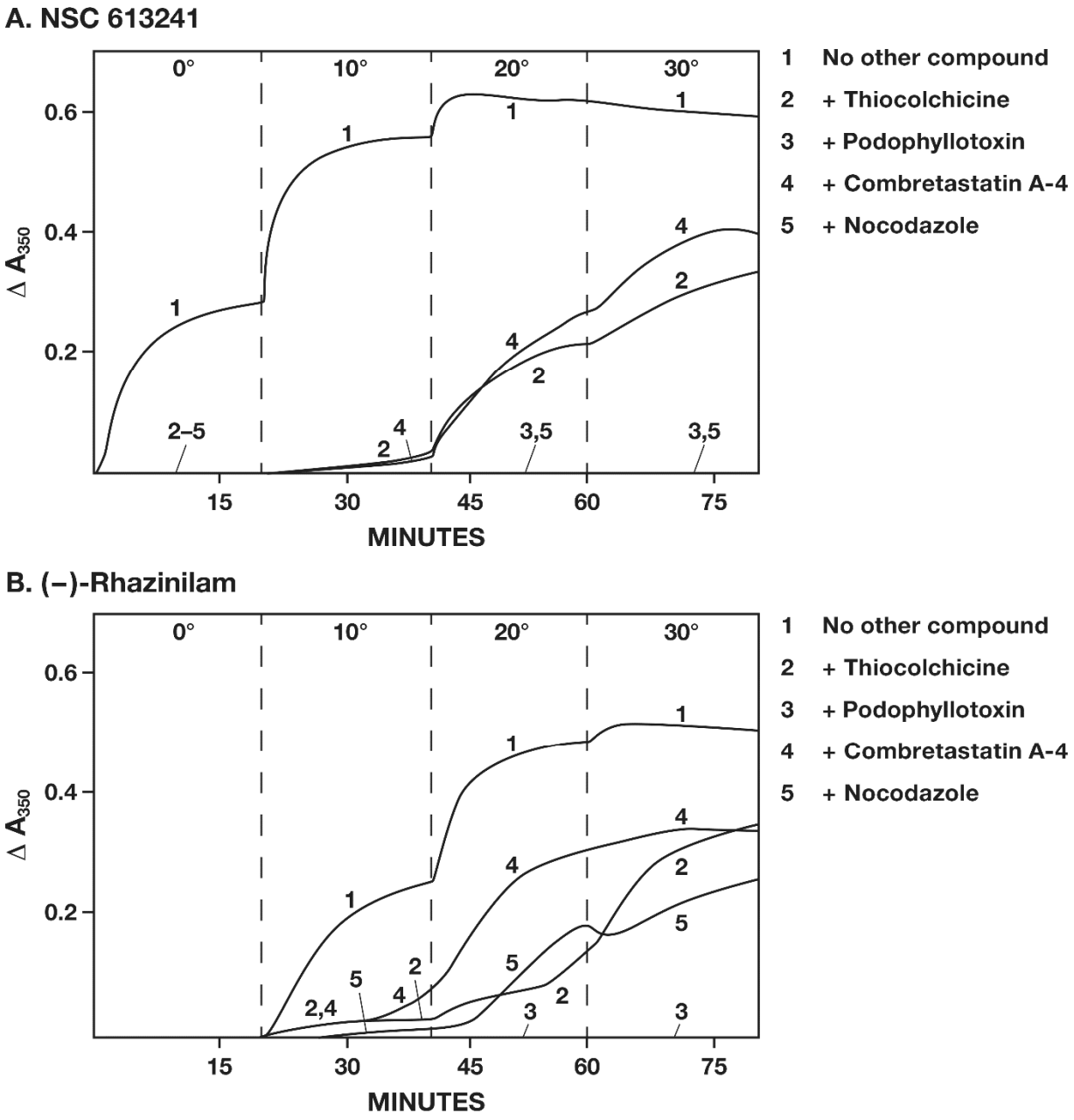
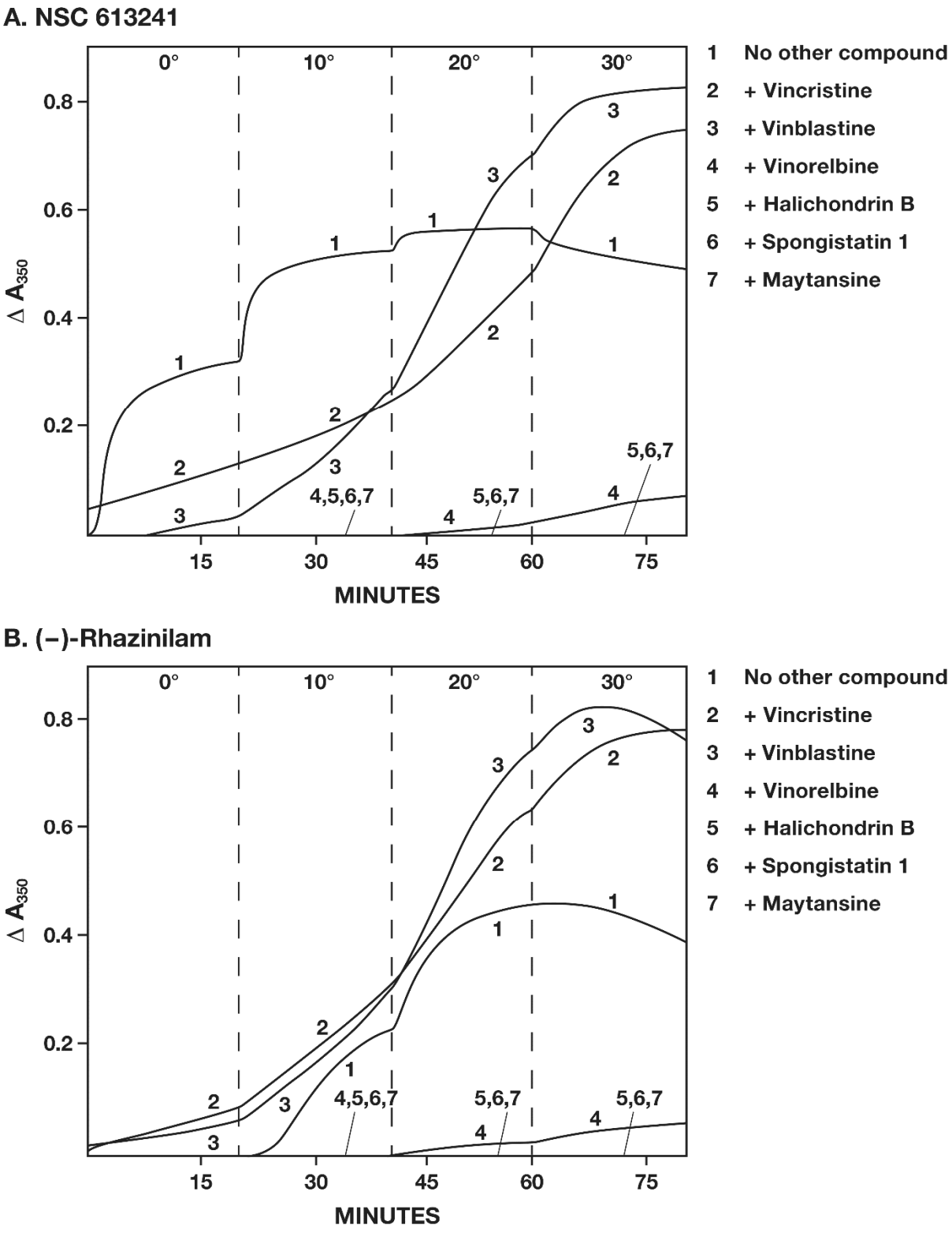


Supplementary Materials Figure 1: Vinca-induced tubulin structure with individual coils prominent. The reaction mixture contained 10 µM tubulin, 0.75 M monosodium glutamate, 20 µM vinblastine, and 10 µM GTP. The tubulin used in this study was not subjected to gel filtration chromatography.



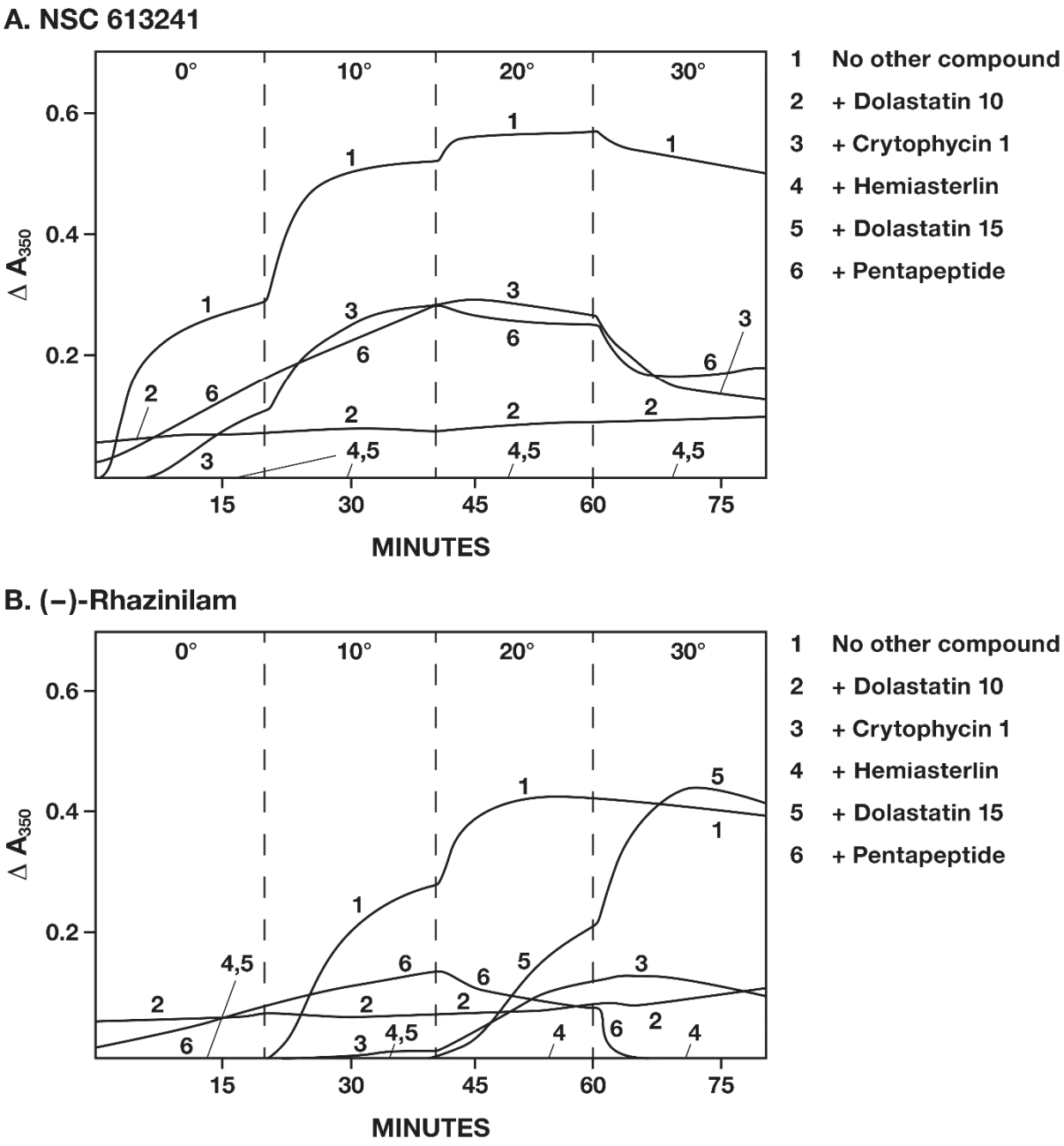
Supplementary Materials Figure 2: Inhibitory effects of colchicine site agents on aberrant assembly reactions induced by NSC 613241 (A) or by (-)-rhazinilam (B). Reaction mixtures contained the components described in the **Materials and methods** section, except for NSC 613241 or (-)-rhazinilam. The inhibitory drug was added to the other reaction components, including tubulin, in cuvettes held at 0 °C. The temperature was maintained at 0 °C for 20 min (no significant change in turbidity occurred in any sample), and at zero time either NSC 613241 (A) or (-)-rhazinilam (B) was added at 10 µM. Temperature changes were made at the times indicated by the dashed lines, with the temperature shown at the right of the dashed lines.

Inhibitory drugs, in both panels, as follows: curves 1, none; curves 2, 50 µM thiocolchicine (note that a preformed thiocolchicine-tubulin complex was used, as described for Table 1); curves 3, 50 µM podophyllotoxin; curves 4, 50 µM combretastatin A-4; curves 5, 50 µM nocodazole.



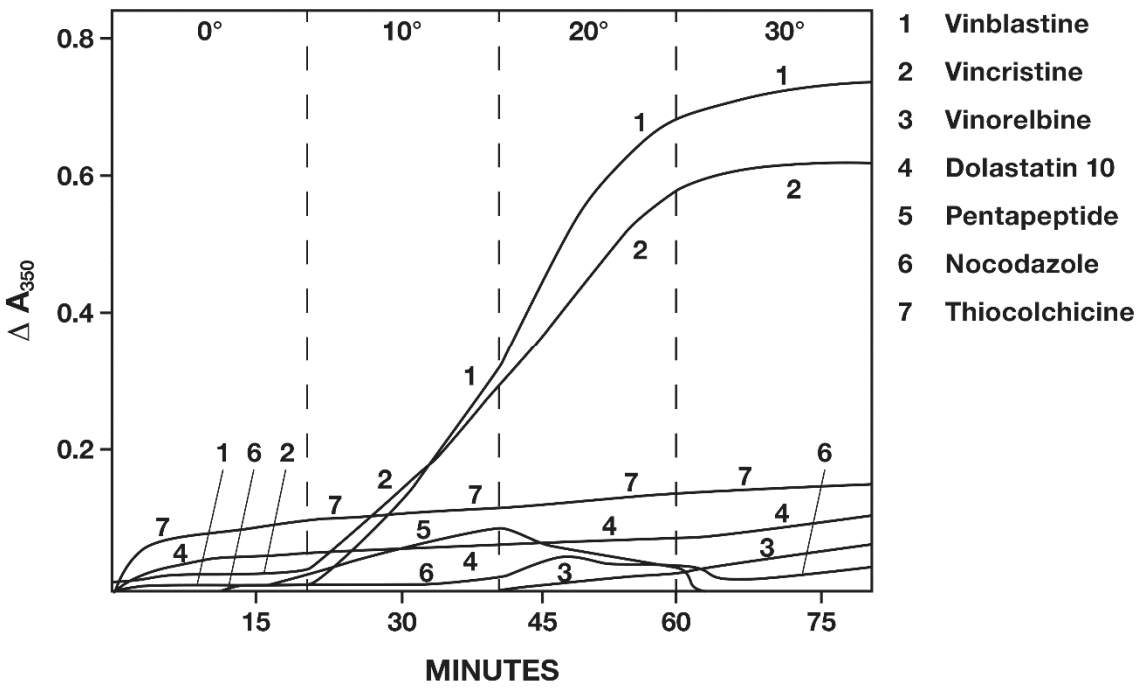
Supplementary Materials Figure 3: Inhibitory effects of compounds that inhibit vinca alkaloid binding to tubulin on aberrant assembly reactions induced by NSC 613241 (A) or by (-)-rhazinilam (B). Reaction mixtures contained the components described in the **Materials and methods**

section, except for NSC 613241 or (-)-rhazinilam. The inhibitory drug was added to the other reaction components, including tubulin, in cuvettes held at 0 °C. The temperature was maintained at 0 °C for 20 min (no significant change in turbidity occurred in any sample, except as indicated by an initial reading in the figure above the baseline), and at zero time either NSC 613241 (A) or (-)-rhazinilam (B) was added at 10 µM. Temperature changes were made at the times indicated by the dashed lines, with the temperature shown at the right of the dashed lines. Inhibitory drugs, in both panels, as follows: curves 1, none; curves 2, 50 µM vincristine; curves 3, 50 µM vinblastine; curves 4, 50 µM vinorelbine; curves 5, 50 µM halichondrin B; curves 6, 50 µM spongistatin 1; curves 7, 50 µM maytansine.

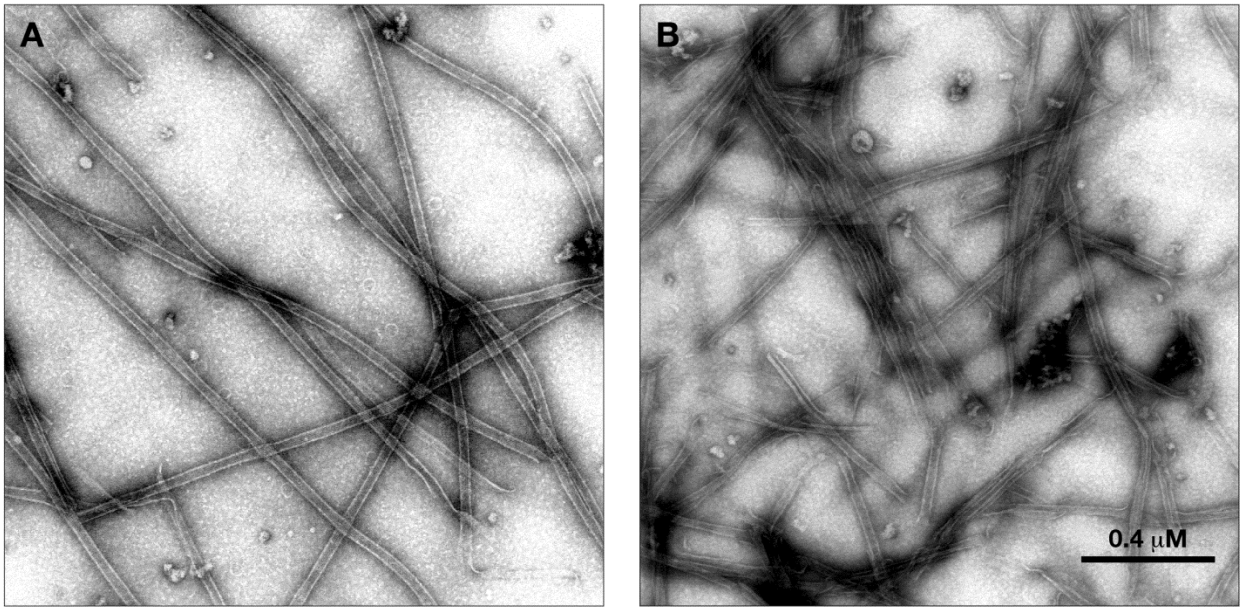


Supplementary Materials Figure 4: Inhibitory effects of antimitotic peptides on aberrant assembly reactions induced by NSC 613241 (A) or by (-)-rhazinilam (B). Reaction mixtures contained the components described in the **Materials and methods** section, except for NSC 613241 or (-)-rhazinilam. The inhibitory drug was added to the other reaction components, including tubulin, in cuvettes held at 0 °C. The temperature was maintained at 0 °C for 20 min (no significant change in turbidity occurred in any sample, except as indicated by an initial reading in the figure above the baseline), and at zero time either NSC 613241 (A) or (-)-rhazinilam (B) was added at 10 µM. Temperature changes were made at the times indicated by the dashed lines, with the temperature shown at the right of the dashed lines. Inhibitory drugs, in both panels, as follows: curves 1, none;

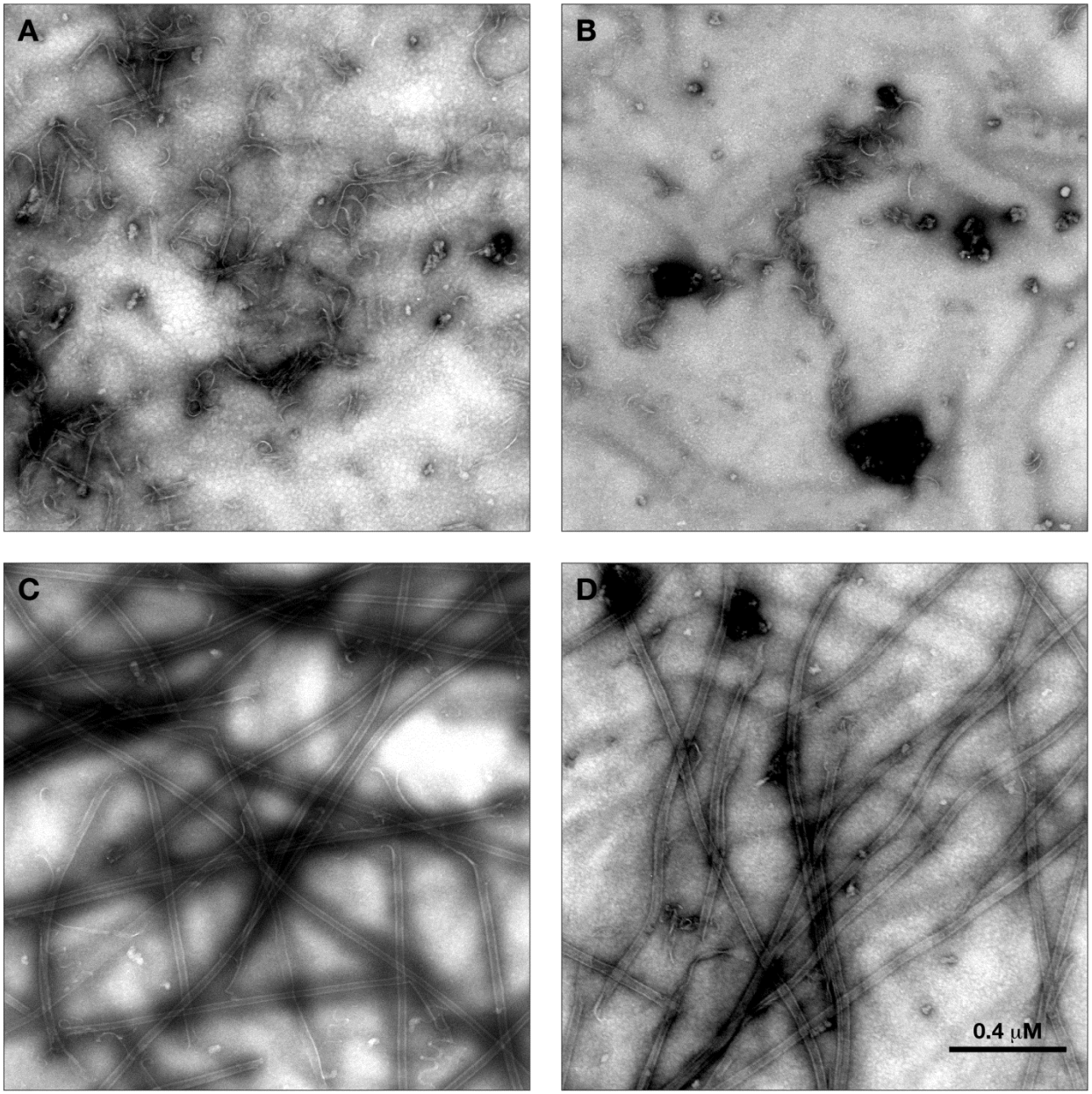
curves 2, 50 µM dolastatin 10; curves 3, 50 µM cryptophycin 1; curves 4, 50 µM hemiasterlin; curves 5, 50 µM dolastatin 15; curves 6, 50 µM *N*,*N*-dimethylvalyl-valyl-*N*-methylvalyl-prolyl-proline (pentapetide).



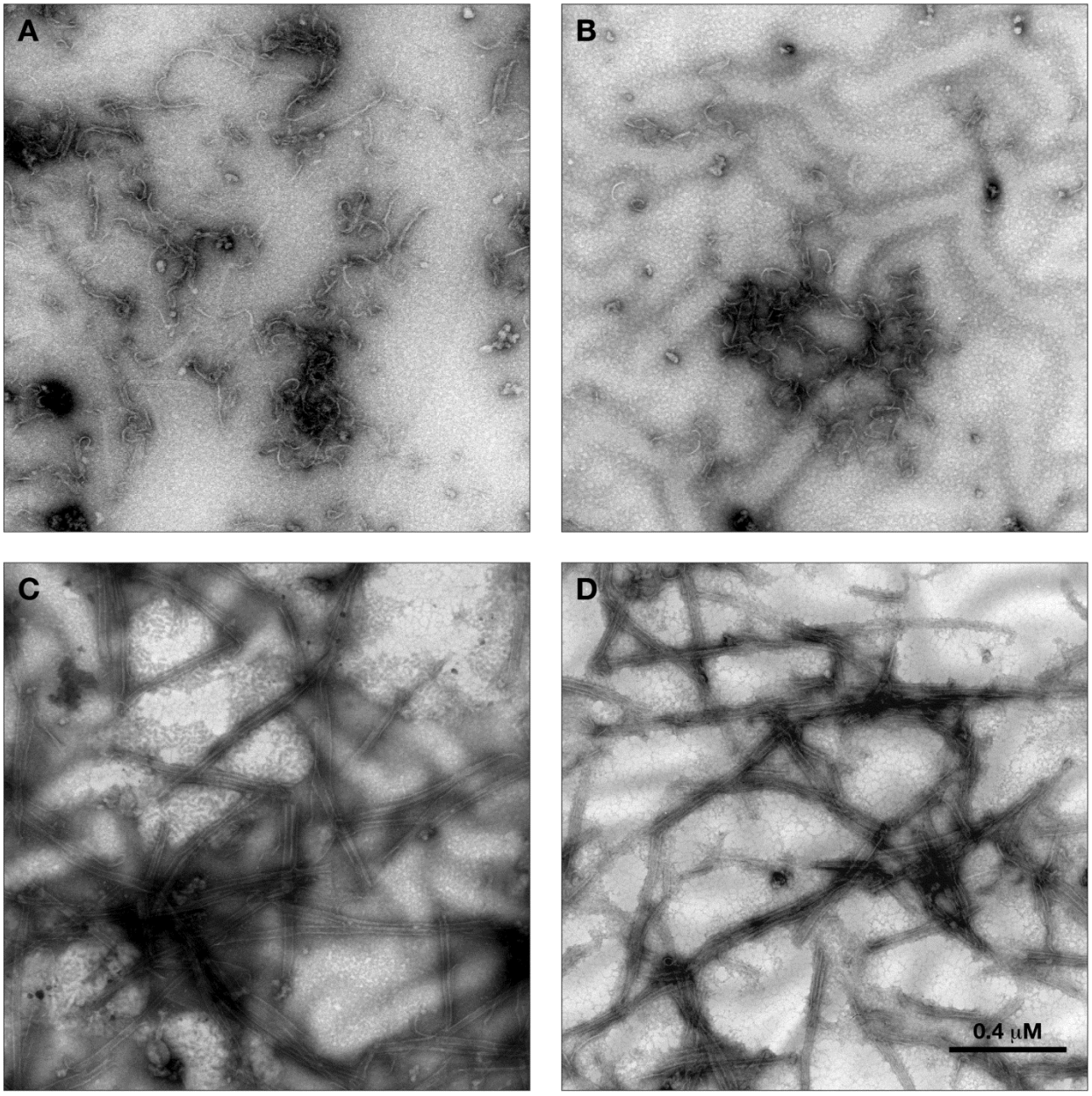
Supplementary Materials Figure 5: Turbidity patterns observed when assembly inhibitors were added to reaction mixtures containing tubulin but no NSC 613241 or (-)-rhazinilam. Reaction mixtures contained the components described in the **Materials and methods** section, and baselines were established after the addition of 10 µM tubulin at 0 °C. At zero time, the inhibitor was added to the reaction mixture. Temperature changes were made at the times indicated by the dashed lines, with the temperature shown at the right of the dashed lines. All inhibitors were at 50 µM. Curve 1, vinblastine; curve 2, vincristine; curve 3, vinorelbine; curve 4, dolastatin 10; curve 5, *N*,*N*-dimethylvalyl-valyl-*N*-methylvalyl-prolyl-proline (pentapeptide); curve 6, nocodazole; curve 7, thiocolchicine. There was no significant change in turbidity with the following inhibitors at 50 µM: podophyllotoxin, combretastatin A-4, halichondrin B, spongistatin 1, maytansine, dolastatin 15, hemiasterlin, or cryptophycin 1.



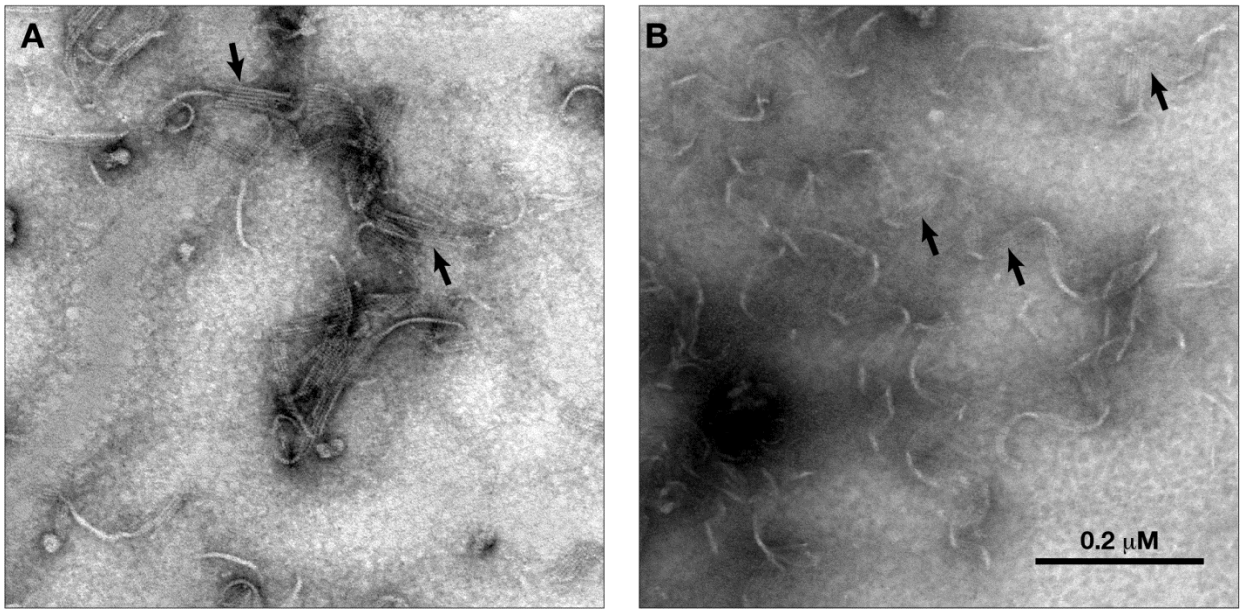
Supplementary Materials Figure 6: Predominant microtubule formation of polymer formed in the presence of laulimalide (A) or epothilone B (B). Reaction mixtures (0.25 mL) contained 10 μM tubulin, 0.6 M monosodium glutamate (pH 6.6), 0.5 mM GTP, 1.0 mM $MgCl_2$, 2% dimethyl sulfoxide, and 10 μM compound, with the laulimalide or epothilone B the last component added to the reaction mixture. After an incubation of 20 min at 0 °C, then 20 min at 10 °C, then 20 min at 20 °C, aliquots of each reaction mixture were applied to the grids and processed as described in **Materials and methods**. Magnification, as indicated by the bar shown in panel B, applies to both panels.



Supplementary Materials Figure 7: Effects of sequential drug additions of laulimalide with (-)-rhazinilam or NSC 613241. Reaction mixtures (0.25 mL) contained 10 μM tubulin, 0.6 M monosodium glutamate (pH 6.6), 0.5 mM GTP, 1.0 mM $MgCl_2$, 2% dimethyl sulfoxide, and 10 μM initial compound the last component added to the reaction mixture. After an incubation of 20 min at 0 °C, then 20 min at 10 °C, then 20 min at 20 °C, the second compound at 10 μM was added. The incubation continued an additional 20 min at 20 °C. Aliquots of each reaction mixture were applied to the grids and processed as described in **Materials and methods**. Magnification, as indicated by the bar shown in panel D, applies to all panels. A. (-)-Rhazinilam was the first compound added, laulimalide the second. B. NSC 613241 was the first compound added, laulimalide the second. C. Laulimalide was the first compound added, (-)-rhazinilam the second. D. Laulimalide was the first compound added, NSC 613241 the second.



Supplementary Materials Figure 8: Effects of sequential drug additions of epothilone B with (-)-rhazinilam or NSC 613241. Reaction mixtures (0.25 mL) contained 10 μM tubulin, 0.6 M monosodium glutamate (pH 6.6), 0.5 mM GTP, 1.0 mM $MgCl_2$, 2% dimethyl sulfoxide, and 10 μM initial compound the last component added to the reaction mixture. After an incubation of 20 min at 0 °C, then 20 min at 10 °C, then 20 min at 20 °C, the second compound at 10 μM was added. The incubation continued an additional 20 min at 20 °C. Aliquots of each reaction mixture were applied to the grids and processed as described in **Materials and methods**. Magnification, as indicated by the bar shown in panel D, applies to all panels. A. (-)-Rhazinilam was the first compound added, epothilone B the second. B. NSC 613241 was the first compound added, epothilone B the second. C. Epothilone B was the first compound added, (-)-rhazinilam the second. D. Epothilone B was the first compound added, NSC 613241 the second.



Supplementary Materials Figure 9: Higher power magnification of structures formed following sequential addition of (-)-rhazinilam, then epotholine B (A) or of NSC 613241, then epothilone B (B). Reaction mixtures (0.25 mL) contained 10 μM tubulin, 0.6 M monosodium glutamate (pH 6.6), 0.5 mM GTP, 1.0 mM $MgCl_2$, 2% dimethyl sulfoxide, and 10 μM compound of the indicated initial compound being the last component added to the reaction mixture. After an incubation of 20 min at 0 °C, then 20 min at 10 °C, then 20 min at 20 °C, epothilone B at 10 μM was added with the incubation continued for 20 min at 20 °C. Aliquots of each reaction mixture were applied to the grids and processed as described in **Materials and methods**. Magnification, as indicated by the bar shown in panel B, applies to both panels. Arrows indicate structures with apparent parallel protofilaments.