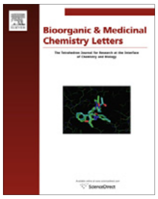
Bioorganic & Medicinal Chemistry Letters 20 (2010) 5277–5281

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl



Synthesis, in vitro and in vivo evaluation of 3-arylisoquinolinamines as potent antitumor agents

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ARTICLE INFO

Article history:
Received 3 May 2010
Revised 23 June 2010
Accepted 28 June 2010
Available online 3 July 2010

Keywords:
3-Arylisoquinolinamines
Antitumor agents
In vivo evaluation
Cytotoxicity
Synthesis
Qsar

ABSTRACT

In the search for potent water-soluble 3-arylisoquinolines, several 3-arylisoquinolinamines were designed and synthesized. Various substituted 3-arylisoquinolinamines exhibited strong cytotoxic activity against eight different human cancer cell lines. In particular, C-6 or C-7 dimethylamino-substituted 3-arylisoquinolinamines displayed stronger potency than the lead compound **7a**. Interestingly, compounds **7b** and **7c** showed more effective activity against paclitaxel-resistant HCT-15 human colorectal cancer cell lines when compared to the original cytotoxic cancer drug, paclitaxel. We analyzed the cell cycle dynamics by flow cytometry and found that treatment of human HCT-15 cells with 3-arylisoquinolinamine **7b** blocked or delayed the progression of cells from G0/G1 phase into S phase, and induced cell death. Treatment with compound 7b also significantly inhibited the growth of tumors and enhanced tumor regression in a paclitaxel-resistant HCT-15 xenograft model.

Cancer is a complex disease that depends on the tissue and original cell types along with many causal factors. Among various targets for the development of antitumor agents, protein kinases,[1,2] and topoisomerases I and II[3–6] have emerged as promising targets for the treatment of tumors. With these targets in mind, it is anticipated that current advances in our understanding of the molecular and structural biology of the cell cycle[7,8] will lead to the discovery of small-molecule inhibitors that target proteins whose improper expression or action has been linked to tumor progression. The cell cycle is considered an attractive target for the development of small-molecule inhibitors for use as both new chemotherapeutics and research probes.[9]

Since the potent antitumor activity of 7,8-dimethoxy-2-methyl-3-(4,5-methylenedioxy-2-vinylphenyl)isoquinolin-1(2*H*)-one (**1**) was first described,[10] we have investigated the structure–activity relationships of 3-arylisoquinolines against human tumor cell lines. Diverse modifications of the 3-arylisoquinoline skeleton provided the indeno[1,2-*c*]isoquinolines **2**,[11,12] isoindolo[2,1-*b*]isoquinolinones **3**,[13] 12-oxobenzo[*c*]phenanthridinones **4**, and benz[*b*]oxepines **5**[14] as the constrained forms of the 3-aryl rings as shown in Figure 1. Most of these arylisoquinoline derivatives exhibited micromolar cytotoxicities with topoisomerase I inhibitory activities. The rigidification of the flexible 3-aryl ring flattened the molecule so that it could work as a DNA intercalator in the topoisomerase I–DNA ternary complex. However, we could not find any compounds that exceeded the cytotoxicity of the original hit compound **1**, even in the SAR study of 3-arylisoquinolines **6**. Next our interest was focused on finding potent water-soluble 3-arylisoquinolines. The designed 3-arylisoquinolinamines **7** were expected to maintain the cytotoxicity of the 3-arylisoquinolines because the nitrogen atom is considered the bioisostere of the oxygen of the amide carbonyl group.[15] In this work, we synthesized various 3-arylisoquinolinamines based on the previously reported 3-arylisoquinolinamine **7a**, which exhibited sub-micromolar cytotoxicities against several cancer cell lines.[16] One feasible mechanism of these compounds was suggested to be inhibition of topoisomerase I. However, the precise mechanism of action of these analogs remains unclear. In order to understand the mode of action of 3-arylisoquinolinamines, as well as their SAR, we synthesized diverse substituents of the lead compound **7a** (Table 1).

For the synthesis of 3-arylisoquinoliamines **7**, we used the previously reported lithiated toluamide–benzonitrile cycloaddition method.[17] *N*-Diethyl-*o*-toluamides **8** were treated with *n*-BuLi to give the anions, which were then reacted with benzonitrile **9** to afford the 3-arylisoquinolines **6** in moderate yield. Treatment of **6** with $POCl_3$ provided the corresponding imine chloride **10**, which was then reacted with *p*-methoxybenzylamine and K_2CO_3 in DMF

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doi:10.1016/j.bmcl.2010.06.132

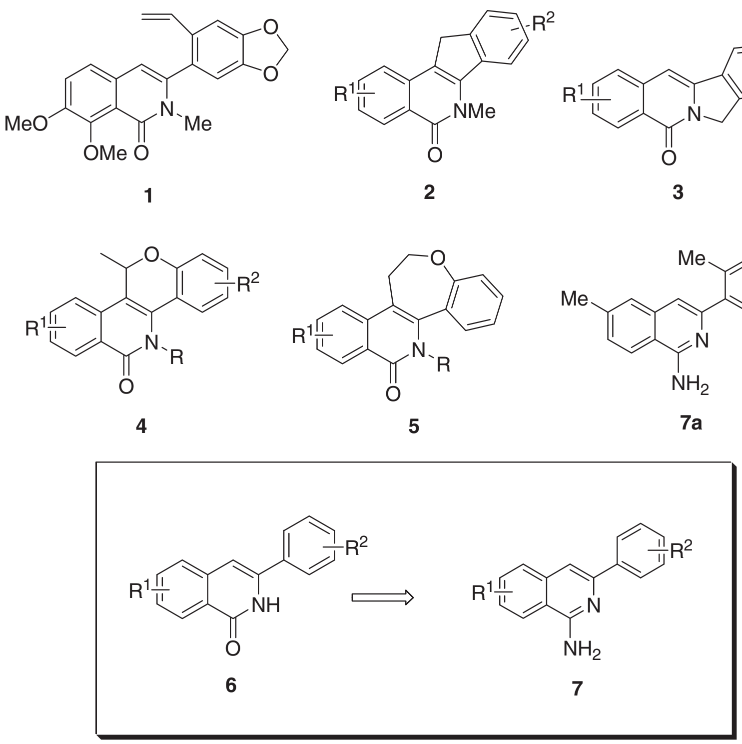


Figure 1. Structure of potent antitumor agent **1** constrained structures of 3-arylisoquinolines; indeno[1,2-*b*]isoquinoline **2**, isoindolo[2,1-*b*]isoquinoline **3**, 12-oxobenzo[*c*]phenanthridine **4**, and benz[*b*]oxepine **5**. Structural modification of **6** to 3-arylisoquinolinamines **7**.

Table 1
Chemical yield and melting points of the 3-arylisoquinolinamines **7a**–**s**

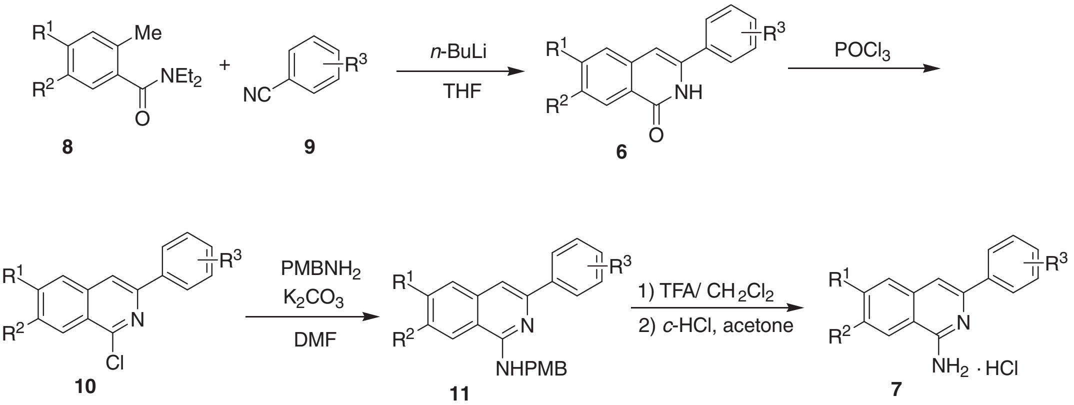
No.	Compound	R^1	R^2	R^3	Yield[b]	Mp (°C)
1	**7a**	$-CH_3$	H	2-Methyl	68	269–270
2	**7b**	H	$-N(CH_3)_2$	3-Methoxy	62	202–206 (dec)
3	**7c**	H	$-N(CH_3)_2$	3-Methyl	63	223–227 (dec)
4	**7d**	H	$-N(CH_3)_2$	3,4-Dimethoxy	76	249–260 (dec)
5	**7e**	H	$-N(CH_3)_2$	3,5-Dimethoxy	35	251–252 (dec)
6	**7f**	$-N(CH_3)_2$	H	3-Methoxy	67	265–276 (dec)
7	**7g**	$-CH_3$	H	2-Methoxy	64	226–232
8	**7h**	$-CH_3$	H	3-Methoxy	68	147–148
9	**7i**	$-CH_3$	H	4-Methoxy	60	134–139
10	**7j**	$-CH_3$	H	3,5-Dimethoxy	90	248–257 (dec)
11	**7k**	$-CH_3$	H	2-Fluoro	69	127–128
12	**7l**	H	$-CH_3$	2-Methoxy	75	195–211 (dec)
13	**7m**	H	$-CH_3$	2-Fluoro	79	114–121
14	**7n**	H	$-CH_3$	2,6-Dimethyl	68	166–167 (dec)
15	**7o**	H	$-CH_3$	3,4-Methylenedioxide	70	172–175
16	**7p**	H	$-CH_3$	3-Methyl	97	75–85[a]
17	**7q**	H	$-CH_3$	5-Methoxy-2-methyl	77	148–159
18	**7r**	$-OCH_3$	$-OCH_3$	2,6-Dimethyl	82	262–273 (dec)
19	**7s**	$-OCH_3$	$-OCH_3$	3-Methyl	78	259–266 (dec)

[a] Not HCl salt.
[b] Yield calculated from compound **11**.

to yield the PMB-protected isoquinolinamine **11** in good yield. In the previous work,[16] we introduced benzylamine at the C-1 position, followed by catalytic hydrogenation to obtain the amine in moderate yield. However, deprotection of the benzyl group by catalytic hydrogenation reaction did not work on some substituted analogs. After several attempts under various conditions, deprotection of PMB of **11** was successfully accomplished with trifluoroacetic acid to afford the desired isoquinolinamines **7** in moderate yield as shown in Scheme 1. The free amines were then treated with *c*-HCl in acetone to provide the HCl salt forms of the amines.

The in vitro cytotoxicity experiments were performed with the synthesized compounds against various human cancer cell lines such as MDA-MB-231 (breast), PC3 (prostate), HCT-15 (colon), HCT 116 (colon), OVCAR-3 (ovary), Caki-1 (kidney), PANC-1 (pancreas), SNB-19 (glioblastoma), and SK-MEL-28 (melanoma) cells from the American Type Culture Collection (ATCC) (Manassas, VA).

The growth inhibition assay of representative 3-arylisoquinolinamine derivatives against human cancer cell lines was performed using the sulforhodamine B method.[18] Absorbance was measured at 530 nm using Benchmark Plus Microplate reader (Bio-Rad Laboratories, Hercules, CA). The drug concentration which inhibited the cell growth by 50% (IC_{50}) was calculated using Kaledia Graph software program (Synergy software, Reading, PA).



Scheme 1. The synthesis of 3-arylisoquinolinamines **7a–s**. (a) R^1 = Me, R^2 = H, R^3 = 2-Me; (b) R^1 = H, R^2 = NMe_2, R^3 = 3-MeO; (c) R^1 = H, R^2 = NMe_2, R^3 = 3-Me; (d) R^1 = H, R^2 = NMe_2, R^3 = 3,4-$(OMe)_2$; (e) R^1 = H, R^2 = NMe_2, R^3 = 3,5-$(OMe)_2$; (f) R^1 = NMe_2, R^2 = H, R^4 = 3-MeO; (g) R^1 = Me, R^2 = H, R^3 = 2-MeO; (h) R^1 = Me, R^2 = H, R^3 = 3-MeO; (i) R^1 = Me, R^2 = H, R^3 = 4-MeO; (j) R^1 = Me, R^2 = H, R^3 = 3,5-$(OMe)_2$; (k) R^1 = Me, R^2 = H, R^3 = 2-F; (l) R^1 = H, R^2 = Me, R^3 = 2-MeO; (m) R^1 = H, R^2 = Me, R^3 = 2-F; (n) R^1 = H, R^2 = Me, R^3 = 2,6-$(Me)_2$; (o) R^1 = H, R^2 = Me, R^3 = 3,4-(OCH_2O); (p) R^1 = H, R^2 = Me, R^3 = 3-Me; (q) R^1 = H, R^2 = Me, R^3 = 5-MeO-2-Me; (r) R^1 = OMe, R^2 = OMe, R^3 = 2,6-$(Me)_2$; (s) R^1 = OMe, R^2 = OMe, R^3 = 3-Me.

Surprisingly, most 3-arylisoquinolinamine derivatives exhibited potent cytotoxicities in dose-dependent manner, as shown in Table 2, against eight cancer cell lines, suggesting that the potency of these compounds was not highly dependent on the substitution pattern of the modified phenyl ring. Among these compounds, eight compounds, **7b**, **7c**, **7d**, **7e**, **7f**, **7h**, 7n, and **7q**, showed equal or better growth inhibitory activity against human cancer cells compared to **7a**. In particular, compounds **7b** and **7c** inhibited the cell growth at IC_{50} values ranged from 14 nM to 32 nM in the human cancer cells tested, which are 5–13 times more active than compound **7a**.

From the viewpoint of structure–activity relationships, the general features could be summarized as follows. The dimethylamino group on C-6 or C-7 position of the A ring contributed to significant increases in cytotoxic potency with the preference at C-7. But methyl-substituted compounds on C-6 or C-7 of the A ring did not show the relationship between substitution and activity.

Next, the in vitro antitumor effects of compounds **7b** and **7c** were tested against paclitaxel-resistant HCT-15 human colorectal cancer cells and HCT 116 colon cancer cells, and their antitumor activities were compared with those of paclitaxel (Taxol®). As shown in Table 3, compounds **7b** and **7c** showed potent antiproliferative activities in vitro with IC_{50} values in the low nanomolar range in both cell types and higher antitumor activities than that of paclitaxel against paclitaxel-resistant HCT-15 colorectal cancer cells. When IC_{50} values were compared in both colon cancer cell lines, the activity of paclitaxel was decreased by 70-fold in HCT-15 cells but both compounds **7b** and **7c** displayed still the strong growth inhibition of these cells.

Table 3
IC_{50} of compounds **7b**, **7c**, and paclitaxel in HCT-15 and HCT 116 cells

	7b	**7c**	Paclitaxel
HCT-15	15 nM	21 nM	140 nM
HCT 116	17 nM	23 nM	2 nM
Resistant indices[a]	0.88	0.91	70

[a] The resistant indices are defined by IC_{50} (HCT-15)/IC_{50} (HCT 116).

Although some 3-arylisoquinoline derivatives showed moderate topoisomerase I inhibitory activities in a previous study,[16] no confirmed cytotoxic mechanism were clarified. To better understand the cytotoxic mechanism of the compounds, cell cycle dynamics were analyzed by flow cytometry. Briefly, HCT-15 cells were plated at a density of 1×10^6 cells per 100-mm culture dish

Table 2
Inhibition of cell growth (IC_{50}, μM) by 3-arylisoquinolinamine compounds **7a–s** against human cancer cell lines

Compound	MDA-MB-231 (breast)	PANC-1 (pancreas)	HCT 116 (colon)	PC3 (prostate)	OVCAR-3 (ovary)	SK-MEL-28 (melanoma)	Caki-1 (kidney)	SNB19 (glioblastoma)
7a[a]	0.15	0.24	0.19	0.24	0.15	0.35	0.12	0.26
7b	0.021	0.019	0.017	0.019	0.014	0.032	0.022	0.032
7c	0.021	0.018	0.023	0.024	0.016	0.032	0.023	0.028
7d	0.15	0.19	0.18	0.23	0.14	0.33	0.17	0.25
7e	0.027	0.026	0.029	0.048	0.025	0.045	0.038	0.057
7f	0.059	0.11	0.064	0.19	0.071	0.11	0.068	0.094
7g	0.44	0.93	0.67	>3.0	0.49	0.80	0.35	1.13
7h	0.14	0.31	0.19	0.25	0.18	0.34	0.13	0.29
7i	0.93	2.27	1.36	2.98	0.79	1.80	0.75	2.23
7j	0.18	0.38	0.24	0.43	0.18	0.41	0.16	0.47
7k	0.55	1.59	0.70	1.84	0.57	1.07	0.46	1.51
7l	0.32	0.68	0.47	1.01	0.51	0.67	0.22	0.78
7m	0.72	2.14	1.22	2.76	0.91	1.62	0.59	2.20
7n	0.051	0.079	0.074	0.12	0.075	0.12	0.048	0.12
7o	0.15	0.37	0.18	0.25	0.23	0.42	0.13	0.38
7p	0.21	0.69	0.28	0.71	0.29	0.50	0.44	0.53
7q	0.030	0.058	0.042	0.070	0.042	0.051	0.047	0.052
7r	0.25	0.54	0.79	0.68	0.16	0.63	0.53	0.42
7s	0.79	2.27	2.32	2.50	0.58	1.12	1.78	1.56

[a] Compound **7a** was previously reported[16] and was therefore used as a reference molecule for cytotoxicity comparison in this Letter.

and incubated for 24 h. Fresh media containing test samples were added to the culture dishes. After 24 h and 48 h, the cells were harvested (trypsinization and centrifugation), fixed with 70% ethanol, and incubated with a staining solution containing 0.2% NP-40, RNase A (30 μg/ml), and propidium iodide (50 μg/ml) in phosphate–citrate buffer (pH 7.2). Cellular DNA content was analyzed by flow cytometry using a Becton Dickinson laser-based flow cytometer. At least 20,000 cells were used for each analysis, and the results were displayed as histograms. In cell cycle analysis using HCT-15 cells (see Fig. 2), treatment of 1 nM of compound **7b** displayed a significant increase in G0/G1 phase at 24 h with a decrease in G2/M phase, but the increase of G0/G1 phase at 48 h was not significant. At higher concentration of compound **7b** (10 nM), there were a significant increase in G0/G1 phase and decrease in G2/M phase, and an emergence of sub-G1phase, at both 24 h and 48 h. These data indicated that compound **7b** blocked or delayed the progression of cells from G0/G1 phase into S phase, and induced cell death.

In order to observe the inhibition of tumor growth in an animal model, an ex vivo xenograft study of nude mice was conducted utilizing compound **7b**. Paclitaxel-resistant HCT-15 cell suspension (1×10^6 cells in 0.2 ml of RPMI) was injected subcutaneously into the right flank of six-week-old female athymic mice (BALB/c nu/nu) on day 0. Animals with tumors in the proper size range were assigned to various treatment groups. Paclitaxel was used as a positive control. Compound **7b** and paclitaxel were dissolved in 5% Cremophor and 5% ethanol in PBS, and solvent alone served as a vehicle control. All study medications (vehicle control; paclitaxel: 10 mg/kg/day; compound **7b**: 10 mg/kg/day) were given by intraperitoneal injection three times per week starting on day 10 and ending on day 29 after inoculation of HCT-15 cells. To quantify tumor growth, three perpendicular diameters of the tumors were measured with calipers every 3–5 days, and the body weights of the mice were monitored to assess toxicity. Tumor volume (mean ± SEM) in each group of animals is presented in Figure 3, which shows the measurement of tumor volume as an indicator of the efficacy of compound 7b against HCT-15 human colon carcinoma xenografts. Compound **7b** treatment was well tolerated with no deaths and body weight fluctuations of <1 g observed. As a result of the measurement of antitumor activity against HCT-15 human colorectal cancer in nude mice at day 29, **7b** was found to have higher antitumor efficacy (69.2% inhibition) than the control drug, paclitaxel (48.8% inhibition), as shown in Figure 3.

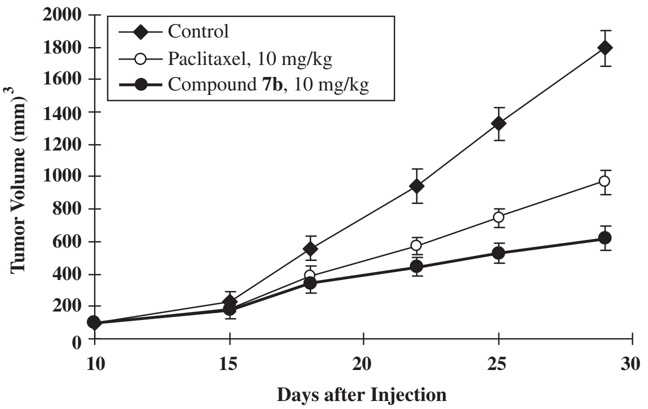


Figure 3. Antitumor activity of compound **7b** against HCT-15 human colon carcinoma xenografts. Paclitaxel-resistant HCT-15 cell suspension (1×10^6 cells in 0.2 ml of RPMI) was injected subcutaneously into the right flank of six-week-old female athymic mice (BALB/c nu/nu) on day 0. Animals with tumors in the proper size range were assigned to various treatment groups. Palcitaxel was used as a positive control. Compound **7b** and paclitaxel dissolved in 5% Cremophor and 5% ethanol in PBS were given by intraperitoneal injections three times per week starting from day 10 and ending on day 29 after inoculation of HCT-15 cells. The tumor volume (mean ± SEM) was calculated using the formula: tumor volume (mm^3) = (width) × (length) × (height) × π/6.

In conclusion, we synthesized various C-6 or C-7 substituted 3-arylisoquinolinamines based on the lead compound **7a**, which exhibited 0.17 μM cytotoxicity against A549,[16] and their antitumor activity was examined in eight different human cancer cell lines using SBR assay. Among them, eight compounds including C-6 or C-7 dimethylamino-substituted 3-arylisoquinolinamines displayed stronger potency than the lead compound **7a**. Our further in vitro studies showed that compounds **7b** and **7c** are potent antiproliferative agents with low nanomolar range of IC_{50} against paclitaxel-resistant HCT-15 cells, and this cell growth inhibition by com-

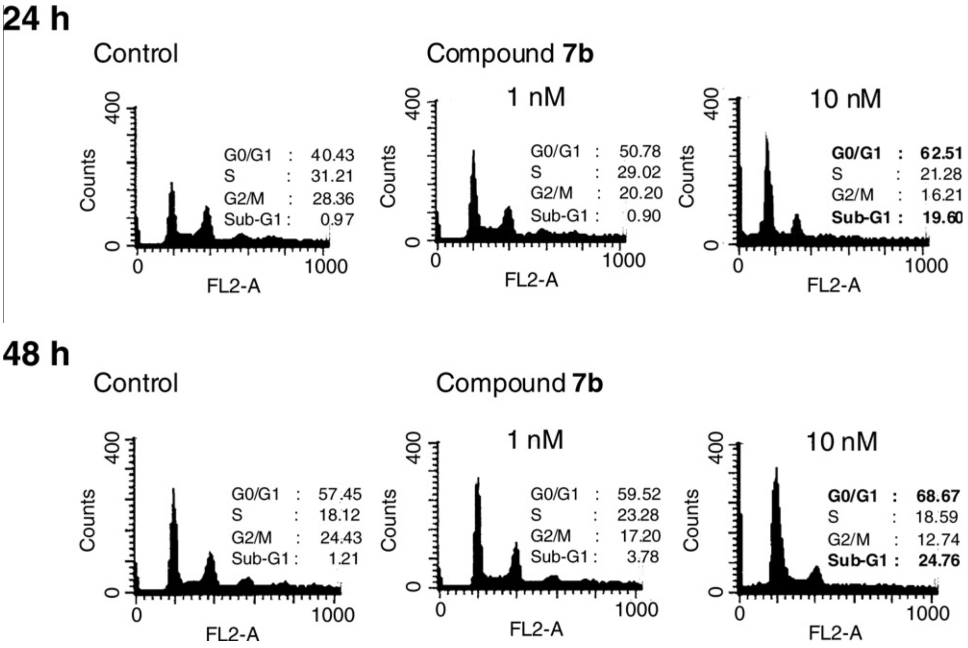


Figure 2. The effect of **7b** on cell cycle progression in HCT-15 cells. Cells were treated with **7b** (10 nM) for 24 h or 48 h and then the cell cycle was analyzed by flow cytometry analysis.

pound **7b** results from G0/G1-phase arrest. Moreover, in vivo study demonstrated that compound **7b** inhibited the tumor formation in paclitaxel-resistant HCT-15 xenograft model to suggest a potential use of compound **7b** in drug-resistant tumor treatment. Thus higher antitumor efficacy of 3-arylisoquinolineamine **7b** and related analogs could provide a new research area for the development of novel antitumor chemotherapeutic candidates.

Acknowledgment

This work was supported by Korea Research Foundation Grant (NRF-2009-0071379) and Rexahn Pharmaceuticals, Inc.

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