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Structure–Activity Relationships of Phenylcyclohexene and Biphenyl Antitubulin Compounds against Plant and Mammalian Cells

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Abstract—Phenylcyclohexenes (PCHs) [e.g., *trans*-4-nitro-5-(2,3,4-trimethoxyphenyl)cyclohexene, **2d**] were found to bind weakly to the colchicine site of bovine tubulin, but are the first mimics of colchicine found to have high activity towards plant cells. Structure–activity relationships for PCHs and biphenyl AC-ring analogues of colchicine (e.g., 2,3,4,4′-tetramethoxy-2′-methyl-1,1′-biphenyl, **3e**) are discussed.

Tubulin is the major component of microtubules and is the target of various anticancer drugs[1] (paclitaxel, *Vinca* alkaloids and compounds interacting at the colchicine binding site) and herbicides[2] (dinitroanilines, *N*-phenylcarbamates, amiprofos-methyl, pronamide, and dithiopyr) that inhibit mitosis. Although tubulin is highly conserved between organisms, mammalian and plant tubulins differ greatly in their interaction with antitubulin agents.[3] Colchicine (**1**, Fig. 1), one of the best studied antitubulin compounds, is known to be much more potent toward mammalian cells than toward plant cells.[3]

Random screening identified **2a** (Fig. 1) as a weak herbicide lead with pre-emergence grass activity. Synthesis[4,5] of several dozen analogues of **2a**, including **2b–e**, led to the identification of **2d** and the cyano analogue **2e** as substantially more potent herbicides than **2a** in greenhouse tests.[5]

The symptoms exhibited by plants treated in the greenhouse with **2d** and other phenylcyclohexenes (PCHs) were similar to those seen in plants treated with the herbicides pronamide and trifluralin, suggesting that the PCHs function by disrupting mitosis. In an in vitro assay for inhibition of tobacco root growth,[6] **2d** did not prevent seed germination but strongly inhibited root elongation and produced swollen root tips. These symptoms are typical of an antimitotic herbicide.[2] Compound **2d** ($EC_{50} = 0.27$ μM) was more potent than the commercial herbicides pronamide ($EC_{50} = 0.72$ μM) and trifluralin ($EC_{50} = 1.32$ μM) in the tobacco root assay. Cytological staining of chromosomes in tobacco suspension-cultured cells[6] treated with **2d** showed an increase in the mitotic index (Fig. 2) resulting from an accumulation of cells in arrested metaphase, confirming the inhibition of mitosis. The increase in mitotic index produced by **2d** was of similar magnitude to that obtained with **1**, however the concentration of **2d** required to arrest mitosis in tobacco was more than three orders of magnitude lower than the concentration needed for **1** (Fig. 2).

By contrast, colchicine was >4000-fold more active than **2d** and **2e** towards HCT-116 tumor cells,[7] and in experiments using tubulin isolated from bovine brain **2d** and **2e** weakly inhibited microtubule assembly[8] at 200 μM (Fig. 3), whereas colchicine strongly inhibited assembly at 2 μM. Although much less potent than colchicine towards mammalian tubulin, **2d** was found to be a competitive inhibitor of the binding of ^{3}H-colchicine to bovine brain tubulin[9] (Fig. 4) indicating a common binding site.

Biphenyl colchicine mimics, including **3e** and **3f** (Fig. 1), which are active towards mammalian tubulin and bear

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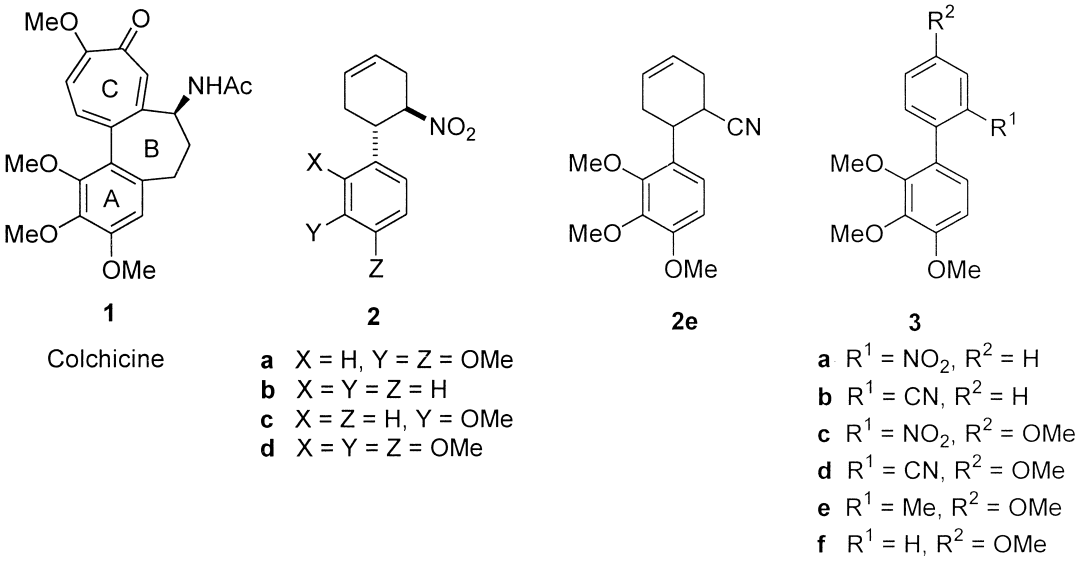

Figure 1. Compounds.

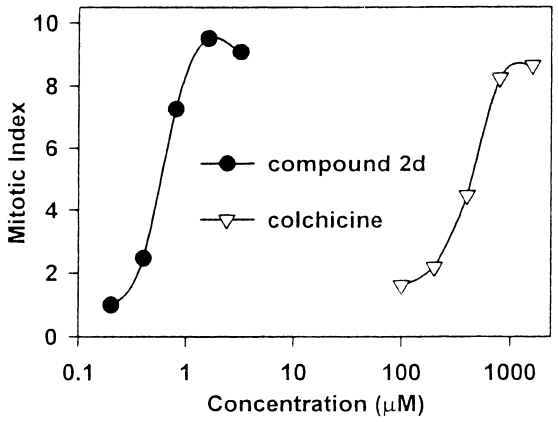

Figure 2. Arrest of mitosis in tobacco suspension-cultured cells.

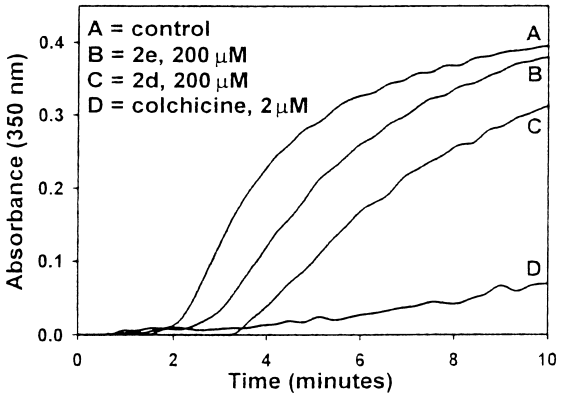

Figure 3. Inhibition of microtubule assembly.

an intriguing structural similarity to the PCHs have been described in the literature.[10–13] Compound **3c** has been described in the literature[14] but its biological activity has not been reported. Thus, it was of interest to synthesize biphenyls **3a**–**f**[15] and compare the structure–activity relationships of colchicine, **2b**–**e**, and **3a**–**f** on both plant and mammalian cells.

The results given by colchicine, **2b**–**e**, and **3a**–**f** in three assays are shown in Table 1. Colchicine and its known AC-ring biphenyl mimic **3e**[10] showed much stronger activity against HCT116 colon cancer cells and as inhibitors of bovine microtubule assembly than against tobacco. In contrast, PCH **2d** was very active toward tobacco but showed at best weak activity against HCT116 cells and as an inhibitor of bovine microtubule assembly. The 2,3,4-trimethoxy substitution pattern in the A ring appears to be critical for optimal activity in colchicine based on structure–activity studies[16] of analogues of 5-(trimethoxyphenyl)tropolone methyl ether, which has similar potency to colchicine[17] but lacks the B-ring. Similarly, the trimethoxy substitution on the phenyl ring of the PCHs was important for activity toward tobacco (**2d** > **2c** > **2b**) and also towards HCT116 cells, although to a lesser extent.

Consistent with their potent herbicidal activity, **2d** and **2e** were highly active in the tobacco root assay. Against HCT116 cells, **2d** and **2e** were weakly active and comparable in activity. To better characterize the activity of **2d** and **2e**, they were separated into their individual enantiomers by preparative HPLC on a chiral column.[18] The *trans* stereoisomers of **2e** were much more active than the *cis* stereoisomers towards tobacco. The faster eluting enantiomer **2d**-*trans*-**I** was almost 20-fold more potent towards tobacco than the slower eluting enantiomer **2d**-*trans*-**II**, while they were equipotent against HCT116 cells. The faster eluting *trans* enantiomer in the cyano series **2e**-*trans*-**I** was 10-fold more potent on tobacco than its enantiomer **2e**-*trans*-**II**; however, **2e**-*trans*-**II** was more potent than **2e**-*trans*-**I** against HCT116 cells. Furthermore, the slower eluting *cis* enantiomer **2e**-*cis*-**II** was about equipotent to **2e**-*trans*-**II** against HCT116 cells.

Biphenyls **3a** and **3b**, the fully aromatic analogues of PCHs **2d** and **2e**, were substantially less active towards tobacco than the corresponding PCHs, but gained potency against HCT116 cells.

Itoh and co-workers reported that a methoxy group in place of H at the 4′-position in the biphenyl system (i.e., **3**, $R^2 = OMe$) increased activity towards mammalian cells.[10] Their most active compounds were **3e** and **3f**. Hence, it was of interest to prepare **3c** and **3d** and compare them with **3e** and **3f**. Against HCT116 cells, **3c** was found to be less active than **3e** and **3f**; however, **3d** showed increased potency. In the tobacco root assay, the 4′-methoxy group (**3c**) abolished all activity.

PCHs **2d** and **2e** are apparently the first examples of antitubulin colchicine mimics with potent activity toward plants. Our results suggest that the binding pocket for the colchicine A-ring is conserved between plant and mammalian tubulin while the tropolone C-ring binding regions differ significantly. Molecular modeling is underway to shed further light on the binding site differences. X-ray crystallography is in progress to determine the absolute configuration of **2e**-*trans*-**I**.

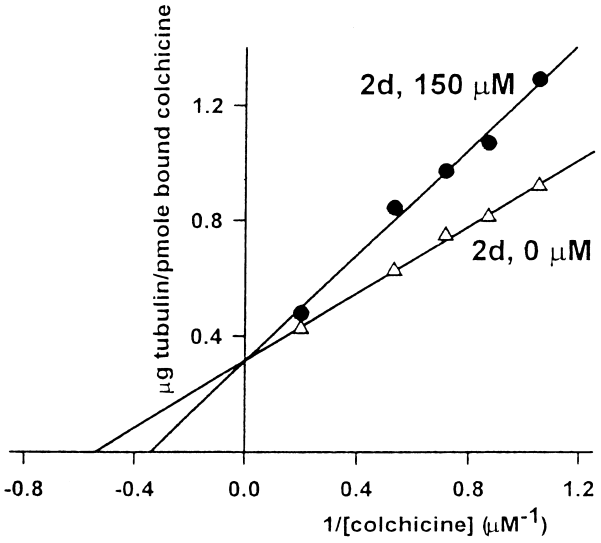

Figure 4. Competitive inhibition of [^{3}H]-colchicine binding to bovine tubulin by **2d**.

Table 1. Structure–activity relationships

Compd	Tobacco root assay EC_{50} (μM)	HCT116 tumor cell assay EC_{50} (μM)	Bovine microtubule assembly EC_{50} (μM)
1	356	0.020	0.75–1.25
2b[a]	>100	174	>100
2c[a]	3.78	143	>100
2d[a]	0.27	85	>100
2e[b]	2.29	113	>100
2d-*trans* enantiomer **I**[c]	0.100	79	>100
2d-*trans* enantiomer **II**[c]	1.96	71	>100
2e-*cis* enantiomer **I**[c]	>25	142	>100
2e-*cis* enantiomer **II**[c]	>25	45	>100
2e-*trans* enantiomer **I**[c]	0.667	136	>100
2e-*trans* enantiomer **II**[c]	7.17	47	>100
3a	52.0	19.6	>100
3b	>100	13.8	50–100
3c	>100	2.65	6.25–12.5
3d	>100	0.58	6.25–12.5
3e	>100	0.78	6.25–12.5
3f	>100	1.52	6.25–12.5

[a]Racemic mixture of the *trans* diastereomer.
[b]Racemic mixtures of both the *cis* and *trans* diastereomers.
[c]Stereoisomers were separated by semi-preparative HPLC on a chiral column. See ref 18.

Acknowledgements

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4. Synthesis of **2b–d** was accomplished by Diels–Alder reaction of the appropriate *trans*-β-nitrostyrene with butadiene sulfone yielding only the *trans*-4-nitro-5-phenylcyclohexene derivatives following the literature procedure: Bryce, M. R.; Gardiner, J. M. *Tetrahedron* **1988**, *44*, 599. Synthesis of **2e**, as a 1:1 mixture of the *cis* and *trans* cyclohexene isomers, was accomplished by Diels–Alder reaction of ethyl 2-cyano-3-(2,3,4-trimethoxyphenyl)propenoate with butadiene sulfone followed by saponification and decarboxylation. Spectral data for the new compounds **2d** and **2e** are reported below in ref 19.
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8. Inhibition of microtubule assembly was assayed turbidimetrically as described in ref 6 using a 1 h incubation at 37 °C of the test compound with bovine brain tubulin before initiation of assembly.
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15. Biphenyls **3a–f** were synthesized by Suzuki coupling of 2,3,4-trimethoxybenzeneboronic acid with the appropriate bromobenzene under standard conditions Fu, J.-M.; Zhao, B. P.; Sharp, M. J.; Snieckus, V. *Can. J. Chem.* **1994**, *72*, 227. Spectral data for new compounds **3a**, **3b**, and **3d** are reported below in ref 19.

16. Banwell, M. G.; Cameron, J. M.; Collis, M. P.; Crisp, G. T.; Gable, R. W.; Hamel, E.; Lambert, J. N.; Mackay, M. F.; Reum, M. E.; Scoble, J. A. *Aust. J. Chem.* **1991**, *44*, 705.
17. Fitzgerald, T. J. *Biochem. Pharmacol.* **1976**, *25*, 1383.
18. A semi-preparative Chiralcel® OC column from Chiral Technologies, Inc., 250×4.6 mm was used with isooctane–ethanol (95:5, by volume) as the mobile phase.
19. *trans*-4-Nitro-5-(2,3,4-trimethoxyphenyl)cyclohexene (**2d**): white solid, mp 99–100 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.2–2.5 (m, 2H), 2.7–2.9 (m, 2H), 3.70 (m, 1H), 3.81 (s, 3H), 3.84 (s, 3H), 3.95 (s, 3H), 5.12 (m, 1H), 5.65–5.85 (m, 2H), 6.72 (d, J=8.6 Hz, 1H), 6.83 (d, J=8.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 31.2, 32.5, 38.8, 55.7, 60.4, 61.0, 85.8, 107.1, 122.0, 122.3, 125.4, 126.7, 142.0, 151.8, 152.8; IR ($CDCl_3$) 1603, 1548, 1498, 1421, 1368 cm^{-1}. Anal. calcd $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53; N, 4.78. Found: C, 61.38; H, 6.47; N, 4.73. 4-Cyano-5-(3,4-dimethoxyphenyl)cyclohexene (**2**, 1:1 mixture of diastereomers): oily solid; ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.9, 29.2, 29.7, 29.8, 30.7, 31.7, 32.0, 33.1, 36.6, 55.9 (double height), 60.6, 60.7, 61.1, 61.2, 107.1, 107.4, 121.0, 121.2, 121.9, 122.7, 123.4, 127.2, 127.7, 141.9, 142.1, 151.3, 151.8, 152.9, 153.0; IR ($CHCl_3$) 3030, 2930, 2850, 2240, 1655, 1600 cm^{-1}; MS (ESI) m/z 273 (M^+). Anal. calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12. Found: C, 70.37; H, 6.87; N, 4.96. *cis*-4-Cyano-5-(3,4-dimethoxyphenyl)cyclohexene (**2e**-*cis*-**I**, **2e**-*cis*-**II**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.22 (m, 1H), 2.30–2.45 (1H), 2.55–2.80 (2H), 3.14 (m, 1H), 3.38 (m, 1H), 3.87 (s, 3H), 3.88 (s, 3H), 3.90 (s, 3H), 5.70 (m, 1H), 5.94 (m, 1H), 6.70 (d, J=8.7 Hz, 1H), 7.13 (d, J=8.7 Hz, 1H). The measured coupling constant between C_4HCN and C_5HAr $J_{4,5}$ was 4 Hz. *trans*-4-Cyano-5-(3,4-dimethoxyphenyl)cyclohexene (**2e**-*trans*-**I**, **2e**-*trans*-**II**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.25 (m, 1H), 2.40 (m, 1H), 250 (m, 2H), 3.05 (m, 1H), 3.30 (m, 1H), 3.85 (s, 3H), 3.86 (s, 3H), 3.95 (s, 3H), 5.70 (m, 1H), 5.82 (m, 1H), 6.67 (d, J=8.7 Hz, 1H), 6.85 (d, J=8.7 Hz, 1H). The measured coupling constant between C_4HCN and C_5HAr $J_{4,5}$ was 10.3 Hz. The chemical shift and multiplicity in the ^{1}H NMR spectrum of this compound matched those in **2d**. 2′-Nitro-2,3,4-trimethoxy-1,1′-biphenyl (**3a**): yellow solid, mp 85–87 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.65 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 6.75 (d, J=8.6 Hz, 1H), 6.98 (d, J=8.6 Hz, 1H), 7.39 (d, J=7.6 Hz, 1H), 7.45 (m, 1H), 7.60 (m, 1H), 7.92 (d, J=8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 55.8, 60.4, 60.8, 104.9, 107.1, 123.5, 123.8, 124.3, 127.7, 132.4, 132.5, 141.5, 149.1, 150.3, 154.0; IR ($CDCl_3$) 1611, 1599, 1524, 1502, 1435, 1414, 1358 cm^{-1}. Anal. calcd for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.46; H, 5.35; N, 4.75. 2′-Cyano-2,3,4-trimethoxy-1,1′-biphenyl (**3b**): white solid, mp 87–89 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.73 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 6.76 (d, J=8.6 Hz, 1H), 7.01 (d, J=8.6 Hz, 1H), 7.38–7.52 (2H), 7.61 (m, 1H), 7.73 (d, J=8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 55.9, 60.9, 61.0, 107.0, 112.9, 118.5, 124.8, 125.0, 127.2, 130.9, 132.1, 132.7, 142.0, 142.1, 151.0, 154.2; IR ($CDCl_3$) 2220 cm^{-1}; MS (EI) m/z 270 $(M+1)^+$, 254, 223, 140. Anal. calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.16; H, 5.61; N, 5.08. 2′-Cyano-2,3,4,4′-tetramethoxy-1,1′-biphenyl (**3d**): white solid, mp 78–80 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71 (s, 3H), 3.85 (s, 3H), 3.90 (s, 3H), 3.92 (s, 3H), 6.75 (d, J=8.6 Hz, 1H), 6.99 (d, J=8.6 Hz, 1H), 7.15 (dd, J=8.6, 2.6 Hz, 1H), 7.22 (d, J=2.6 Hz, 1H), 7.37 (d, J=8.6 Hz); ^{13}C NMR ($CDCl_3$) δ 55.4, 55.8, 60.8, 60.9, 106.9, 113.3, 116.9, 118.4, 118.7, 124.5, 125.0, 132.1, 134.4, 142.0, 151.2, 153.9, 158.1; IR ($CDCl_3$) 2225 cm^{-1}; MS (EI) m/z 300 $(M+1)^+$, 285, 253, 170. Anal. calcd for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.72; N, 4.68. Found: C, 68.04; H, 5.76; N, 4.68.