Bioorganic & Medicinal Chemistry Letters, Vol.1, No.9, pp. 471-476, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

STRUCTURAL REQUIREMENTS FOR THE BINDING OF COLCHICINE ANALOGS TO TUBULIN: THE ROLE OF THE C-10 SUBSTITUENT

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(*Received* 2 *July* 1991)

Abstract: Derivatives of colchicine and the bicyclic colchicine analog 2-methoxy-5-(2',3',4'-trimethoxyphenyl)tropone were tested for inhibition of tubulin polymerization. The nature of the tropone substituent had little effect on the efficacy of the colchicine series, with some exceptions. In contrast, the potency of the bicyclic analogs varied greatly with the tropone substituent.

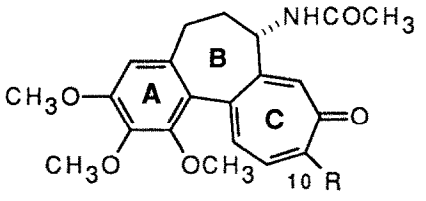
Tubulin is the target protein for a variety of antimitotic drugs, which possess a wide range of therapeutic utilities. A number of these agents (including colchicine, steganacin, podophyllotoxin, and combretastatin) exert their biological effects by binding to a single site on the tubulin heterodimer (M_r = 100 kdal) which has come to be known as the colchicine binding site.[1] While the colchicine binding site appears to accommodate a diversity of structures, it is also remarkably sensitive to changes in the tropone C ring of colchicine. Colchicine itself binds to tubulin with moderately high affinity ($K_a = 10^6 - 10^7$ M^{-1} at 37° C)[2], while isocolchicine, a colchicine analog in which the tropone methoxy and carbonyl substituents are interchanged, binds with 1000 fold less affinity than colchicine.[3] When the C ring of colchicine is a tropolone as in colchiceine, the molecule's affinity for the colchicine site on tubulin is also greatly decreased, and the molecule binds to tubulin at sites other than the colchicine binding site.[4]

Colchicine is believed to interact with tubulin through up to three binding site subdomains: the A ring subdomain, which recognizes the trimethoxyphenyl portion of the molecule, the C ring subdomain, which accommodates the tropone portion of the molecule, and possibly a site related to the substitution pattern at the chiral C-7 on the B ring.[5,6] The B ring and substituents are known to affect the kinetic[5] and spectroscopic features[6] of colchicinoids binding to tubulin, but in colchicine analogs investigated to date the effect of the B ring on the ligand's binding affinity appears to be minor (1-2 kcal/mol).[7-9] In this work we have examined the role of the tropone substituent in colchicine and bicyclic colchicine analogs binding to tubulin. Our results show that the colchicine binding site can accommodate relatively diverse tropone substituents in the colchicine series, but only a more limited range of structures in the absence of the B ring. These results support the hypothesis that the B ring and/or C-7 substituent contribute positively to the affinity of colchicinoids for tubulin, and define the characteristics of the optimal tropone substituent for high affinity binding to tubulin.

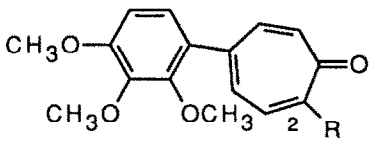
The effect of the tropone substituent of colchicine derivatives (series I) and bicyclic derivatives (series II) on *in vitro* microtubule assembly is shown in Table I. It has been shown previously that a substituent other than

hydrogen α to the tropone carbonyl is required for inhibitory activity.[10] In the series of C-10 analogs tested here, only two showed decreased activity relative to colchicine. The two less active analogs, **2** and **7**, contain substituents that are ionizable near physiological pH,[11] and decreased activity may be related to the presence of anionic forms of the two molecules.

Table I. Inhibition of microtubule assembly by 10-substituted colchicine derivatives (**I**) and 2-substituted-5-(2',3',4'-trimethoxyphenyl)tropones (**II**).



I



II

Substituent (R)	Colchicine Derivatives (I) Compound	I_{50}, μM[a]	Bicyclic Derivatives (II) Compound	I_{50}, μM	Substituent Volume, cm^3/mol[b]
OMe	**1**	7.5	**10**	3.8	17.37
OH	**2**	20	**11**	~200[c]	8.04
OEt	**3**	----	**12**	5.7	20.81
NH_2	**4**	1.9	**13**	625	10.54
NHMe	**5**	2.6	**14**	2.7	21.75
$N(Me)_2$	**6**	4.3	**15**	22	31.67
SH	**7**	55	**16**	----[c]	14.8
SMe	**8**	2.5	**17**	7.0	24.47
H	**9**	NA[d,e]	**18**	NA[e]	3.2
Et			**19**	34	17.11
Cl			**20**	~500[c]	11.65

[a] I_{50} is the concentration required to effect a 50% reduction in microtubule protein polymerization. The experimental procedure used is described in reference 4. [b] van der Waals volume, from reference 12. [c] Low solubility. [d] From reference 10. [e] NA = not active.

The remaining colchicine derivatives retained high activity in inhibiting microtubule assembly and were in fact more potent than colchicine in this assay. The differences in potency were relatively minor, and it is concluded from this analysis that unless the substituent is ionizable, the steric and electronic effects of the C-10

substituent on tubulin binding are small.

In the bicyclic series, however, the nature of the tropone substituent had a dramatic effect on the activity of the compound. Like colchicine, the tropone (**18**) and tropolone (**11**) derivatives were poor inhibitors of tubulin assembly. Unlike colchicine, the other tropone derivatives tested exhibited widely varying activities. It appears that in the absence of the B ring of colchicine, the tropone substituent becomes of major importance in the affinity of the ligand for the colchicine binding site. These data support the hypothesis of a three subdomain binding site on tubulin and indicate that the B ring is intimately involved in determining the energetics of colchicinoids binding to tubulin. It is not clear whether the B ring skeleton is the major structural feature that overrides the importance of the tropone substituent, perhaps by locking in the most favorable conformation of the A and C rings in the binding site, or whether an electrostatic interaction between tubulin and the C-7 substituent serves to stabilize the colchicinoid-tubulin complexes.

The data for series II may be used to assess the optimal features of the tropone substituent that stabilize the complex. The size of the substituent seems to be important, since small groups such as $-NH_2$ and -Cl had reduced activity, as did the more bulky $-N(Me)_2$. Examination of the group of bicyclic compounds with similar and intermediate molecular size (-OMe, -OEt, -NHMe, -SMe, and -Et) indicates that the electronic nature of the substituent contributes to the efficacy of the ligand, but is somewhat less important in the efficacy of the ligand than its size. An unshared pair of electrons in the atom directly bonded to the tropone ring enhances activity, implying that an electrostatic interaction may contribute to the stability of the complex.

The inhibition of [^{3}H]-colchicine binding to tubulin was quantitatively evaluated for a few of the most active series I and II compounds (Table II). The opposing effects of the nature of the tropone substituent on the affinity of the different structures for tubulin serves to further emphasize that there are pronounced differences in the manners in which the colchicine and bicyclic compounds interact with tubulin.

Table II. Competitive inhibition of [^{3}H]-colchicine binding to tubulin by colchicine and bicyclic colchicine derivatives.

Substituent	Colchicine Derivatives (I) Compound	K_I, μM[a]	Bicyclic Derivatives (II) Compound	K_I, μM
OMe	**1**	2.5	**10**	11.5
NHMe	**5**	0.75	**14**	2.5
SMe	**8**	0.70	**17**	22.0

[a] K_I is the inhibition constant found in a competitive binding assay with [^{3}H]-colchicine, which was performed as described in reference 4.

Colchicine was purchased from the Aldrich Chemical Co. Colchicine derivatives **2** - **6** and **8** were prepared by published procedures[14-17], and the bicyclic analog of colchicine (**10**) was synthesized according to Fitzgerald.[18] The other analogs used in these studies were synthesized by several different approaches. The tropone methoxy of either colchicine (**1**) or compound **10** was displaced by nucleophilic reagents to produce

analogs **7** and **13** - **17**. These nucleophiles were gases at room temperature, which were condensed and added to the substrate for reaction in a sealed bomb. Desulfurization of **17** with Raney nickel led to **18**. Reaction of **10** with dilute hydrochloric acid produced **11**, which was then reacted with oxalyl chloride to produce **20**. Treatment of **10** with ethyl Grignard reagent produced both **12** and **19**. Compound **12** was apparently formed through reaction of the Grignard reagent with a small amount of ethyl acetate which was not removed from **10** prior to the reaction. A similar reaction occurs with 10-demethyl-10-tosylcolchicine (M.E. Staretz and S.B. Hastie, unpublished observation). The structure and purity of each compound was confirmed by 360 MHz NMR, mass spectrometry and TLC analysis.[21] Syntheses and spectra are detailed in a note with this communication.[22].

Acknowledgment: This work was supported by National Institutes of Health Research Grant DK32453 (to T.L.M.) and National Science Foundation Grant DMB 90-05614 (to S.B.H.)

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21. NMR spectra were obtained with a Bruker AM-360 spectrometer with $(CH_3)_4Si$ as the internal standard and $CDCl_3$ as solvent unless otherwise noted. Electronic-ionization mass spectra (EIMS) and chemical ionization mass spectra (CIMS) were obtained with a Finnegan MAT 46000 mass spectrometer.
22. *Compound* **7** *from* **1**: catalytic TosOH·H_2O and excess H_2S in MeOH, 14 days rt, silica chro. (EtOH:EtOAc, 2:8), 18%: NMR δ 7.48 (d, 1H, J = 10.1 Hz), 7.34 (s, 1H), 7.28 (d, 1H, J = 10.1 Hz), 6.52 (s, 1H), 6.39 (broad d, 1H, J = 7.2 Hz), 4.64 (d of t, 1H, J = 7.2, 11.2 Hz), 3.90 (s, 3H), 3.88 (s, 3H), 3.64 (s, 3H), 2.54 (d of d, 1H, J = 13.7, 6.8 Hz), 2.2-2.46 (m, 2H), 2.03 (s, 3H), 1.76-1.87 (m, 1H); EIMS, m/e 401, 373.

    Compound **18** *from* **17**: Raney nickel was refluxed 11 h in acetone, then **17** was added, reflux 12 h, preparative TLC (EtOAc:hexane, 1:1), <10% by TLC: NMR δ 7.35 (d of d, 1H, J = 12.6, 1.8 Hz), 6.94-7.24 (complex overlapping multiplets, 4H), 6.97 (d, 1H, J = 7.6 Hz), 6.73 (d, 1H, J = 7.6 Hz), 3.92 (s, 3H), 3.90 (s, 3H), 3.76 (s, 3H); CIMS (CH_4), m/e 273.

    Compound **20** *from* **11**: 10 equiv. oxalyl chloride in benzene, 2 h reflux, silica chro. (EtOAc:hexane, 7:3), 47%: NMR δ 7.99 (d, 1H, J = 10.0 Hz), 7.51 (d of d, 1H, J = 12.8, 1.8 Hz), 7.17 (d, 1H, J = 12.8 Hz), 7.12 (d, 1H, 8.7 Hz), 6.93 (d, 1H, 8.7 Hz), 3.80 (s, 3H), 3.88 (s, 3H), 3.93 (s, 3H); EIMS, m/e 308(M+2), 306, 278.

    Compound **11** *from* **10**: THF:0.1 M HCl (1:1), 24 h reflux, recrystallize MeOH, 95%: NMR δ 7.45 (2H, d, J=11.7 Hz), 7.37 (2H, d, J=11.7 Hz), 6.97 (1H, d, J=8.6 Hz), 6.74 (1H, d, J=8.6 Hz), 3.93 (3H, s), 3.91 (3H, s), 3.70 (3H, s); EIMS, m/e 288,260.

    Compounds **12** *and* **19** *from* **10**: 12 equiv. EtMgBr, 4 h rt, silica chro. (Hexane:EtOAc, 5:3), **19** (18%): NMR δ 7.34 (d of d, 1H, J = 12.6, 2.2 Hz), 7.30 (d, 1H, J = 9.0 Hz), 7.08 (d, 1H, J=12.6 Hz), 7.04 (broad d, 1H, J = 9.0 Hz), 6.97 (d, 1H, J = 7.9 Hz), 6.73 (d, 1H, J = 7.9 Hz), 3.93 (s, 3H), 3.91 (s, 3H), 3.75 (s, 3H), 2.72 (q, 2H, J = 7.2 Hz), 1.23 (t, 3H, J = 7.2 Hz); EIMS, m/e 300, 272, 257.

    12 (25%): NMR δ 7.43 (d of d, 1H, J = 12.6, 1.8 Hz), 7.24 (d, 1H, J = 12.6 Hz), 7.14 (d of d, 1H, J = 10.8, 1.8 Hz), 6.97 (d, 1H, J = 8.3 Hz), 6.82 (d, 1H, J = 10.8 Hz), 6.73 (d, 1H, J = 8.3 Hz), 4.18 (q, 2H, J = 6.8 Hz), 3.93 (s, 3H), 3.91, (s, 3H), 3.73 (s, 3H), 1.55 (t, 3H, J = 6.8 Hz); EIMS, m/e 316, 301, 288.

    Compound **13** *from* **10**: excess NH_3 in MeOH, sealed vessel 6 h 100° C followed by rt overnight, silica chro. (EtOAc), 99%: NMR (acetone-d_6) δ 7.40 (d of d, 1H, J = 12.2, 1.8 Hz), 7.24 (d of d, 1H, J = 10.8, 1.8 Hz), 3.89 (s, 3H), 3.85 (s, 3H), 3.67 (s, 3H); CIMS (CH_4), m/e 288.

    Compound **14** *from* **10**: excess NH_2CH_3 in MeOH, sealed vessel 78 h rt, recrystallize EtOAc/hexane, 61%: NMR (DMSO-d_6, 80° C), δ 7.60 (broad s, 1H), 7.32 (d of d, 1H, J = 11.9, 1.8), 7.3 (d of d, 1H, J = 10.8, 1.8 Hz), 6.94 (d, 1H, J = 9.0 Hz), 6.92 (d, 1H, J = 11.9 Hz), 6.83 (d, 1H, J = 9.0 Hz), 6.58 (d, 1H, J = 10.8 Hz), 3.83 (s, 3H), 3.78 (s, 3H), 3.59 (s, 3H), 3.59 (s, 3H); EIMS, m/e 301.

    Compound **15** *from* **10**: catalytic TosOH·H_2O and excess $NH(CH_3)$ in MeOH, 3 days rt, silica chro. (EtOAc), 98%: NMR δ 7.28 (d of d, 1H, J = 11.5, 2.9 Hz), 7.14 (d of d, 1H, J = 10.8, 2.9 Hz), 6.98 (d, 1H, J = 11.5 Hz), 6.94 (d, 1H, J = 7.6 Hz), 6.72 (d, 1H, J = 7.6 Hz), 6.57 (d, 1H, J = 10.8 Hz), 3.93 (s, 3H), 3.90 (s, 3H), 3.72 (s, 3H), 3.14 (s, 6H); EIMS, m/e 315, 300.

*Compound **16** from **10**:* catalytic TosOH·H_2O and excess H_2S in MeOH, 3 days rt, silica chro. (hexane:EtOAc, 1:1), approx. 50% by TLC: NMR δ 7.36 (d, 1H, J = 12.6 Hz), 7.34 (d, 1H, J = 10.4 Hz), 7.0 (d, 1H, J = 10.4 Hz), 6.90 (d, 1H, J = 12.6 Hz), 6.82 (d, 1H, J = 8.3 Hz), 6.64 (d, 1H, J = 8.3 Hz), 3.69 (s, 3H), 3.66 (s, 3H), 3.54 (s, 3H); EIMS, m/e 304, 276.

*Compound **17** from **10**:* excess CH_3SH in $CHCl_3$, sealed vessel 5.5 h 80° C, 45%: NMR δ 7.45 (d, 1H, J = 12.6 Hz), 7.18 (d, 1H, J = 10.8 Hz), 7.08 (1H, d, J = 12.6 Hz), 7.04 (d, 1H, J = 10.8 Hz), 6.97 (d, 1H, J = 7.9 Hz), 6.74 (d, 1H, J = 7.9 Hz), 3.94 (s, 3H), 3.92 (s, 3H), 3.74 (s, 3H), 2.44 (s, 3H); EIMS, m/e 318, 290, 257, 228.