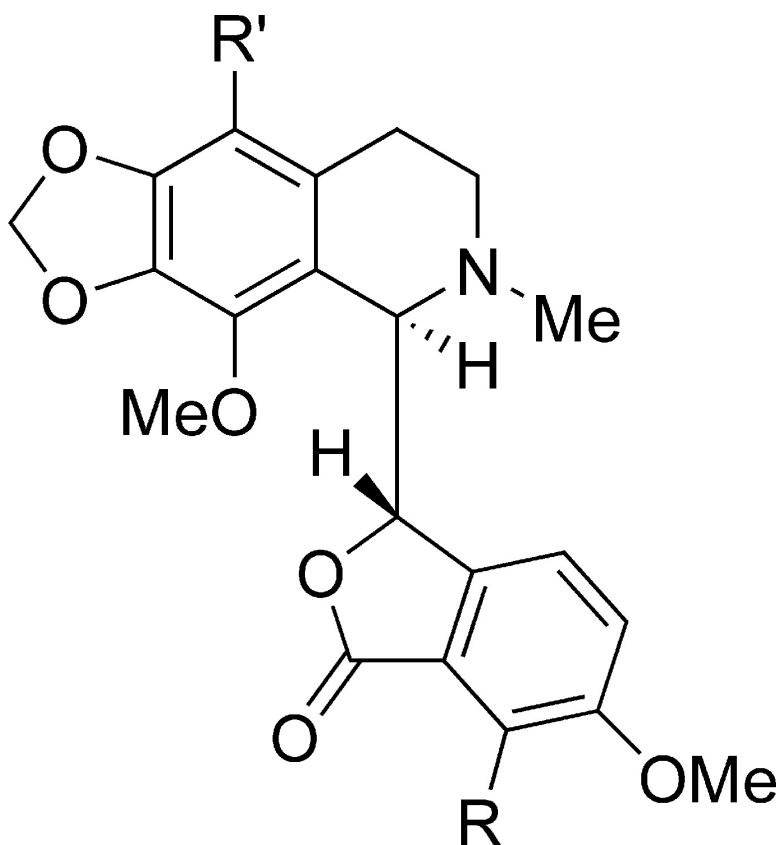Letter

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Identification of Novel and Improved Antimitotic Agents Derived from Noscapine

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Abstract: Analogues of the natural product noscapine were synthesized and their potential as antitumor agents evaluated. The discovery of a novel regioselective O-demethylation facilitated the synthesis of the potent aniline **6**, which arrests mammalian cells in the G2/M phase of the cell cycle at 0.1 μM and also affects tubulin polymerization. Aniline **6** is orally bioavailable and is 250-fold more potent than noscapine in reducing cell proliferation in rapidly dividing cells.

Antitumor agents that affect microtubule dynamics are of great medical interest and are now commonly used in current chemotherapy regimens.[1,2] However, several problems associated with existing cancer therapeutics of this type remain, such as (1) myelosuppression, (2) neuropathy, (3) alopecia,[3] (4) increased drug resistance in tumors,[4] (5) poor bioavailability that results in a need for intravenous delivery, and (6) poor solubility, necessitating the use of agents (i.e., Cremophor) that can possess undesirable qualities.[5] Thus, there is still a need for effective microtubule-directed drugs with improved solubility and therapeutic index. Moreover, it would be desirable to have an orally active drug of this type that might facilitate patient dosing.

Noscapine **1** is a naturally occurring phthalideisoquinoline alkaloid obtained from opium. It has been used orally in humans as an antitussive agent and displays a favorable toxicity profile.[6] Additionally, it has been known for some time that noscapine can act as a weak anticancer agent in certain in vivo models.[7] Recently, Joshi et al. have performed several studies to evaluate the mechanism of action of this anticancer effect and found that noscapine can disrupt tubulin dynamics.[8] Although noscapine appears to be a weak inhibitor of microtubule polymerization, its low cost and ready availability allow for further exploratory medicinal chemistry of this natural product. Accordingly, our goal was to identify *more potent and orally active analogues of noscapine* as potential anticancer agents.

In an earlier communication, we reported an easily scalable synthesis of phenol **2** as a single diastereomer.[9] We examined **2** in a cell cycle assay that measures DNA content, and it showed nearly complete G2/M arrest of HEK293 at 1 μM (Figure 1 and Table 1). In addition, **2**
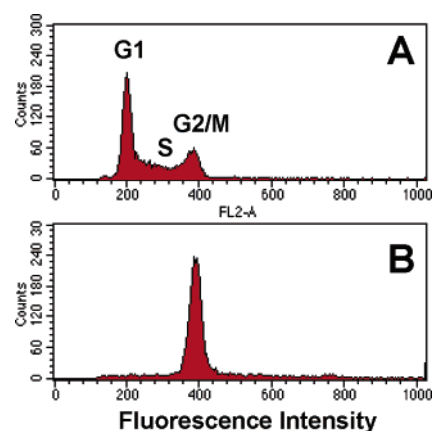
Figure 1. FACS cell cycle analysis of HEK293 treated for 16 h with (A) 0.5% DMSO or (B) 1 μM **2**.
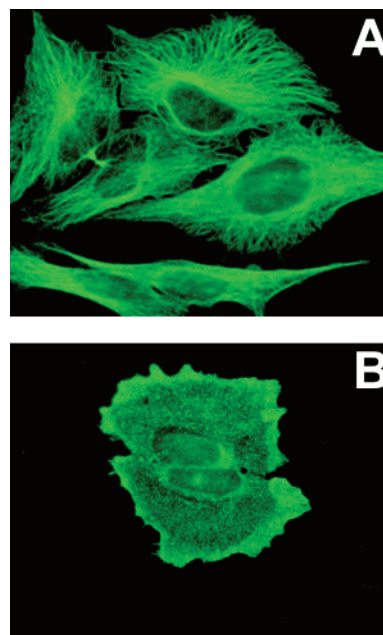

Figure 2. HeLa cells were incubated for 30 min with (A) 0.5% DMSO or (B) 10 μM **2** and then fixed and stained for the presence of microtubules.

Table 1. Cell Cycle Results for HEK293 Cells

| drug	R	R′	concn (μM)[a]	% G1	% S	% G2/M
DMSO			0.5%	48	39	13
colchicine			10	3	14	83
1, noscapine	OMe	H	50	19	31	50
2	OH	H	1	3	9	88
3	OTf	H	50	56	35	9
5	NHBn	H	5	8	29	63
6	NH$_2$	H	0.1	1	7	92
7	NH$_2$	Br	1	4	15	81
8	NHMe	H	0.5	8	25	67
9	SH		5	3	7	90
10	H		1	4	6	90

[a] Concentrations 2- to 10-fold lower did not show a difference in the FACS profile when compared to DMSO control.

was observed to disrupt the microtubule cytoskeleton when HeLa cells were stained for microtubules using a fluorescent-labeled anti-tubulin antibody (Figure 2B). A direct effect on microtubule dynamics was confirmed in an in vitro tubulin polymerization assay, where **2**

Table 2. Tubulin Polymerization and A549 Cell Growth Assays

| drug	tubulin polymerization assay, EC_{50} (μM)	MTS assay, EC_{50} (μM)	colony-forming assay, EC_{50} (μM))
vinblastine	0.2	0.0009	0.0005
noscapine	>50	25	not determined
2	0.7	0.6	0.5
6	0.3	0.097	0.084

Scheme 1. Palladium-Catalyzed Amination[a]
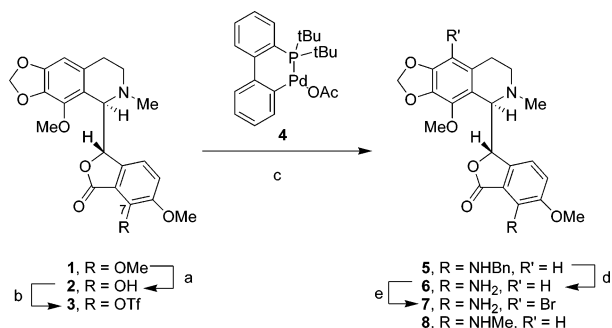
[a] Reaction conditions: (a) MeMgBr, BnOH, toluene−THF, 120 °C, 60%; (b) Tf$_2$O, pyridine, CH$_2$Cl$_2$, 0 °C, 93%; (c) BnNH$_2$ (or aqueous MeNH$_2$), **4** (4 mol %), Ba(OH)$_2$·8H$_2$O, α,α,α-trifluorotoluene−H$_2$O (2:1), 110 °C, 80% for **5** (51% for **8**); (d) 10% Pd/C, H$_2$, MeOH, 43%; (e) Br$_2$, H$_2$O, 48% HBr, 50%.

had an EC$_{50}$ of 0.7 μM. These pronounced effects on mammalian cells suggested that phenol **2** would also inhibit cell growth, and proliferation of the lung epithelial cell line A549 was assessed using two different assays. In an MTS assay,[10] **2** had an EC$_{50}$ of 0.6 μM, while it showed an EC$_{50}$ of 0.5 μM in a colony forming assay (Table 2). These effects were specific for dividing cells, since an MTS assay using **2** in dividing Swiss 3T3 cells gave an EC$_{50}$ of 3 μM while nondividing Swiss 3T3 cells were not affected at 50 μM (data not shown).

The readily accessible phenol **2** provided new opportunities for functionalization via conversion to its triflate **3**, which itself was not active at 50 μM in the cell cycle assay. We first explored palladium-catalyzed amination chemistry[11] on triflate **3** to give nitrogen analogues of noscapine at the 7-position (Scheme 1). Initially, amination using benzylamine proceeded with epimerization of the phthalide stereocenter (∼15−25%), most likely due to the strong base NaOtBu.[12,13] However, the diastereomers could be separated on small scale using reversed-phase HPLC. The pure (natural) stereoisomer *N*-benzyl derivative **5** showed moderate G2/M arrest activity (63% at 5 μM, Table 1), differing from the *O*-benzyl analogue that showed S-phase arrest.[9] The activity was dramatically improved when the benzyl group was removed to give aniline **6**, which arrested HEK293EBNA cells in the G2/M phase at 0.1 μM (Table 1).

The problem of racemization in the triflate amination was solved after the discovery of improved reaction conditions. Replacing NaOtBu with barium hydroxide and using a two-phase system consisting of either α,α,α-trifluorotoluene−H$_2$O[14] or toluene−H$_2$O proved to be best when using benzylamine. Other bases were explored such as LiOtBu, K$_2$CO$_3$, Cs$_2$CO$_3$, and aqueous KOH (biphasic),[15] but aminations using benzylamine were very sluggish. Other ammonia equivalents such as benzophenone imine,[16] LiHMDS,[17] HMDS, and *tert*-

Scheme 2. Triflate Displacement and Reduction[a]
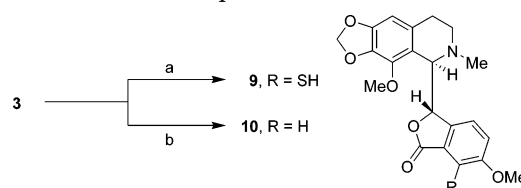
[a] Reaction conditions: (a) NaSH, NMP, 80 °C (19%); (b) Pd(OAc)$_2$, 1,3-bis(diphenylphosphino)propane, MeOH, Et$_3$N, DMSO, 80 °C (58%).

butyl carbamate[18] were investigated, but only HMDS gave any desired amination product albeit at very low conversion (∼5%). An optimal catalyst−ligand system was found in the conveniently prepared palladacycle **4**.[19] The amination was highly efficient and proceeded to completion within 15−30 min at 110 °C. More importantly, no epimerization was detected using the optimized conditions, and the reaction could be easily performed on a multigram scale (up to 20 g of starting **3**).

Aniline **6** was observed to inhibit tubulin polymerization in vitro (Table 2). Further examination of **6** in the MTS (EC$_{50}$ = 97 nM) and colony-forming (EC$_{50}$ = 84 nM) assays indicated that it was more active than **2** and at least 2 orders of magnitude more potent than noscapine (Table 2). Like the phenol **2**, aniline **6** was specific for dividing Swiss 3T3 cells (EC$_{50}$ ≈ 50 nM) with no effects on nondividing Swiss 3T3 cells at 3 μM.

Interestingly, stereochemistry was much more important for activity for noscapine-derived G2/M inhibitors than for S-phase inhibitors.[9] The phthalide diastereomer of **6** was isolated and showed significantly less activity in the cell-cycle assay (partial G2/M arrest at 25 μM).

Compound **6** was easily brominated using a known protocol[20] to give analogue **7**, which was less potent than the parent compound (Table 1). The 7-NHMe analogue of noscapine, **8**, was synthesized by the amination of **3** using aqueous MeNH$_2$ with catalyst **4** and Ba(OH)$_2$· 8H$_2$O.

Compound **8** showed G2/M arrest activity at 0.5 μM. Interestingly, the facile displacement of triflate **3** with sodium hydrosulfide gave the thiophenol analogue **9** (with ∼10% epimerization of the phthalide ring), which also showed G2/M arrest at 5 μM (Scheme 2). The source of activity was postulated to be from an H-donor group at the 7-position. However, this hypothesis was refuted when **10** displayed good G2/M arrest at 1 μM (Scheme 2, Table 1).[21]

As noted, it would be desirable to have orally active drugs that affect cancer cell growth through an alteration of microtubule dynamics. The in vivo pharmacokinetics of aniline **6** and phenol **2** were investigated in mice and compared with noscapine (Table 3). Noscapine and **6** showed similar exposure, volume of distribution (V_d), half-life, and clearance. Compound **2** appeared to have an increased half-life and volume of distribution relative to the other two compounds. All three compounds showed readily measurable oral bioavailability ranging from 11% to 22%, although **2** appeared to have somewhat better oral absorption. It should be noted that the bioavailability of noscapine in humans has been

Table 3. In Vivo Pharmacokinetics in Mice[a]

drug	route	AUC (mg·h/L)	V_d (L/kg)	$T_{1/2}\beta$ (min)	CL ((mL/min)/kg)	F (%)
noscapine	iv	0.345	15.53	50.0	96.8	
noscapine	po	0.524				14.1
2	iv	0.331	28.12	167.7	100.6	
2	po	0.740				22.0
6	iv	0.350	8.47	62.6	95.3	
6	po	0.391				11.4

[a] Dosing: 2 mg/kg (iv); 20 mg/kg (po).

measured to be ~30%.[6] Thus, it is possible that **2** and **6** may show greater oral absorption in humans than in mice.

In conclusion, we have synthesized several potent derivatives of noscapine. In particular, phenol **2** and aniline **6** showed significant improvement in microtubule inhibition and cytotoxicity relative to noscapine. Like noscapine, both **2** and **6** appear to be orally absorbed in mice. Importantly, the more potent **6** can be synthesized in only four steps from noscapine, which is abundant and inexpensive. The potent activity of these natural product derivatives and their ready synthesis lend hope that one or more novel anticancer agents may emerge from these efforts. Toward this goal, select noscapine derivatives described here will be examined in established cancer models.

Supporting Information Available: Experimental procedures and details. This material is available free of charge via the Internet at http://pubs.acs.org.

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