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Oxadiazole derivatives as a novel class of antimitotic agents: Synthesis, inhibition of tubulin polymerization, and activity in tumor cell lines

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Abstract—Oxadiazole derivatives were synthesized and evaluated for their ability to inhibit tubulin polymerization and to cause mitotic arrest in tumor cells. The most potent compounds inhibited tubulin polymerization at concentrations below 1 μM. Lead analogs caused mitotic arrest of A431 human epidermoid cells and cells derived from multi-drug resistant tumors (**10**, EC_{50} = 7.8 nM). Competition for the colchicine binding site and pharmacokinetic properties of selected potent compounds were also investigated and are reported herein, along with structure–activity relationships for this novel series of antimitotic agents.

Antimitotic drugs have been successfully used as chemotherapeutic agents in the treatment of cancer.[1] Most antimitotic drugs target microtubules—dynamic elements of the cell cytoskeleton responsible for the formation of the mitotic spindle, required for proper chromosomal separation during cell division. Agents that affect tubulin polymerization impair microtubule dynamics and consequently arrest cells during mitosis.[2] Evidence that microtubules are a validated cancer target comes mainly from the successful use of natural products such as Taxanes and Vincas as chemotherapeutic agents to clinically treat various tumors. However, the complex synthesis, difficult formulation, lack of oral availability and, more importantly, acquired and intrinsic resistance to Taxol® and Vinca alkaloids render these drugs suboptimum for clinical treatment of cancer.[3] Hence, there is considerable interest in the discovery and development of novel small molecule inhibitors of tubulin polymerization that can circumvent the difficulties seen with natural products.[4]

Many synthetic small molecule tubulin inhibitors have been reported and several are currently in clinical trials.[5] Two small molecule clinical candidates, D-24851 and ABT-751, deserve notice for their unique characteristics (Fig. 1). Indole based D-24851 demonstrated impressive efficacy in a variety of tumor models including multiple-drug resistant (MDR) xenographs.[6] This compound does not bind to any of the characterized binding sites on tubulin nor does it seem to cause neurotoxicity in mice. ABT-751, a pyridine containing sulfonamide, binds to the colchicine site on tubulin and shows anti-vascular effects in addition to its predicted antimitotic activity.[7] Both these compounds are orally bioavailable, an advantage over existing therapies.

We have previously reported a triazole class of tubulin inhibitors with potent antimitotic activity in tumor cells

Keywords: Tubulin polymerization inhibitor; 1,3,4-Oxadiazole; Antimitotic agent; Multiple-drug resistance.
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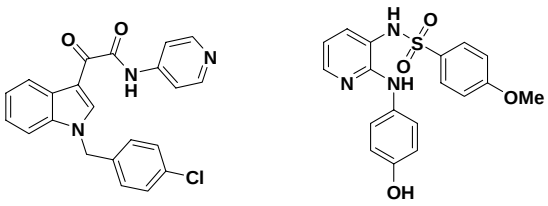


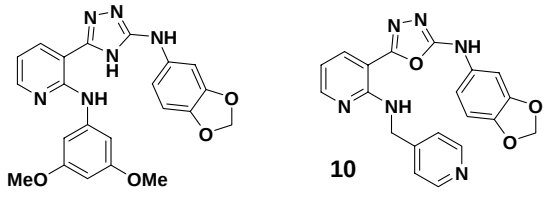


Figure 1. Structures of D-24851, ABT-751, triazole, and oxadiazole.

and have shown that the presence of the electron-rich 3,5-dimethoxyphenyl moiety is necessary for cellular potency (Fig. 1).[8] In an effort to improve the physicochemical properties and pharmacokinetic profile of these compounds, we designed a series of oxadiazoles where the 3,5-dimethoxyaniline group is not required for potent antimitotic activity in cells (i.e., **10** in Fig. 1). The present report describes the synthesis and evaluation of oxadiazoles as a novel class of tubulin inhibitors that are potent and effective against tumor cells including a cell line with MDR. Structure–activity relationships (SARs) and pharmacokinetic studies of this novel tubulin inhibitor class are reported herein.

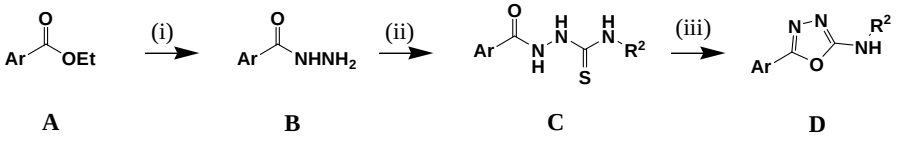
Oxadiazole compounds in this report were synthesized according to Method **I** described by Sunder (Scheme 1).[9] Ester **A** was treated with hydrazine monohydrate to yield hydrazide **B**, which was reacted further with an isothiocyanate to form thiourea intermediate **C**. Finally, **C** was cyclized to produce oxadiazole **D** by heating with DCC. Oxadiazole analogs similar to (**10**) (Fig. 1) were synthesized by the displacement of chlorine atom from 2-chloronicotinic acid ethyl ester by a variety of amines under thermal conditions. As a result, the 2-alkylaminonicotinic acid ethyl ester **E** was generated and then converted via Method **I** to the oxadiazole analogs listed in Table 1 (Scheme 2).

Compounds listed in Table 1 can also be prepared via a very efficient protocol utilizing cyclization of commercially available 2-fluoronicotinic acid with a thiosemicarbazide provided oxadiazole intermediate **F**, which is substituted with an amine of choice to give a final oxadiazole (Scheme 3). Therefore, oxadiazole derivatives could be obtained in two easy steps without purification by column chromatography.

Compounds of the general formula given in Table 1 were evaluated in two assays. Compounds that showed inhibition of tubulin polymerization in vitro were evaluated for inducing cell cycle arrest in A431 human cancer cells (Table 1). To identify an alternative group to 3,5-dimethoxyaniline, we initially kept R^2 constant as either 2,3-dihydro-benzo[1,4]dioxin-6-yl or benzo[1,3]dioxol-5-yl groups (preferred substitutions at R^2 position obtained from the triazole series[8]) and used various alkylamines ($R^1CH_2NH_2$) to determine the effect of R^1 on activity (**1**–**21**, Table 1). This strategy proved to be successful as compounds with a variety of groups at R^1 were active. Aryl groups were the most favorable as R^1 substituents: compounds displayed double digit nanomolar activity in cellular assays when R_1 was alkoxylphenyl (**1**–**3**), fluorophenyl (**4**–**6**), methanesulfonamidophenyl (**7**–**8**), pyridyl (**9**–**14**), pyrazinyl (**15**) or furanyl (**17**) moiety. R^1 alkyl groups generally demonstrated a weaker activity than aromatic analogs. For example, tetrahydrofuran analogs (**19**) and (**20**) had EC_{50}s >150 nM. Compounds with polar groups at R^1 (i.e., **21**) lost functional and cellular activities. Noteworthy, compounds with a 2,3-dihydro-benzo[1,4]dioxin-6-yl group at R^2 are often more potent than closely related analogs with a benzo[1,3]dioxol-5-yl group. Benzodioxin analogs (**5**), (**7**), (**11**), (**13**), (**15**), and (**17**) are at least twice as potent as their corresponding benzodioxole analogs (**6**), (**8**), (**12**), (**14**), (**16**), and (**18**), indicating that a benzodioxin group is the preferred substitution at R^2.

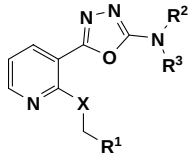
In contrast, our attempts to find a replacement for the benzodioxin or benzodioxole groups at the R^2 position were unsuccessful. While keeping R^1 constant, various substituted phenyl groups at R^2 were evaluated. Only a 4-methoxyphenyl analog (**22**) demonstrated appreciable activity, whereas 3-methoxyphenyl, 4-methylphenyl, 3-methyl, and 4-hydroxyphenyl analogs were inactive, indicating potential hydrogen bond that involved the oxygen atom at the *para* position. Compounds containing substitutions at the R^2 phenyl ring, including electron-donating groups such as 3,4-dimethylamino, amino, and electron-withdrawing groups such as cyano, difluoro, and nitro, had diminished activity in tubulin polymerization assays (see supporting information section). Difluoro-benzodioxole analog (**23**) displayed much weaker activity than the corresponding benzodioxole analog (**14**).

Biaryl ether analogs such as compound (**24**, X = O), synthesized by a modified procedure at step (i) in



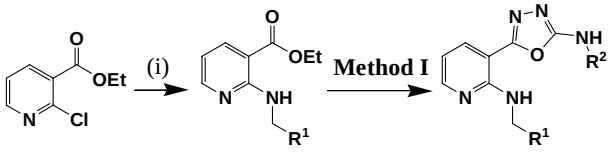
Scheme 1. Method I. Reagents and conditions: (i) hydrazine monohydrate, isopropanol, 120 °C, 2–4 h, 60–90%; (ii) R^2NCS, $CHCl_3$, 30–60 °C, 2–6 h, 80–95% (iii) DCC, toluene, 110 °C, 8–16 h, 60–80%.

Table 1. Effects of various R^1 and R^2 groups on activity



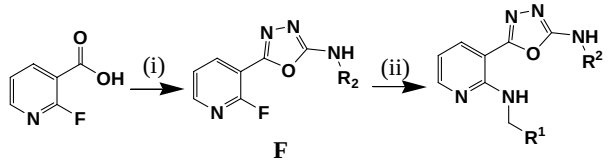
Compound	R^1	R^2	R^3	X	ITP[a] IC_{50} (μM)	G2/M block[b] EC_{50} (μM)
CA4[c]					2.1 ± 0.24	0.0035 ± 0.0008
Taxol					NA	0.009 ± 0.0023
1	2,3-Dihydro-benzo[1,4]dioxin-6-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	1.8 ± 0.3	0.057 ± 0.009
2	Benzo[1,3]dioxol-5-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	0.5 ± 0.1	0.018 ± 0.002
3	4-Methoxyphenyl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	1.6 ± 0.26	0.023 ± 0.002
4	4-Fluorophenyl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	5.0 ± 0.5	0.060 ± 0.012
5	3,4-Difluorophenyl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	0.7 ± 0.1	0.015 ± 0.003
6	3,4-Difluorophenyl	Benzo[1,3]dioxol-5-yl	H	NH	2.2 ± 0.4	0.051 ± 0.001
7	4-Methanesulfonamidophenyl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	2.0 ± 0.4	0.023 ± 0.008
8	4-Methanesulfonamidophenyl	Benzo[1,3]dioxol-5-yl	H	NH	3.8 ± 0.6	0.071 ± 0.037
9	Pyridin-4-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	29.9 ± 1.8	0.018 ± 0.0024
10	Pyridin-4-yl	Benzo[1,3]dioxol-5-yl	H	NH	1.0 ± 0.1	0.020 ± 0.003
11	Pyridin-3-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	0.7 ± 0.03	0.013 ± 0.002
12	Pyridin-3-yl	Benzo[1,3]dioxol-5-yl	H	NH	8.8 ± 0.7	0.031 ± 0.001
13	Pyridin-2-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	1.9 ± 0.1	0.016 ± 0.001
14	Pyridin-2-yl	Benzo[1,3]dioxol-5-yl	H	NH	1.7 ± 0.2	0.049 ± 0.011
15	Pyrazin-2-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	2.2 ± 0.4	0.031 ± 0.001
16	Pyrazin-2-yl	Benzo[1,3]dioxol-5-yl	H	NH	9.6 ± 0.44	0.21 ± 0.019
17	Furan-2-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	2.0 ± 0.4	0.045 ± 0.007
18	Furan-2-yl	Benzo[1,3]dioxol-5-yl	H	NH	4.9 ± 0.03	0.13 ± 0.027
19	Tetrahydro-furan-2-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	1.7 ± 0.25	0.16 ± 0.003
20	Tetrahydro-furan-2-yl	Benzo[1,3]dioxol-5-yl	H	NH	15.5 ± 2.0	0.33 ± 0.052
21	1-Methyl-pyrrolidin-2-ylamine	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	NH	>30	>10
22	Pyridin-2-yl	4-Methoxyphenyl	H	NH	1.5 ± 0.1	0.020 ± 0.004
23	Pyridin-2-yl	2,2-Difluoro-benzo[1,3]dioxol-5-yl	H	NH	13.3 ± 1.9	0.78 ± 0.19
24	Pyridin-2-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	H	O	18.1 ± 0.08	0.26 ± 0.024
25	Pyridin-2-yl	2,3-Dihydro-benzo[1,4]dioxin-6-yl	CH_3	NH	>30	NT

NA, not applicable; NT, not tested.
[a] Compound concentration required for 50% inhibition of maximum tubulin assembly.
[b] Compound concentration required for 50% of A431 cells to accumulate at the G2/M phase of the cell cycle.
[c] CA4 = Combretastatin A4.



E

Scheme 2. Reagents and conditions: (i) $R^1CH_2NH_2$, isopropanol, 80–100 °C, 2–10 h, 40–90%.



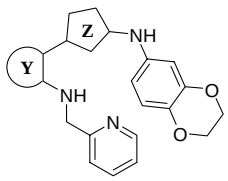
F

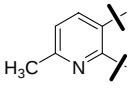
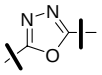
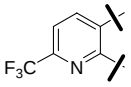
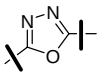
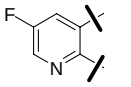
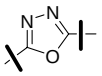
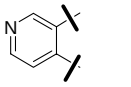
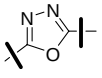
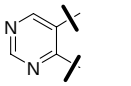
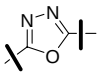
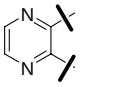
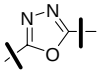
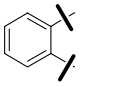
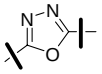
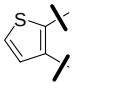
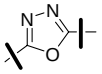
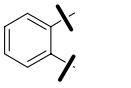
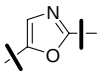
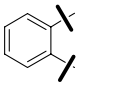
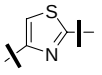
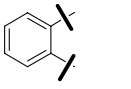
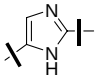
Scheme 3. Reagents and conditions: (i) $R^2NHC(S)NHNH_2$, EDCI, DCM, 25 °C, 12 h, 64–78%; (ii) $R^1CH_2NH_2$, isopropanol, sealed tube, 100 °C, 8–24 h, 89–94%.

Scheme 1,[10] were less active than their amine analogs (**13**). The importance of the NH functionality between the oxadiazole ring and the R^2 group was demonstrated by alkylation of (**13**) where the presence of a methyl group at R^3 abolished its activity (**25**).

Table 2 lists our SAR investigation on the two central ring moieties connecting R^1 and R^2. First, we synthesized pyridine, pyrimidine, and pyrazine analogs (**26**–**31**), using previously reported procedures similar to that described in Scheme 1 where the corresponding commercially available ring chloro derivatives were used instead of ethyl-2-chloro-nicotinate **E**. We demonstrated that only small substituents such as methyl or fluoro groups on pyridine ring Y were tolerated (**26**, **28**), while bulkier groups such as trifluoromethyl (**37**) lowered compound activity, presumably, due to steric limitations around the Y ring. The inactive regioisomeric pyridine analog (**29**) and pyrimidine analog (**30**) showed that a basic nitrogen *para* to the NH is not desirable, while nitrogen atoms *ortho* and *meta* to NH are allowed, as demonstrated by the potent pyrazine analog (**31**). When

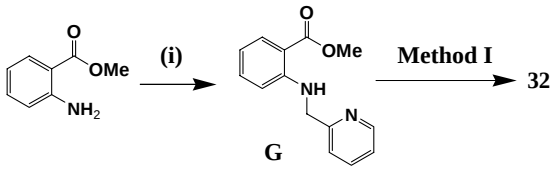
Table 2. Effect of central rings Y and Z on activity[a]



Compound	Y	Z	ITP (μM)	G2/M block (μM)
26			2.4 ± 0.47	0.099 ± 0.012
27			>30	NT
28			3.4 ± 0.41	0.082 ± 0.010
29			>30	NT
30			>30	NT
31			2.0 ± 0.2	0.075 ± 0.013
32			1.2 ± 0.2	0.014 ± 0.002
33			4.4 ± 0.3	0.022 ± 0.001
34			1.4 ± 0.15	0.035 ± 0.003
35			>30	NT
36			>30	NT

[a] See Table 1 for footnotes for the assay description.

pyridine ring Y was replaced by a phenyl ring, corresponding compounds had similar or enhanced potency, as exemplified by compound (**32**). Compound (**32**) was synthesized using conditions listed in Scheme 4: 2-amino-benzoic acid methyl ester was alkylated to give ester intermediate **G**, which was then converted to its final form (**32**) following Method **I**. Using a similar methodology, analog (**33**) with a thiophene Y ring was synthesized and demonstrated good cellular potency. Other compounds with alternative Y rings such as imidazole, pyrazole, and isothiazole analogs were less potent or



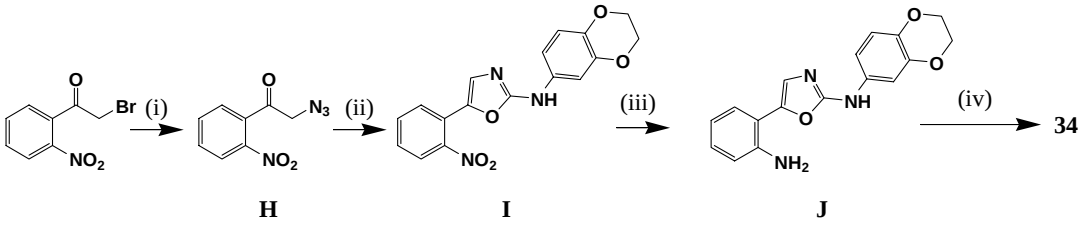
Scheme 4. Reagents and conditions: (i) pyridine-2-carboxaldehyde, AcOH, toluene, 100 °C, 12 h, then $NaBH(OAc)_3$, 12 h, 85%. Method I, see Scheme 1 (R^2NCS = 6-isothiocyanato-2,3-dihydro-benzo[1,4]-dioxine).

inactive (synthetic schemes are not shown, see supporting information section).

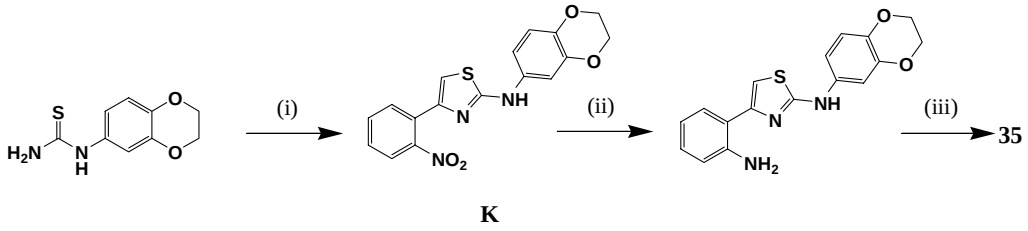
Making use of synthetic accessibility and keeping the Y ring as a phenyl group, we were able to explore some modifications of the oxadiazole Z ring with other five-membered heterocycles. 1,3-Oxazole analog (**34**) was the most successful replacement, while imidazole (**36**) and thiazole (**35**) derivatives were inactive. Oxazole analog (**34**) was prepared according to Scheme 5. Commercially available 2-bromo-1-(2-nitro-phenyl)-ethanone was first converted to an azido intermediate **H**, which was reacted with an isothiocyanate in the presence of PPh_3 to give rise to the 1,3-oxazole intermediate **I**.[11] The nitro group of **I** was reduced to amino compound **J**, which was further reductively alkylated by an aldehyde to provide analog (**34**). The thiazole analog (**35**) was prepared as described in Scheme 6. 2-Bromo-1-(2-nitro-phenyl)-ethanone was reacted with thiourea to form the nitro-thiazole intermediate **K** that was converted to final form (**35**) as shown in Scheme 5 for intermediate **I**. The imidazole compound (**36**) was analogous to the thiazole analog (**35**), except for a protection step needed for the transformation (Scheme 7).

Once SAR trends were established, we proceeded with further evaluation of our most active compounds. In the NCI/ADR breast cancer MDR positive cell line that is resistant to Taxol®, selected compounds were assayed for their antimitotic activity. Most of the compounds tested were shown to be highly active in arresting MDR cells with EC_{50}s as low as 7.8 nM. Taxol® was completely inactive in this assay (Table 3). The binding site of our compounds on tubulin was also determined. When these compounds are tested in a colchicine competition binding assay,[12] representative analogs competed for the colchicine binding site on tubulin with an IC_{50} = 0.01–0.4 μM (Table 3).

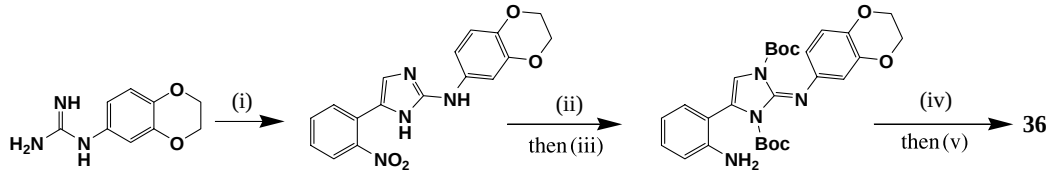
Pharmacokinetic properties of several analogs were evaluated (Table 4). When dosed ip, selected compounds showed good in vivo exposure with AUC values ranging from 6 to 100 μM h. Compounds (**10**), (**13**), and (**33**) achieved significant AUC values (30–100 μM h). Such an exposure suggests that even at moderate doses, efficacious compound concentrations are likely to be achieved in vivo. It is noteworthy that compounds of the oxadiazole series reported here have longer half-lives than previously reported triazoles[8] (data not shown). In addition, some oxadiazoles (i.e., **13**) demonstrated oral exposure (AUC = 8.8 μM h). The desirable pharmacokinetic



Scheme 5. Reagents and conditions: (i) NaN_3, acetone, H_2O, 50 °C, 0.5 h, 88%; (ii) PPh_3, 6-isothiocyanato-2,3-dihydro-benzo[1,4]dioxine, dioxane, 95 °C, 0.4 h, 15%; (iii) H_2, Pd/C, MeOH, 50 °C, 4 h, 87%; (iv) pyridine-2-carboxaldehyde, $NaBH(OAc)_3$, AcOH, toluene, 70 °C, 22 h, 66%.



Scheme 6. Reagents and conditions: (i) 2-Bromo-1-(2-nitro-phenyl)-ethanone, TEA, THF, −78–25 °C, 12 h, 81%; (ii) H_2, Pd/C, EtOH, 60 °C, 4 h, 83%; (iii) pyridine-2-carboxaldehyde, $NaBH(OAc)_3$, AcOH, DCE, 25 °C, 16 h, 49%.



Scheme 7. Reagents and conditions: (i) 2-Bromo-1-(2-nitro-phenyl)-ethanone, DBU, CH_3CN, 50 °C, 18 h, 25%; (ii) $(Boc)_2O$, DMAP, 25 °C, 24 h, 93%; (iii) H_2, Pd/C, EtOH, 40 °C, 4 h, 97%; (iv) pyridine-2-carboxaldehyde, $NaBH(OAc)_3$, AcOH, toluene, 60 °C, 6 h, 83%; (v) TFA, DCM, 25 °C, 1 h, 93%.

Table 3. In vitro data for selected compounds and standards

Compound	EC_{50} (μM) MDR cells[a]	IC_{50} (μM) colchicine binding[b]
Taxol	>10	NA
Combretastatin A4	0.0015 ± 0.0003	0.03 ± 0.02
10	0.0078 ± 0.0019	0.03 ± 0.005
11	0.019 ± 0.003	0.03 ± 0.001
13	0.027 ± 0.003	0.19 ± 0.04
14	0.256 ± 0.0002	0.03 ± 0.0058
19	0.128 ± 0.028	0.39 ± 0.06
32	0.037 ± 0.009	0.39 ± 0.06
33	0.046 ± 0.0012	0.075 ± 0.004
34	0.103 ± 0.017	0.01 ± 0.001

[a] Compound concentration required for 50% of the NCI/ADR cells (MDR) to accumulate at the G2/M phase of the cell cycle.
[b] Colchicine competition binding assay, with tubulin at 40 nM and [^{3}H]colchicine at 65 nM.

Table 4. Pharmacokinetic properties for selected compounds in mice[a]

Compound	C_{max} (μM)	$T_{1/2}$ (h)	AUC (0–4 h) (μM*h)
Triazole	7.3	0.62	13.6
Triazole[b]	BLD[c]	NA	NA
10	15.1	2.6	52.6
11	6.5	0.8	6.7
13	12.5	2.7	31.2
13[b]	3.8	2.2	8.8
14	4.9	4.1	21.5
19	16.4	0.78	26.3
32	6.4	1.5	17.2
33	59.2	0.75	102
34	4.6	2.43	14.4

[a] IP dosing at 60 mg/kg in DMSO/cremophore.
[b] PO dosing at 60 mg/kg in ethanol/Tween 80/PEG400.
[c] BLD, below the level of detection.

profile for this series strongly supports further evaluation of oxadiazoles in mouse tumor models. Efficacy studies in mouse xenograft models are currently in progress and will be reported in due course.

In conclusion, a series of oxadiazole derivatives has been discovered as a novel class of tubulin polymerization inhibitors that bind to the colchicine site on tubulin.[13] Structure–activity relationships have been established for this class of compounds in terms of inhibition of tubulin polymerization in vitro and mitotic arrest of tumor cells. Selected compounds displayed nanomolar potency against tumor cells including those displaying a MDR phenotype. Compounds from this series demonstrated substantial plasma levels in mice via both oral and intraperitoneal administration. In vivo evaluation of the oxadiazole series as antimitotic agents is in progress.

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.11.094.

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