Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 5154–5159
Bioorganic & Medicinal Chemistry Letters

Synthesis and structure–activity relationships of 1,2,4-triazoles as a novel class of potent tubulin polymerization inhibitors

Xiaohu Ouyang,[a,*] Xiaoling Chen,[a] Evgueni L. Piatnitski,[a] Alexander S. Kiselyov,[a] Hai-Ying He,[a] Yunyu Mao,[a] Vatee Pattaropong,[a] Yang Yu,[a] Ki H. Kim,[a] John Kincaid,[a] Leon Smith, II,[a] Wai C. Wong,[a] Sui Ping Lee,[a] Daniel L. Milligan,[a] Asra Malikzay,[b] James Fleming,[b] Jason Gerlak,[b] Dhanvanthri Deevi,[c] Jacqueline F. Doody,[b] Hui-Hsien Chiang,[b] Sheetal N. Patel,[b] Ying Wang,[b] Robin L. Rolser,[b] Paul Kussie,[b] Marc Labelle[a] and M. Carolina Tuma[b]

[a]*Department of Chemistry, ImClone Systems, 710 Parkside Avenue, Suite 2, Brooklyn, NY 11226, USA*
[b]*Department of Protein Science, ImClone Systems, 180 Varick Street, New York, NY 10014, USA*
[c]*Department of Experimental Therapeutics, ImClone Systems, 180 Varick Street, New York, NY 10014, USA*

Received 16 July 2005; revised 19 August 2005; accepted 22 August 2005
Available online 28 September 2005

Abstract—A novel triazole-containing chemical series was shown to inhibit tubulin polymerization and cause cell cycle arrest in A431 cancer cells with EC_{50} values in the single digit nanomolar range. Binding experiments demonstrated that representative active compounds of this class compete with colchicine for its binding site on tubulin. The syntheses and structure–activity relationship studies for the triazole derivatives are described herein.

Microtubules (MT) are among the most successful targets for anticancer therapy.[1] Composed of dynamic polymers of tubulin, microtubules are elements of the cell cytoskeleton that play key roles in cell division and cell shape. During mitosis, tightly regulated microtubule dynamics is essential for spindle formation and chromosomal separation. Therefore, rapidly dividing cells are more susceptible to inhibitors of tubulin polymerization than non-dividing cells. Indeed, targeting tubulin is a successful strategy for cancer therapy.[2] There are three well-characterized drug binding sites on tubulin: taxol, vinca, and colchicine sites. Drugs that bind to the first two sites, such as *Paclitaxel* (*Taxol*®) and *Vinblastine*®, have been successfully used in clinics as chemotherapeutics to treat various tumor types; however, their use is limited by multiple drug resistance (MDR). Several colchicine site binders, on the other hand, have been shown to inhibit MDR tumors, instilling renewed interest in the search for novel antimitotic agents that bind to the colchicine binding site on tubulin.[3]

Several classes of colchicine site binders are currently in clinical trials. Candidates T138067, A-289099, and A-105972 demonstrate antitumor activity against *Paclitaxel* and *Vinblastine*® resistant tumors[4–6] with the added benefit of oral administration. A new approach to the use of these colchicine site binders is the targeting of tumor vasculature (vascular disrupting agents or VDAs). VDAs preferentially disrupt microtubules in tumor endothelial cells, causing tumor vascular damage that leads to massive tumor necrosis even when dosed at a fraction of their maximum tolerated doses (MTD).[7,8] Examples of such agents currently undergoing clinical trials are ZD-6126 and *Combretastatin A4P* (CA4P), derived from colchicine and *Combretastatin A4* (CA4), respectively (Fig. 1).[9,10]

Novel triazole-containing tubulin inhibitors were discovered by screening our internal compound library using an in vitro tubulin polymerization assay. At 10 μM, compound **1** inhibited bovine brain tubulin polymerization in vitro by 80% and depolymerized

Keywords: Tubulin inhibitor; Tubulin polymerization; 1,2,4-Triazole; Vascular disrupting agent; Tumor vasculature; Antimitotic.
* Corresponding author. Tel.: +1 347 523 0018; e-mail: shawn_ouyang@yahoo.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.08.056

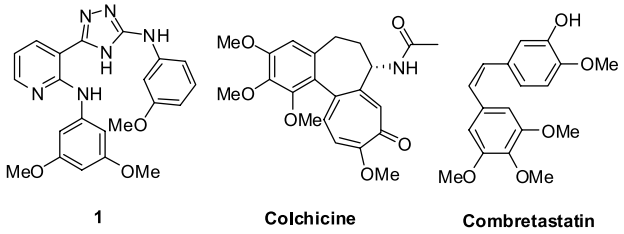
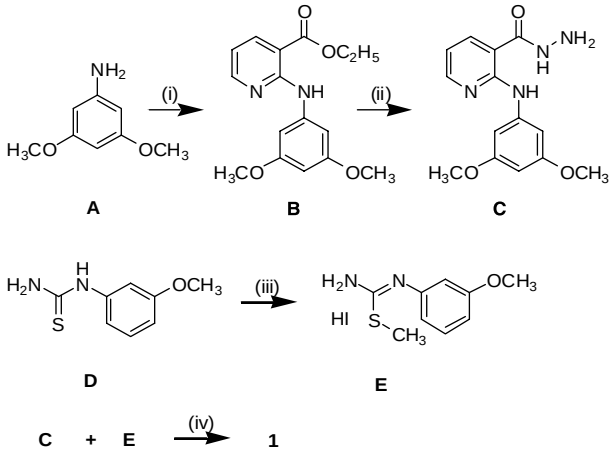


Figure 1. Structures of compound (**1**), colchicine and combretastatin.

microtubules in cultured A431 cells within 20 min (Fig. 2). Compound **1** also demonstrated antiproliferative activity against several tumor cell lines (Table 1) and induced G2/M arrest of A431 human cancer cells. Given its potency, small molecular weight, and potential for significant diversity at various regions of the molecule, we proceeded to synthesize analogs of compound **1**. Compounds were evaluated for inhibition of tubulin polymerization in vitro and capacity to arrest cancer cells during the G2/M phase of the cell cycle for activity determination and discrimination from general cytotoxic compounds. Representative syntheses and SAR studies of this novel tubulin inhibitor class are reported herein.

Compound **1** was synthesized by a condensation of a substituted arylhydrazide **C** with a substituted methylated thiourea **E** according to a previously reported procedure[11] (Scheme 1). Intermediate **C** was synthesized by heating aniline **A** with ethyl-2-chloronicotinate to yield **B** which was reacted further with hydrazine mono hydrate to provide **C**. An intermediate **E** was formed by reacting the substituted thiourea **D** with methyl iodide. A variety of substituted anilines and amines replaced aniline **A** to produce compounds which are listed in Table 2.



Scheme 1. Reagents and conditions: (i) Ethyl-2-chloronicotinate, DMF, 140 °C, 20 h; (ii) hydrazine monohydrate, *i*-PrOH, 120 °C, 10 h; (iii) CH_3I, acetone, reflux, 4 h; (iv) pyridine, sealed tube, 140 °C, 4 h.

To identify structural elements required for activity, we generated a set of compounds following Scheme 1 with varying R^1 groups. Compounds that showed inhibition of tubulin polymerization were then tested for G2/M block activity in A431 cells (Table 2). By keeping R^2 constant, compounds **1** and **2** demonstrated that *meta* or *para* alkoxylanilines in the R^1 position are superior in inhibiting tubulin polymerization and cycling cells than an unsubstituted aniline analog **3**. However, the presence of dimethoxyanilines (**4**–**6**) leads to reduced activity. A similar effect was also observed for anilines substituted with other functional groups (**7**–**9**). It is noteworthy that compounds **10** and **11**, with cyclized alkoxyl moieties, showed potency similar to that of compound **1** in the G2/M assay. Cyclized alkoxyl moieties were later found to be optimal regardless of modifications made to other

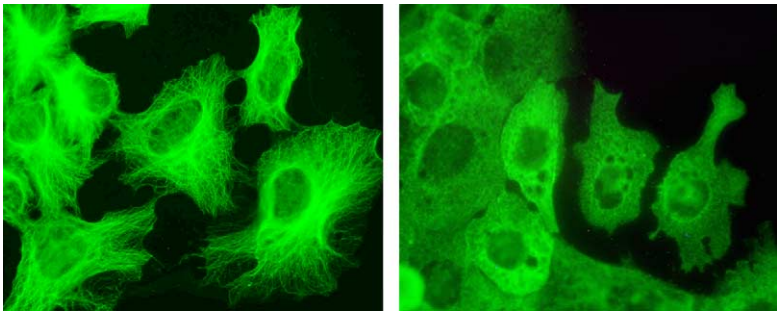


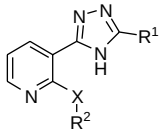
Figure 2. Disruption of A431 cellular microtubules by (**1**). Left, 0.1% DMSO. Right, 10 μM compound (**1**). Cells were stained with an α-tubulin antibody.

Table 1. Antiproliferative activity[a] by (**1**) against various tumor cells

Cell line	A431	A498	DLD1	DU145	NCI-H460
GI_{50} (μM)[b]	0.05	0.060	0.07	0.02	0.01
Cell line	LoVo	HCT116	SNB19	SKOV3	NCI/ADR-RES[c]
GI_{50} (μM)	0.02	0.40	0.15	0.220	0.150

[a] Cytotoxicity measured after treatment for 24 h.
[b] GI_{50} value for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using sulfurhodamine B assay. Values are the means of three independent determinations (SD < 10%).
[c] MDR-1 positive breast cell line.

Table 2. Effects of R^1 and R^2 on tubulin activity



Compound	R^1	R^2	X	% ITP[a]	% G2/M block[b]	EC_{50}(μM)[c]
CA4				95	88	0.0035
Colchicine				80	80	0.0028
1	3-Methoxyphenylamino	3,5-Dimethoxyphenyl	NH	80	72	0.090
2	4-Methoxyphenylamino	3,5-Dimethoxyphenyl	NH	73	68	0.083
3	Phenylamino	3,5-Dimethoxyphenyl	NH	5	22	>10
4	2,5-Dimethoxyphenylamino	3,5-Dimethoxyphenyl	NH	33	20	>10
5	2,4-Dimethoxyphenylamino	3,5-Dimethoxyphenyl	NH	47	17	NT
6	3,5-Dimethoxyphenylamino	3,5-Dimethoxyphenyl	NH	45	NT	NT
7	4-*N*,*N*-Dimethylamino-phenylamino	3,5-Dimethoxyphenyl	NH	35	NT	NT
8	3,5-Dichlorophenylamino	3,5-Dimethoxyphenyl	NH	44	NT	NT
9	4-Cyanophenylamino	3,5-Dimethoxyphenyl	NH	34	NT	NT
10	Benzo[1,3]dioxol-5-ylamino	3,5-Dimethoxyphenyl	NH	87	85	0.062
11	2,3-Dihydro-benzo[1,4]dioxin-6-ylamino	3,5-Dimethoxyphenyl	NH	95	83	0.117
12	3-Methoxy-benzylamino	3,5-Dimethoxyphenyl	NH	100	71	0.12
13	4-Methoxy-benzylamino	3,5-Dimethoxyphenyl	NH	88	22	NT
14	(Tetrahydro-furan-2-yl)-methylamino	3,5-Dimethoxyphenyl	NH	97	36	NT
15	3-Methoxyphenyl	3,5-Dimethoxyphenyl	NH	15	NT	NT
16	3-Methoxybenzyl	3,5-Dimethoxyphenyl	NH	9	NT	NT
17	3-Methoxyphenylamino	3-Methoxyphenyl	NH	9	NT	NT
18	3-Methoxyphenylamino	Phenyl	NH	4	NT	NT
19	3-Methoxyphenylamino	3,5-Difluorophenyl	NH	0	NT	NT
20	Benzo[1,3]dioxol-5-ylamino	3,5-Difluorophenyl	NH	28	NT	NT
21	2,3-Dihydro-benzo[1,4]dioxin-6-ylamino	3-(2-Oxo-pyrrolidin-1-yl)-propyl	NH	21	NT	NT
22	2,3-Dihydro-benzo[1,4]dioxin-6-ylamino	2-(Pyridin-3-yl)ethyl	NH	23	NT	NT
23	Benzo[1,3]dioxol-5-ylamino	Pyridin-4-yl-methyl	NH	84	70	0.066
24	Benzo[1,3]dioxol-5-ylamino	3,4-Difluorobenzyl	NH	84	23	NT
25	2,3-Dihydro-benzo[1,4]dioxin-6-ylamino	3,4-Difluorobenzyl	NH	84	32	NT
26	2,3-Dihydro-benzo[1,4]dioxin-6-ylamino	4-Methoxybenzyl	NH	68	30	NT
27	3-Methoxyphenylamino	3,5-Dimethoxyphenyl	O	100 (100[d])	83	0.021
28	Benzo[1,3]dioxol-5-ylamino	3,5-Dimethoxyphenyl	O	98 (91[d])	83	0.0072
29	3-Hydroxyphenylamino	3,5-Dimethoxyphenyl	O	100 (83[d])	86	0.010
30	2,3-Dihydro-benzo[1,4]dioxin-6-ylamino	3,5-Dimethoxyphenyl	O	100 (88[d])	89	0.0036
31	3,5-Dimethoxyphenylamino	3,5-Dimethoxyphenyl	O	99 (73[d])	87	0.023
32	4-Dimethylamino-phenylamino	3,5-Dimethoxyphenyl	O	98 (76[d])	86	0.031
33	4-Cyanophenylamino	3,5-Dimethoxyphenyl	O	97 (57[d])	27	NT
34	3,5-Difluorophenylamino	3,5-Dimethoxyphenyl	O	87 (51[d])	27	NT
35	Pyridine-3-amino	3,5-Dimethoxyphenyl	O	49[d]	61	0.066
36	3-Methoxy-benzylamino	3,5-Dimethoxyphenyl	O	100	32	NT
37	4-Methoxy-benzylamino	3,5-Dimethoxyphenyl	O	86	28	NT
38	Piperonylamino	3,5-Dimethoxyphenyl	O	90[d]	24	NT
39	Pyridin-4-yl-methylamino	3,5-Dimethoxyphenyl	O	68	44	NT
40	(3-Methoxy-phenyl)-methyl-amino	3,5-Dimethoxyphenyl	O	5[d]	NT	NT
41	(3-Methoxy-benzyl)-methyl-amino	3,5-Dimethoxyphenyl	O	28[d]	NT	NT
42	4-Methoxy-benzamido	3,5-Dimethoxyphenyl	O	8[d]	NT	NT
43	Benzo[1,3]dioxol-5-ylamino	3,5-Difluorophenyl	O	97[d]	23	NT
44	Benzo[1,3]dioxol-5-ylamino	3,5-Dimethylphenyl	O	52[d]	83	0.010
45	Benzo[1,3]dioxol-5-ylamino	3-Hydroxy-5-methoxyphenyl	O	80[d]	83	0.012
46	Benzo[1,3]dioxol-5-ylamino	3,4,5-Trimethoxyphenyl	O	89[d]	86	0.016
47	Benzo[1,3]dioxol-5-ylamino	3-Dimethylamino-phenyl	O	53[d]	63	0.011
48	Benzo[1,3]dioxol-5-ylamino	Pyridin-3-yl	O	43[d]	21	NT
49	Benzo[1,3]dioxol-5-ylamino	3-Fluorophenyl	O	43[d]	37	NT

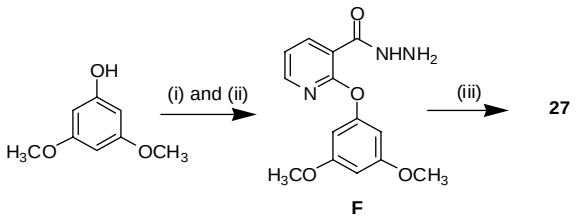
NT, not tested.

[a] % ITP = percent inhibition of tubulin polymerization; 30 μM tubulin and 10 μM compounds were used. Measured as percent inhibition of tubulin assembly compared to that of DMSO control. Average of three experiments with SD < 15%. Compounds that showed >55% inhibition were tested in the G2/M block assay.

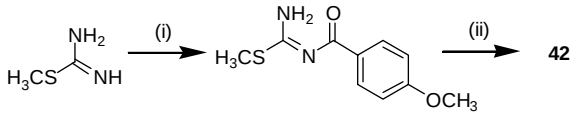
[b] Induction of cell cycle arrest, expressed as percent of cells in the G2/M phase. A431 cells were treated with 0.1 μM compound for 24 h and then analyzed by flow cytometry. Average of two experiments with SD < 10%. Only compounds that showed G2/M cell arrest in single point measurements were tested for EC_{50}s.

[c] Dose–response induced by cell cycle arrest in the G2/M phase, expressed as the compound concentration that causes 50% cells to arrest. Average of three experiments with SD < 10%.

[d] 3.33 μM compounds were used in the assay. Average of three experiments with SD < 10%. At this concentration, only compounds with 40% inhibition or higher were tested in the G2/M block assay.



Scheme 2. Reagents and conditions: (i) Ethyl-2-chloronicotinate, K_2CO_3, DMF, 100 °C, 18 h; (ii) hydrazine monohydrate, *i*-PrOH, 70 °C, 18 h; (iii) **E** from Scheme 1, pyridine, sealed tube, 140 °C, 4 h.

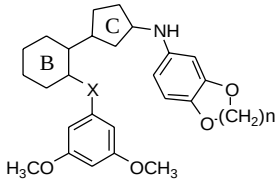


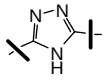
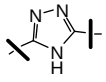
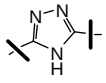
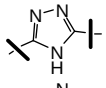
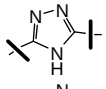
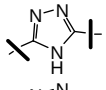
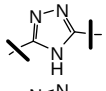
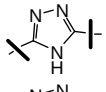
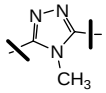
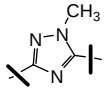
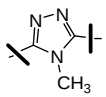
Scheme 3. Reagents and conditions: (i) 4-methoxybenzoyl chloride, TEA, pyridine, 25 °C, 20 min; (ii) **F** from Scheme 2, 125 °C, 12 h.

areas of the molecule. In contrast, results of replacing anilines with alkylamines were disappointing. Compounds **12**–**14** displayed excellent activities in inhibiting tubulin polymerization in vitro; however, only **12** showed good cellular activity. Efforts to remove the NH linker or replace it with a methylene group in the R^1 position resulted in a loss of activity. For example, compounds **15** and **16**, synthesized by condensing hydrazide **C** (Scheme 1) with 3-methoxy-benzamidine or 2-(3-methoxy-phenyl)-acetamidine respectively, were not active. In conclusion, two R^1 moieties, benzo[1,3]dioxol-5-ylamino and 2,3-dihydro-benzo[1,4]dioxin-6-ylamino, as well as *meta* and *para* mono methoxyphenylamino groups gave us the best cellular activity.

While exploring structural requirements at R^2, we found that a 3,5-dimethoxyphenyl moiety was necessary to maintain activity (**17**–**20**). Alkyl groups at R^2 (**21**–**26**)

Table 3. Effects of B and C rings on tubulin activity[a]



Compound	B	C	X	*n*	% ITP	% G2/M block	EC_{50} (μM)
50			O	2	96[c]	86	0.013
51			O	2	69[c]	83	0.044
52			O	2	65[c]	82	0.0066
53			O	2	59[c]	40	0.21
54			NH	1	89[b]	85	0.017
55			NH	1	100[b]	84	0.0094
56			NH	2	100[b]	77	0.0053
57			NH	2	100[b]	68	0.053
58			NH	2	64[c]	25	0.24
59			NH	2	76[b]	26	0.13
60			O	2	30[c]	NT	NT

[a] See Table 2 footnotes for the assay description.
[b] 10 μM compound concentration was used in the polymerization assay.
[c] 3.33 μM compound concentration was used in the polymerization assay.

had little activity, with only benzylamines (**23**–**26**) showing marginal effects in arresting cells in the G2/M phase of the cell cycle. We implemented several strategies for aniline removal from the lower portion of the molecule. Replacing NH with an oxygen atom was the most successful (X = O, Table 2). The biaryl ether analogs were synthesized by replacing aniline **A** with corresponding phenols in a modification of Scheme 1. In step (i), K_2CO_3 was introduced at lower temperatures, in a variation on Scheme 1. Step (ii) was also carried out at reduced temperature to minimize the cleavage of the biaryl ether linkage by hydrazine (Scheme 2). Whilst keeping R^2 constant as a 3,5-dimethoxyphenyl group, compounds **27**–**35**, bearing various aromatic amines at R^1, displayed similar or improved functional activity compared to those of corresponding analogs in Table 2. However, only the compounds with electron-rich aniline or phenol groups at R^2 caused cells to arrest. A similar result was observed for the alkylamines at R^1 where compounds **36**–**39** had no cellular activity. The aniline NH at R^1 appears to be critical for activity, since methylene (**40**, **41**) or acyl (**42**) analogs showed no activity. Compound **42** was prepared according to Scheme 3. In summary, for the SAR at region R^2, only electron-rich substituents provided good functional and cellular activities, as demonstrated by compounds **43**–**49**.

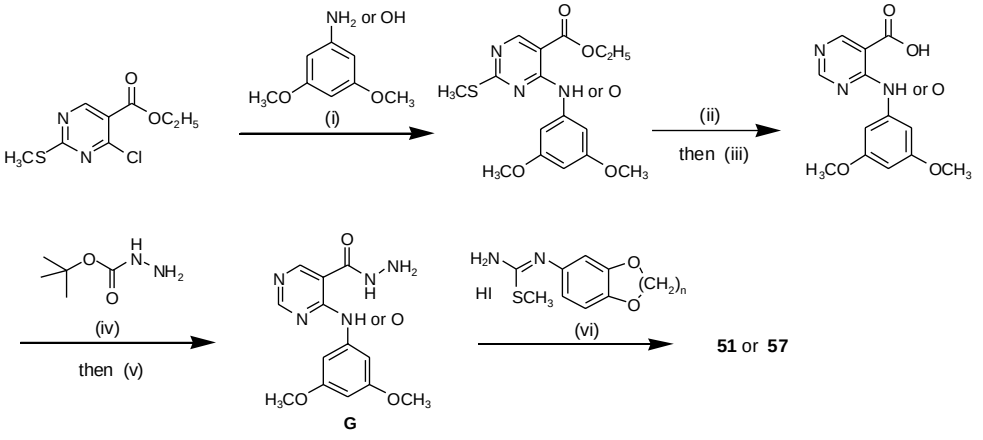
We also investigated the two central ring moieties connecting R^1 and R^2 (Table 3). We demonstrated that the 2,3-disubstituted pyridine at ring B was not required for activity, while a regioisomeric pyridine, pyrimidine, pyrazine or even a phenyl ring was equal to or more effective than the corresponding 2,3-disubstituted pyridine analogs (**50**–**53** compared to **30**; **54** and **55** compared to **10**; **56** and **57** compared to **11**). Pyrimidine containing analogs **51** and **57** were produced according to the general outline in Scheme 4.

Preparation of compounds **50** and **53**–**56** was achieved using procedures similar to that described in Scheme 1 where the corresponding commercially available ring B chloro derivatives were used instead of ethyl-2-chloronicotinate. Compound **52** was synthesized using an alternative procedure depicted in Scheme 5.

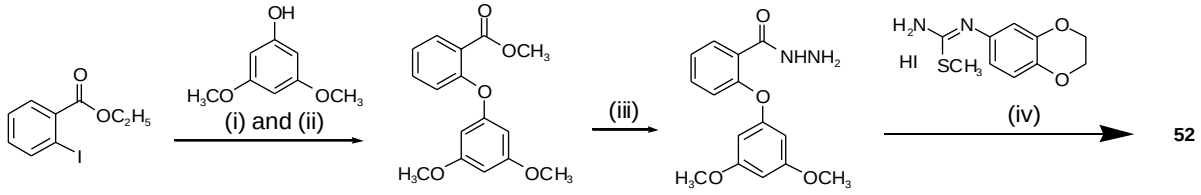
Methyl substitution at two different positions of the triazole ring (**58**–**60**) reduced activity, indicating that either NH groups are critical for binding or steric hindrance plays a role in this region. The synthesis of compounds **58** and **60** is shown in Scheme 6.

N-Methyltriazole analog **59** was prepared by a slightly modified procedure of Scheme 1 where hydrazine is substituted by methylhydrazine.

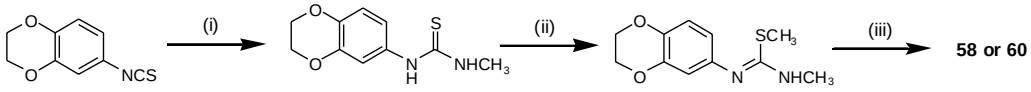
Given that most of our potent compounds possessed structural motifs that mimic colchicine and CA4 (i.e., multiple methoxy groups, Fig. 1), we predicted they would bind to the same tubulin site. Molecular modeling suggested that the binding mode of compound **10** is similar to that of podophyllotoxin, another potent colchicine site binder.[12] Superposition of the compounds suggests that the methoxyphenyl and benzodioxole groups share a similar pharmacophore (Fig. 3). To test our hypothesis, we evaluated these compounds in a colchicine competition binding



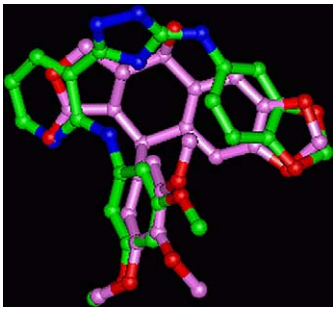
Scheme 4. Reagents and conditions: (i) DMF, K_2CO_3 if phenol starting material, 105 °C; (ii) Raney-Ni, THF, 50 °C; (iii) 2N LiOH, THF; (iv) DCC, HOBt, DCM, 25 °C; (v) TFA, DCM, 0 °C; (vi) pyridine, sealed tube, 140 °C.

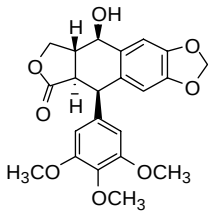


Scheme 5. Reagents and conditions: (i) CuI, Cu, K_2CO_3, water, ultrasound, 40 min, 25 °C; (ii) 1% H_2SO_4 in MeOH, 50 °C, 4 h; (iii) $N_2H_4.H_2O$, *i*-PrOH, 8 h, 120 °C; (iv) pyridine, TEA, 4 h, 140 °C.



Scheme 6. Reagents and conditions: (i) CH_3NH_2, EtOH, 25 °C, 3 h; (ii) CH_3I, acetone, 65 °C, 4 h; (iii) **G** from Scheme 4, pyridine, microwave, 190 °C, 1 h.





Podophyllotoxin

Figure 3. Superposition of the suggested binding mode of **10** (green) and crystal structure of podophyllotoxin (pink) when bound to tubulin.

assay.[13] Indeed, the majority of potent compounds inhibited [^{3}H]colchicine binding to tubulin. Table 4 summarizes the dose dependent results of colchicine competitive binding, and G2/M arrest of cells for selected compounds and standards. Representative triazole derivatives were also evaluated in cytotoxicity assays with NIH 3T3 fibroblasts and showed reduced toxicity compared to tumor cell lines (GI_{50} 10–100× higher, data not shown). The most potent compounds are highly active in all assays, comparable to CA4 and colchinol, the active entities of the VDA prodrugs CA4P and ZD6126, respectively. To evaluate the potential of the triazole derivatives as drug candidates, we plan to investigate their effects in vivo, and the results will be reported in due course.

In conclusion, a series of triazole derivatives has been discovered as a novel class of tubulin polymerization inhibitors that bind to the colchicine site on tubulin.[14,15] Structure–activity relationships have been established for this class of compounds in terms of their capacity to inhibit tubulin polymerization in vitro and cause G2/M arrest in tumor cells. Selected compounds showed biochemical characteristics similar to those of *Combretastatin A4* and colchinol, and are currently being investigated in various in vivo tumor models.

Table 4. K_i for selected compounds and standards

Compound	K_i[a] (μM)	EC_{50}[b] (μM)
CA4	0.03 ± 0.02	0.0035 ± 0.002
Colchinol	1.85 ± 1.68	0.0435 ± 0.033
1	0.10 ± 0.06	0.090 ± 0.035
10	0.06 ± 0.00	0.062 ± 0.015
11	0.16 ± 0.13	0.117 ± 0.007
27	0.05 ± 0.01	0.021 ± 0.012
28	0.01 ± 0.01	0.0072 ± 0.004
30	0.02 ± 0.01	0.0036 ± 0.008

[a] Colchicine competition binding assay, with tubulin at 40 nM and [^{3}H]colchicine at 65 nM.

[b] Compound concentration required for 50% of A431 cells to accumulate at the G2/M phase of the cell cycle.

Acknowledgments

We thank Peter Bohlen, Joel Kawakami, and James Tonra for insightful discussions, and Elizabeth Wu for technical support.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.08.056.

References and notes

1. Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253.
2. Hamel, E. *Biochemistry* **1996**, *16*, 207.
3. Li, Q.; Sham, H. L. *Expert Opin. Ther. Patents* **2002**, *12*, 1663.
4. Shan, B.; Medina, J. C.; Santha, E.; Frankmoelle, W. P.; Chou, T. C.; Learned, R. M.; Narbut, M. R.; Stott, D.; Wu, P.; Jaen, J. C.; Rosen, T.; Timmermans, P. B.; Beckmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5686.
5. Li, Q.; Woods, K. W.; Claiborne, A.; Gwaltney, S. L.; Barr, K. J.; Liu, G.; Gehrke, L.; Credo, R. B.; Hui, Y. H.; Lee, J.; Warner, R. B.; Kovar, P.; Nukkala, M. A.; Zielinski, N. A.; Tahir, S. K.; Fitzgerald, M.; Kim, K. H.; Marsh, K.; Frost, D.; Ng, S.-C.; Rosenberg, S. H.; Sham, H. L. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 465.
6. Szczepankiewicz, B. G.; Liu, G.; Jae, H.-S.; Tasker, A. S.; Gunawardana, I. R.; von Geldern, T. W.; Gwaltney, S. L.; Wu-Wong, J. R.; Gehrke, L.; Chiou, W. J.; Credo, R. B.; Alder, J. D.; Nukkala, M. A.; Zielinski, N. A.; Jarvis, K.; Mollinson, K. W.; Frost, D.; Bauch, J. L.; Hui, Y. H.; Clairborne, A. K.; Li, Q.; Rosenberg, S. H. *J. Med. Chem.* **2001**, *44*, 4416.
7. Tozer, G. M.; Kanthou, C.; Parkins, C. S.; Hill, S. A. *Intl. J. Exp. Pathol.* **2003**, *83*, 21.
8. Thorpe, P. E. *Clin. Cancer Res.* **2004**, *10*, 415.
9. Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, *57*, 1829.
10. Davis, P. D.; Hill, S. A.; Chaplin, D. J.; Dougherty, G. J. *Clin. Cancer Res.* **2000**, *S6*, 282.
11. Okajima, N.; Okada, Y. *J. Heterocycl. Chem.* **1991**, *28*, 177.
12. Ravelli, R. B.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. *Nature* **2004**, *428*, 198 (Protein Data Bank Access Number: 1SA1).
13. Tahir, S. K.; Kovar, P.; Rosenberg, S. H.; Ng, S.-C. *BioTechniques* **2000**, *29*, 156.
14. Ouyang, X.; Kiselyov, A.; Chen, X.; He, H.-Y.; Kawakami, J.; Pattaropong, V.; Piatniski, E.; Tuma, M. C.; Kincaid, J. WO 2005004818, 2005.
15. Piatniski, E.; Kiselyov, A.; Hadari, Y.; Doody, J.; Ouyang, X.; Chen, X. WO 2004052280, 2004.