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THE SYNTHESIS AND EVALUATION OF FUNCTIONALIZED ESTRATROPONES: POTENT INHIBITORS OF TUBULIN POLYMERIZATION

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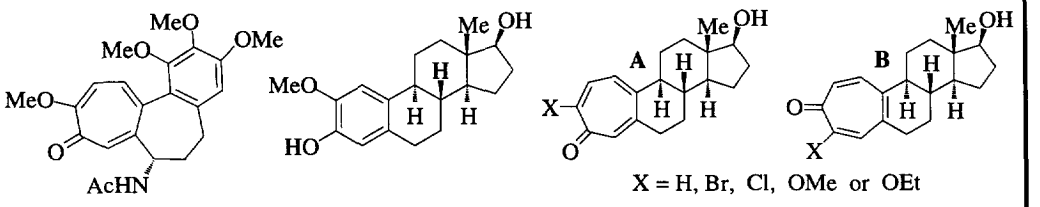
Abstract: The synthesis of several α-substituted estratropones is described. The compounds were evaluated for the inhibition of tubulin polymerization using purified bovine brain tubulin. Several of the compounds are equipotent to colchicine for their ability to inhibit the polymerization of tubulin. © 1997 Elsevier Science Ltd.

Angiogenesis, the process of neovascularization, has been recently recognized as a therapeutic target for a variety of disease states, including neoplastic and ocular diseases and compounds that exhibit antiangiogenic properties have been of increasing interest.[1-6] The disclosure of 2-methoxyestradiol[7-10] and the ensuing structure-activity studies[11] of this structural family have yielded potent antiangiogenic agents that have been proposed to operate through the inhibition of tubulin polymerization. The inhibition of tubulin polymerization has been proposed to be mediated through binding at the colchicine binding site on the tubulin monomer. Ongoing investigation in our laboratory of the relationship between structure and colchicine binding site affinity has lead to the discovery of several potent toxins.[12,13] Recently, our laboratory has achieved the synthesis of A-homo-estrogens, termed estratropones (Figure 1), which inhibit tubulin polymerization, presumably via binding at the colchicine binding site.[12,13] This class of agents was developed on the basis of proposed structural relationships between colchicine and 2-methoxyestradiol, elucidated independently by Folkman *et al*[8] and by Rava *et al.*[10] Our initial investigation of the possible molecular and functional analogy between colchicine

Figure 1. Colchicine, 2-methoxyestradiol, and estratropones **A** and **B**, respectively.

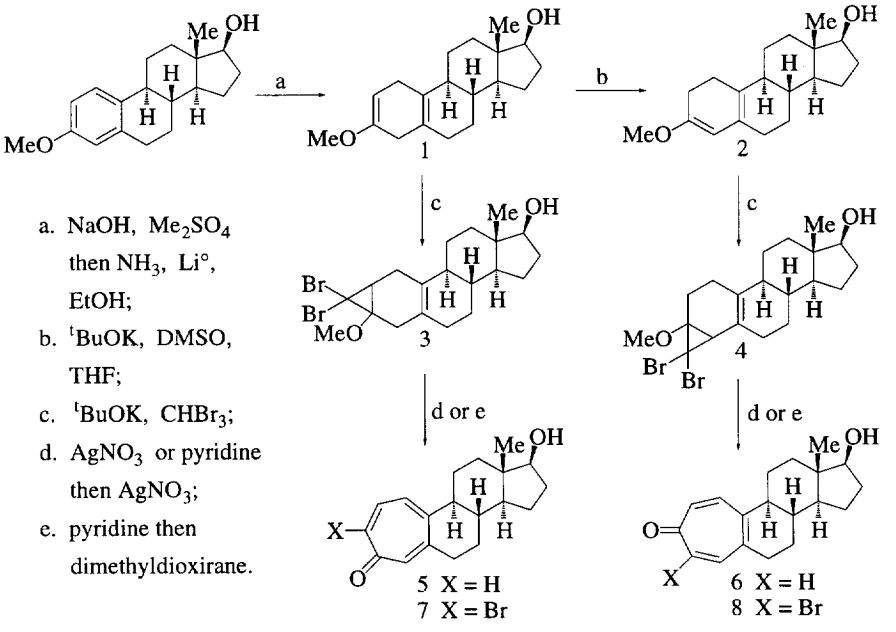


and 2-methoxyestradiol examined the effect of incorporating colchicine's dominating methoxy-tropone functionality into the estrane nucleus. Our studies found estratropone core structures **A** and **B** (Figure 1) to be the most active of the trienone isomers tested for their ability to inhibit tubulin polymerization. Consequently, the synthesis of a variety of functionalized estratropones was desired to further probe the relationship between structure and the inhibition of tubulin polymerization for this promising class of potential antiangiogenic compounds.

Chemistry[14]

The requisite tropones were prepared from estradiol utilizing methodology developed in these and other laboratories (Figure 2).[12,13,15] Thus, selective methylation of estradiol followed by Birch reduction afforded the corresponding 1,4-dihydro adduct **1**, which could be isomerized to the homo-conjugated 1,3-dihydro adduct **2** using ^{t}BuOK in DMSO/THF. Exposure of the dihydro adducts to dibromocarbene afforded the corresponding mono insertion products **3** and **4**, which afforded tropones **5** and **6**, respectively, on exposure to either $AgNO_3$ or refluxing pyridine followed by $AgNO_3$. Bromotropones **7** and **8** were realized by ring expansion with refluxing pyridine followed by mono-epoxidation and silica gel chromatography.

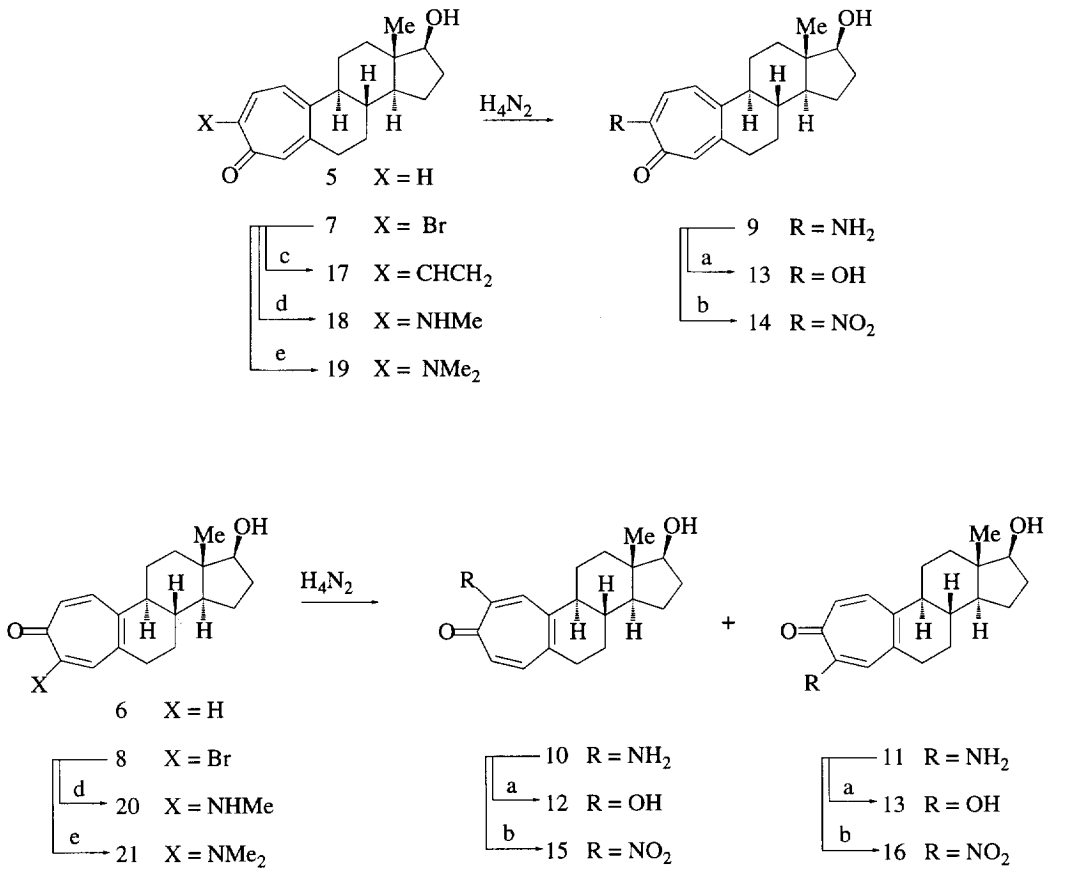
Figure 2. Synthesis of estratropone core structures.



With tropones **5** and **6** in hand (Figure 3), oxidative amination of **5** with hydrazine provided aminotropone **9** exclusively,[16] while similar treatment of **6** afforded **10** and **11** as a 1:1 mixture separable by chromatography. Tropolones **13** and **12** were afforded from the treatment of aminotropones **9** or **11** and **10**, respectively, with NaOH.[16] The conversion of aryl-amines into nitro-aromatics has been previously studied[17] and exposure of

amino tropones **9**, **10**, and **11** to dimethyldioxirane afforded **14**, **15**, and **16**, respectively. The synthesis of vinyl tropone **17** was realized by conjugate addition of vinyl magnesium bromide to **7**.[18,19] The synthesis of amines **18**, **19**, **20**, and **21** was realized by the addition of the corresponding amine to the corresponding α-bromo estratropone.

Figure 3. Synthesis of substituted estratropones.



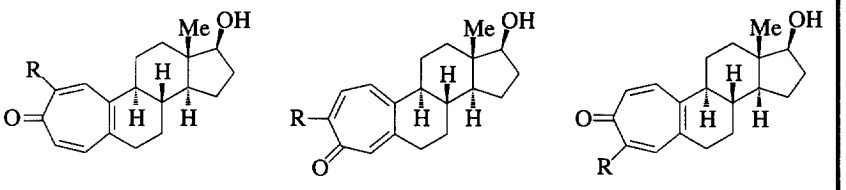
a. NaOH, H_2O; b. dimethyldioxirane (DMD); c. vinyl magnesium bromide (2.2 equiv.);
d. methylamine, toluene; e. dimethylamine, toluene.

Discussion

Data for the inhibition of tubulin polymerization are compiled in Table 1. As noted previously, our preliminary investigations into the structure-activity profile of A-homo-estratropones indicated that estratropones

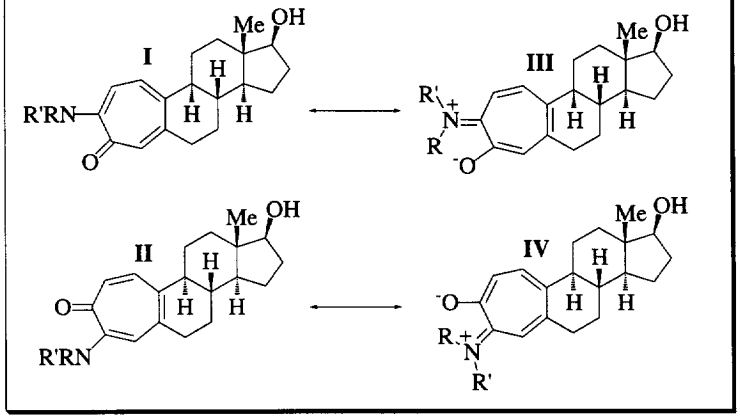
A and **B** (Figure 1) were the most active trienone isomers tested. Isomer **A** showed increased activity with α-substituents that are electron donating, while compounds possessing the **B** trienone core showed greater activity with electron withdrawing α-substituents. The present series of compounds (Figure 4) provides additional, but limited, insight into the structure-activity profile of the estratropones. Many of the congeners inhibit tubulin polymerization to a slightly lesser extent than colchicine. The tropones possessing nitro functionalities displayed either weak inhibition (**14**) or yielded erroneous data (**15** and **16**). In these cases, the nitro-tropones showed polymerization inhibition, however data obtained were nonlinear and inhibition appeared to operate independently of concentration. It is possible that these species are sufficiently electrophilic to react with free sulfhydryl groups on the protein. Finally, one of the congeners (**11**) gave polymerization inhibition activity (IC_{50} = 9.2 μm) which was slightly greater than that of colchicine (IC_{50} = 11.2 μm).

Figure 4. Estratropones with R-substituent at C-2, C-3 , and C-4 respectively.



With the exception of the aforementioned nitrotropones, activity of the compounds studied does not appear to correlate with tropone core structure in an obvious manner. This observation is in contrast to previous studies in these laboratories.[12,13] For the tropolones **12** and **13** the IC_{50} value may represent an average value for the two tautomeric forms. Similarly, the structure-activity profile of tropones possessing amino functionalities may also be attributed to tautomeric forms available to the trienone core as depicted in Figure 5. Aminotropones **19** and **21** may as the result of steric constraints favor structures **I** and **II**, respectively. The remaining

Figure 5. Aminotropones and tautomeric forms. R, R' = H or Me.



aminotropones may favor the corresponding tautomeric isoforms **III** and **IV**. This analysis indicates that tropones in this class that are capable of adopting the trienone configuration represented by **I** and **IV** are the most active for this series. While this rationale may provide a basis for further refining the structure-activity profile of this class of agents, we have not yet addressed the details surrounding these issues experimentally.

Table 1. Inhibition of tubulin polymerization.

Compound #	Polym. IC_{50}[a] (μm)	Relative Activity[b]	C-2 R[c]	C-3 R[c]	C-4 R[c]
colchicine	11.2	1	-	-	-
9	14.4	0.78	H	NH_2	O
10	16.5	0.68	NH_2	O	H
11	9.2	1.2	H	O	NH_2
12	14.9	0.75	OH	O	H
13	22.8	0.49	H	OH	O
14	52	0.21	H	NO_2	O
15	?	-	NO_2	O	H
16	?	-	H	O	NO_2
17	14.2	0.79	H	$CHCH_2$	O
18	60.7	0.18	H	NHMe	O
19	14.3	0.78	H	NMe_2	O
20	19.4	0.58	H	O	NHMe
21	46.8	0.24	H	O	NMe_2

[a]IC_{50} values for three data points were obtained and averaged. Conditions were as follows: Purified bovine brain tubulin (120 μL, 4 mg/mL), PME (240 μL, 1 mM $MgSO_4$, 2 mM EGTA, 100 mM PIPES, pH 6.9), estratropone (32 μL, DMSO), and GTP (8 μL, 50 mM) were allowed to polymerize for 10 min. Absorbancies were recorded with a Varian DMS 90 UV-VIS spectrophotometer at 351 nm in a temperature controlled curvet holder at 30 °C. [b]Relative Activity (IC_{50} colchicine/IC_{50} estratropone). [c]Substituent at the indicated position on the steroid nucleus in Figure 4.

Conclusion

In summary, the synthesis of a second generation of A-homo-estratropones has been achieved. These agents, which may display improved theraputic benefits, represent an effort to increase the potency of the parent, 2-methoxyestradiol, by incorporating stuctural features of colchicine. The compounds were evaluated for their ability the inhibit tubulin polymerization using purified bovine brain tubulin. Several compounds displayed activity similar to that of colchicine, a potent inhibitor of tubulin polymerization. Additionally, the new series of tubulin inhibitors which possess electron donating moieties on the tropone ring display activity which appears to be independent of core structure. Results from our continued study of these and other novel inhibitors of tubulin polymerization will be reported in due course.

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