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Synthesis and Biological Evaluation of 2-Indolyloxazolines as a New Class of Tubulin Polymerization Inhibitors. Discovery of A-289099 as an Orally Active Antitumor Agent

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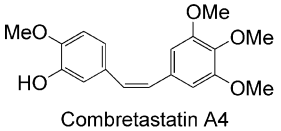
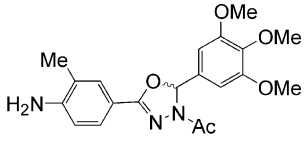
Abstract—A series of indole containing oxazolines has been discovered as a result of structural modifications of the lead compound A-105972. The compounds exert their anticancer activity through inhibition of tubulin polymerization by binding at the colchicine site. A-289099 was identified as an orally active antimitotic agent active against various cancer cell lines including those that express the MDR phenotype. The anticancer activity, pharmacokinetics, and an efficient and enantioselective synthesis of A-289099 are described.

Tubulin binding agents have generated considerable interest among cytotoxic agents, due in part to the success of the taxanes in clinical oncology.[1,2] However, emerging resistance to antimitotic agents such as paclitaxel has limited their ultimate effectiveness.[3] Renewed interest has been generated by the hope that non-MDR substrates that interact with tubulin at sites different from those of vinca alkaloids and taxanes can be discovered.[4] Recently, compounds which bind to tubulin at the colchicine binding site, as represented by combretastatin A4, demonstrate promising activity against various cancer cell lines, including those that express the MDR phenotype.[1,5] The relatively simple structures of these compounds may provide the added advantage of being orally active.



Combretastatin A4



A-105972

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We recently identified the oxadiazoline A-105972 as a colchicine site binder with an IC_{50} of 3.4 μM against tubulin polymerization.[6] As a part of our continuing efforts to identify more stable and orally active compounds, we report here our investigation of indole-based oxazolines, which led to the discovery of A-289099 (**5b-*S***) as a potent and orally active antimitotic agent.[7]

The racemic oxazolines (**5**) were synthesized by coupling of the amino alcohol **1**[7] with either the acids (**2**) in a two-step transformation or the nitriles (**3**) in a one-step reaction as shown in Scheme 1. The required acids (**2**) or nitrile (**3n**) were prepared from known starting materials[8–10] as indicated in Scheme 2.

Synthesis of the pure enantiomers of **5b** proved to be more challenging. Literature methods for the synthesis of optically active 5-aryl oxazolines are limited. We eventually developed the enantioselective oxazoline synthesis shown in Scheme 3. Thus, styrene **14** was enantioselectively dihydroxylated according to the procedure of Ramacciotti et al.[11] using AD-mix-α to give *S*-diol **15**. Monotosylation of **15** followed by nucleophilic displacement of the tosylate with sodium azide provided **16**. Reduction of azide **16** under hydro-

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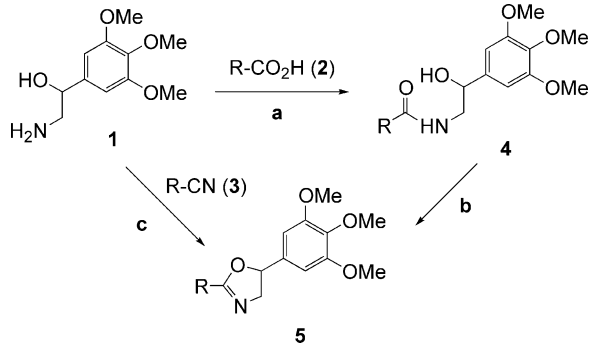
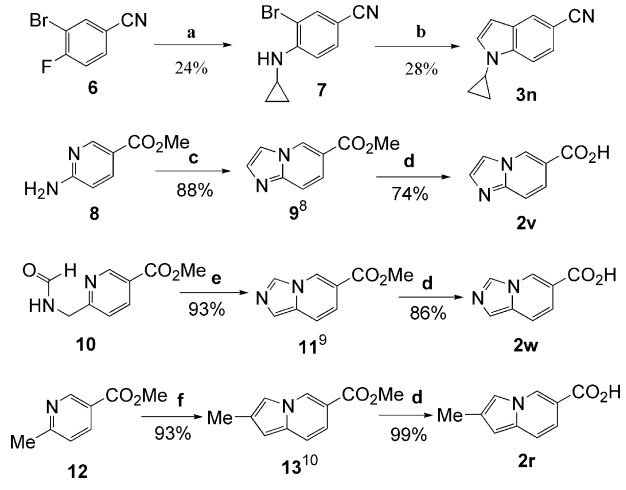
gen in the presence of catalytic amount of 10% Pd/C provided the amino alcohol **17** (95%). Cyclization of the amide of **17** (**4**) using Burgess reagent resulted in partial racemization. Reaction of **17** with methyl indole-5-carboximidate failed to give the desired product. No coupling product could be isolated by condensation of azide **16** with indole-5-carboxaldehyde. Finally, **17** was successfully condensed with cyanoindole **18** to yield the optically pure oxazoline **5b-*S*** in 68% yield and >99% ee.[12] The same reaction sequence was repeated to give the *R*-isomer (**5b-*R***)[13] by replacing AD-mix-α with AD-mix-β. The method described here should be of wide applicability to other 5-substituted chiral oxazolines.

Compounds were evaluated for their antiproliferative activity against the human lung carcinoma cell line NCI-H460 and the MDR positive human colon adenocarcinoma cell line HCT-15.[6a] The most promising analogues were then screened for their oral bioavailability in rat.[14] The 5-indolyl analogue (**5b**) was the most potent among the six regio isomers (Tables 1 and 2). It also possesses good oral bioavailability of 35%.



Scheme 1. (a) EDCI, THF, rt, overnight; (b) Burgess reagent, THF, heat, 2 h; (c) K_2CO_3, ethylene glycol/glycerol (2:1), 140 °C, 40 h.



Scheme 2. (a) Cyclopropylamine, MeOH, 60 °C, 20 h; (b) ethyl ethynyl ether, catecholborane, hexanes, rt, 2 h, reflux, 2 h; then **7**, $Pd(dppf)_2Cl_2$, CsF, THF, reflux, 42 h, H_3O^+; (c) bromoacetaldehyde acetal, HCl, water, 90 °C, 1 h, then **8**, $NaHCO_3$, 60 °C, 1 h; (d) LiOH, THF/H_2O (3:1), rt, 30 min; (e) $POCl_3$, CH_2Cl_2, reflux, 3 h; (f) (i) bromoacetone, acetone, reflux, overnight; (ii) Na_2CO_3, ethanol, reflux, 3 h.

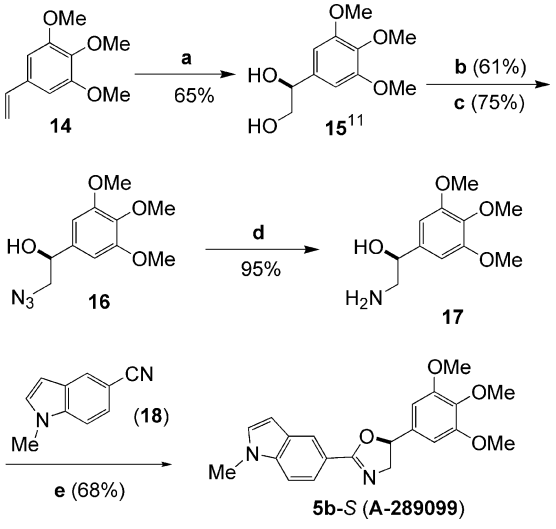
Although *N*-methylation of the indole ring often decreases the observed potency, *N*-methyl analogue **5a** is 10-fold more active than desmethyl compound **5b**. However, *N*-substitution on the 5-indolyl group with substituents larger than methyl resulted in a significant loss of activity (Table 2). The *N*-cyclopropyl analogue (**5n**), which is less metabolically labile, shows improved oral bioavailability (56%).

All attempts to replace the indolyl group of **5b** failed (Table 3), with the closest one being the electron rich indolizine (**5r**), which is only 3-fold less active than the similar indole analogue **5p**.

The two enantiomers of the overall best compound **5b** were evaluated for their antiproliferative activity and pharmacokinetic properties. The *S*-isomer (**5b-*S*,** A-289099) was found to be significantly more potent than the *R*-isomer (**5b-*R***) (Table 4). The IC_{50} values of A-289099 against HCT-15 and NCI-H460 are 8.6 and 6.2 nM, respectively, which are comparable to combretastatin A4 and up to 74-fold more potent than the *R*-isomer. A-289099 is also active against a human lung cancer with mutated β-tubulin (A549-T24[15]) with an IC_{50} of 6.3 nM. As a comparison, paclitaxel exhibits an IC_{50} of >10 μM against the same cell line. A-289099 inhibits tubulin polymerization[6a] with an IC_{50} of 2.3 μM, versus 1.6 μM for combretastatin A4. The binding constant[6a] of A-289099 at the colchicine site is comparable to colchicine at 0.63 μM but is 3-fold higher than that of combretastatin A4.

The ability of A-289099 to depolymerize microtubules in cultured cells[6a] is shown in Figure 1. Microtubule depolymerization in human HCT-15 colon carcinoma cells was observed 4 h after treatment with A-289099 at a concentration of 44 nM.

Molecular modeling suggests that the structure of A-289099 is similar to that of combretastatin A4. The tri-



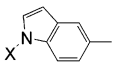
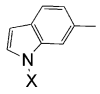
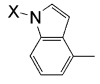
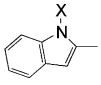
Scheme 3. (a) AD-mix-α, *t*-BuOH/H_2O (1:1), then **14**, 0 °C, 3 h, then rt, overnight; (b) TsCl, Py, 0 °C, 2 h; (c) NaN_3, DMF, 85 °C, 2 h; (d) H_2, 10% Pd/C, rt, overnight; (e) **18**, K_2CO_3, ethylene glycol/glycerol (2:1), 140 °C, 40 h.

methoxylphenyl group is a common pharmacophore. The 2,5-disubstituted oxazoline is a good mimic of the *cis*-double bond. Figure 2 shows the superimposed structures of A-289099 and combretastatin A4.

The pharmacokinetic properties of A-289099 and **5b-*R*** in four different species are summarized in Table 5. Overall, the two enantiomers have similar pharmacokinetic properties with oral bioavailabilities ranging from 6.5 to 44.0%. In mouse and rat, **5b-*R*** displays better oral bioavailabilities than A-289099, but its oral AUCs are lower, especially in mouse, where a 10-fold reduction is observed. The bioavailability of A-289099 in monkey is 18.6%.

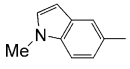
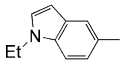
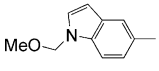
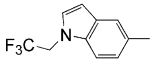
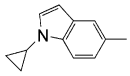
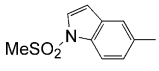
The anticancer activity[6b] of A-289099 was evaluated in the syngeneic M5076 murine ovarian sarcoma flank

Table 1. IC_{50}[6a] (nM) and oral bioavailability[14] of indolyloxazolines (**5**)[a]

Compd	R	X	HCT-15[b]	NCI-H460[c]	F (%)[d]
5a		H	200	100	38.1
5b		Me	16	14	34.7
5c		H	120	330	10.5
5d		Me	280	420	0
5e		H	540	470	0
5f		Me	15,000	150,000	—
5g		H	61	57	0
5f		Me	37	53	0
5g		H	67	82	0
5h		Me	37	87	2.5
5i		H	150	350	40.0
5j		Me	660	1500	—

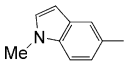
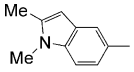
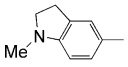
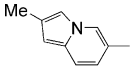
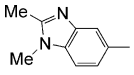
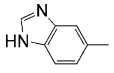
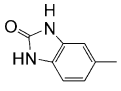
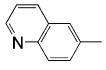
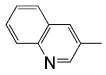
[a]The compounds were tested as free bases of racemic mixtures.
[b]An MDR positive human colon adenocarcinoma cell line.
[c]Human non-small cell lung carcinoma cell line.
[d]Oral bioavailability in rat after a single 10 mg/kg dose.

Table 2. IC_{50}[6a] (nM) and oral bioavailability[14] of *N*-substituted 5-indolyloxazolines (**5**)[a]

Compd	R	HCT-15[b]	NCI-H460[c]	F (%)[d]
5b		16	14	34.7
5k		70	49	11.1
5l		480	680	34.3
5m		3800	4200	19.8
5n		2900	2900	55.8
50		16,300	8180	—

[a,b,c,d]See Table 1.

Table 3. IC_{50}[6a] (nM) of the bicyclic aryloxazolines (**5**)[a]

Compd	R	HCT-15[b]	NCI-H460[c]
5b		16	14
5p		170	210
5q		510	810
5r		520	520
5s		1300	1200
5t		6400	7100
5u		1200	> 100,000
5v		40,000	16,000
5w		28,000	100,000
5x		2400	3200
5y		160,000	270,000
5z		44,000	37,000

[a]The compounds were tested as free bases of racemic mixtures.
[b,c]see Table 1.

Table 4. In vitro characterization of the two enantiomers of **5b**[6a]

Compd	K_i[a] (μM)	ITP[b] IC_{50} (μM)	HCT-15[c] IC_{50} (nM)	NCI-H460[d] IC_{50} (nM)
5b-*S* (A-289099)	0.63	2.3	8.6	6.2
5b-*R*	3.69	740	390	460
Combretastatin A4	0.18	1.6	1.7	3.0
Colchicine	0.78	—	140	46
Vinblastine	> 100	1.0	43	3.4

[a]Binding constants at colchicine site of bovine brain tubulin.
[b]Inhibition of bovine brain microtubule polymerization.
[c,d]see Table 1, footnotes b and c.

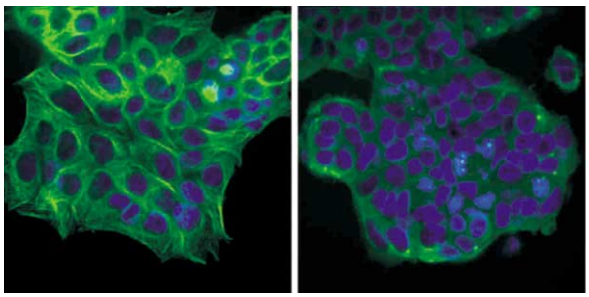


Figure 1. Effect of A-289099 on microtubule depolymerization in HCT-15 cells. Left: control cells. Right: cells treated with 44 nM A-289099 for 4 h. Green: microtubules, Blue: nuclear DNA.

Figure 2. Stereoview of the superimposed structures of A-289099 (green) and Combretastatin A4 (yellow). Their geometries were optimized using DISCOVER force field (CFF98).

Table 5. Pharmacokinetic properties of the two enantiomers of **5b**

Species	PK	**5b-*S*** (A-289099)	**5b-*R***
Mouse[a]	$t_{1/2}$ (h)	1.1	1.3
	AUC (μg h/mL)	5.69	0.40
	F (%)	15.1	21.0
Rat[b]	$t_{1/2}$ (h)	2.1	1.3
	AUC (μg h/mL)	7.18	3.04
	F (%)	17.1	44.0
Dog[b]	$t_{1/2}$ (h)	3.3	2.9
	AUC (μg h/mL)	0.16	0.33
	F (%)	6.5	7.0
Monkey[b]	$t_{1/2}$ (h)	1.3	1.4
	AUC (μg h/mL)	1.75	0.89
	F (%)	18.6	16.2

[a]A single 10 mg/kg dose.
[b]A single 5 mg/kg dose.

Table 6. In vivo efficacy[6b] of A-289099 in comparison with E7010 against M5076 murine ovarian sarcoma in mice (PO, QD, d1-28)

Compd	Dose (mg/kg/day)	Mean tumor delay to 1 g (day)	ILS[a] (%)
A-289099	50	19.0	139
	100[b]	28.4	206
E7010	50	6.4	47
	150[b]	27.4	201

[a]Increase in life span.
[b]Maximum tolerated dose.

tumor model. When dosed QD, d1-28, at the maximum tolerated dose, A-289099 achieved 206% increase in life span and an impressive 28 day delay to 1 g tumor volume in mice with M5076 murine ovarian sarcoma. This efficacy is comparable to that of a previously reported antimitotic agent, E7010[16] (Table 6).

A-289099 exhibits reasonably good physicochemical properties. Its solubilities in 0.1 M HCl and pH 7.4 phosphate buffer are 1.27 mg/mL and 0.7 μg/mL, respectively. A-289099 has a log D of 3.94 and pK_a of 5.0.

In conclusion, a series of indole containing oxazolines has been discovered as a result of structural modifications of the lead compound A-105972. The compounds exert their anticancer activity through inhibition of tubulin polymerization by binding at the colchicine site. A-289099 was identified as an orally active antimitotic agent active against various cancer cell lines, including those that express the MDR phenotype.

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