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Synthesis and Biological Evaluation of N^9-substituted Harmine Derivatives as Potential Anticancer Agents

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ABSTRACT

A series of N^9-substituted harmine derivatives were synthesized and evaluated for their anticancer activity on a panel of cancer cell lines, their apoptosis induction and their cell cycle effects. The results showed that N^9-substituted harmine derivatives had anticancer effects. In particular, N^9-haloalkyl derivatives **9a**–**9c** and N^9-acyl harmine derivatives **11c** and **11d**, with IC_{50} values less than 1 μM, were more potent than doxorubicin against A-549 and/or MCF-7 cell lines. Moreover, structure–activity relationships (SARs) indicated that introducing a haloalkyl or benzenesulfonyl group in the N^9-position of harmine could significantly increase the anticancer activity. The most active compound (**11d**) caused cell cycle arrest in the G2/M phase, and induced cell apoptosis in a dose-dependent manner.

Keywords: Harmine derivatives; Anticancer activities; Structure–activity relationships (SARs);

Apoptosis; Cell cycle arrest

Cancer is one of main diseases that poses a direct threat to human health and life. Approximately seven million people die from cancer every year, and this number is growing rapidly.[1] Most anticancer drugs inhibit the proliferation of cancer cells through inducing apoptosis.[2] However, the resistance of cancer cells to cytotoxic drugs can lead to treatment failure.[3, 4] Therefore, it is of utmost importance to develop new drugs for cancer. Ingenious modification of anticancer compounds could improve their properties and/or decrease their side effects, and is one of feasible ways to search for new drugs.[5]

Many compounds containing an indole or pyridine structure, such as HYL-6d (**1**)[6] and E7010 (**2**),[7] have significant anti-proliferative activity (**Fig. 1**). Harmine, originally isolated from the seeds of *Peganum harmala* in 1847, is a typical *β*-carboline alkaloid, having a core indole structure and a pyridine ring, and is endowed with diverse pharmacological properties, including antiinflammatory,[8] antimalarial,[9] anti-HIV,[10] and anticancer activity.[11] Previous investigations have shown that harmine demonstrates remarkable anticancer potential both *in vitro* and *in vivo*.[12–14] One of the reasons for this is that harmine-mediated inhibition of DYRK1A induces the activation of caspase-9,[15,16] which leads to massive apoptosis in different human cell types. Some studies have also revealed that introducing appropriate substituents in the N^9-position of *β*-carboline, such as in HAC-Y6 (**3**)[17] and 9-phenylpropyl-harmine (**4**),[14] can augment antitumor activity (**Fig. 1**). However, the current research have limitations, and some research groups have presented different views. Cao's group[14] reported that a short alkyl or benzyl substituent at the N^9-position of harmine can increase the anticancer activity significantly, while Ghasemi and Davoudian[18] concluded that a medium-sized

aliphatic alkyl group is favored. In the present study, to improve the anticancer activity of harmine derivatives and to illuminate their antitumor structure–activity relationships (SARs), various N^9-substituted harmine derivatives were synthesized (**Fig. 1**), their anticancer activity evaluated *in vitro* and a preliminary study made of the mechanism of action.

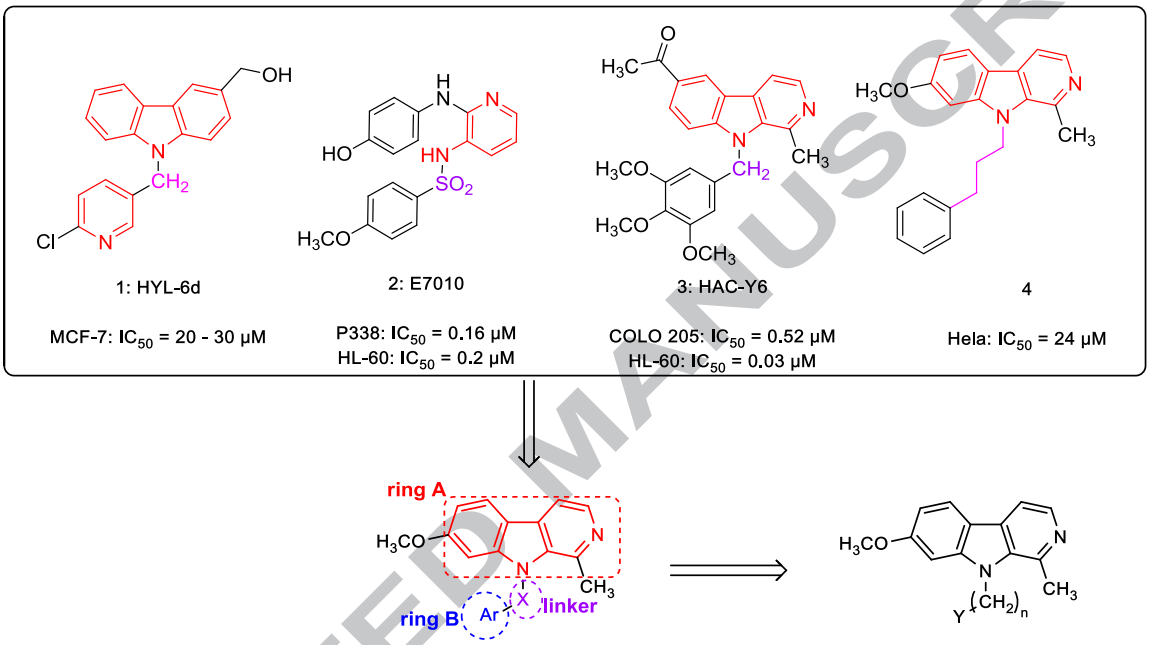
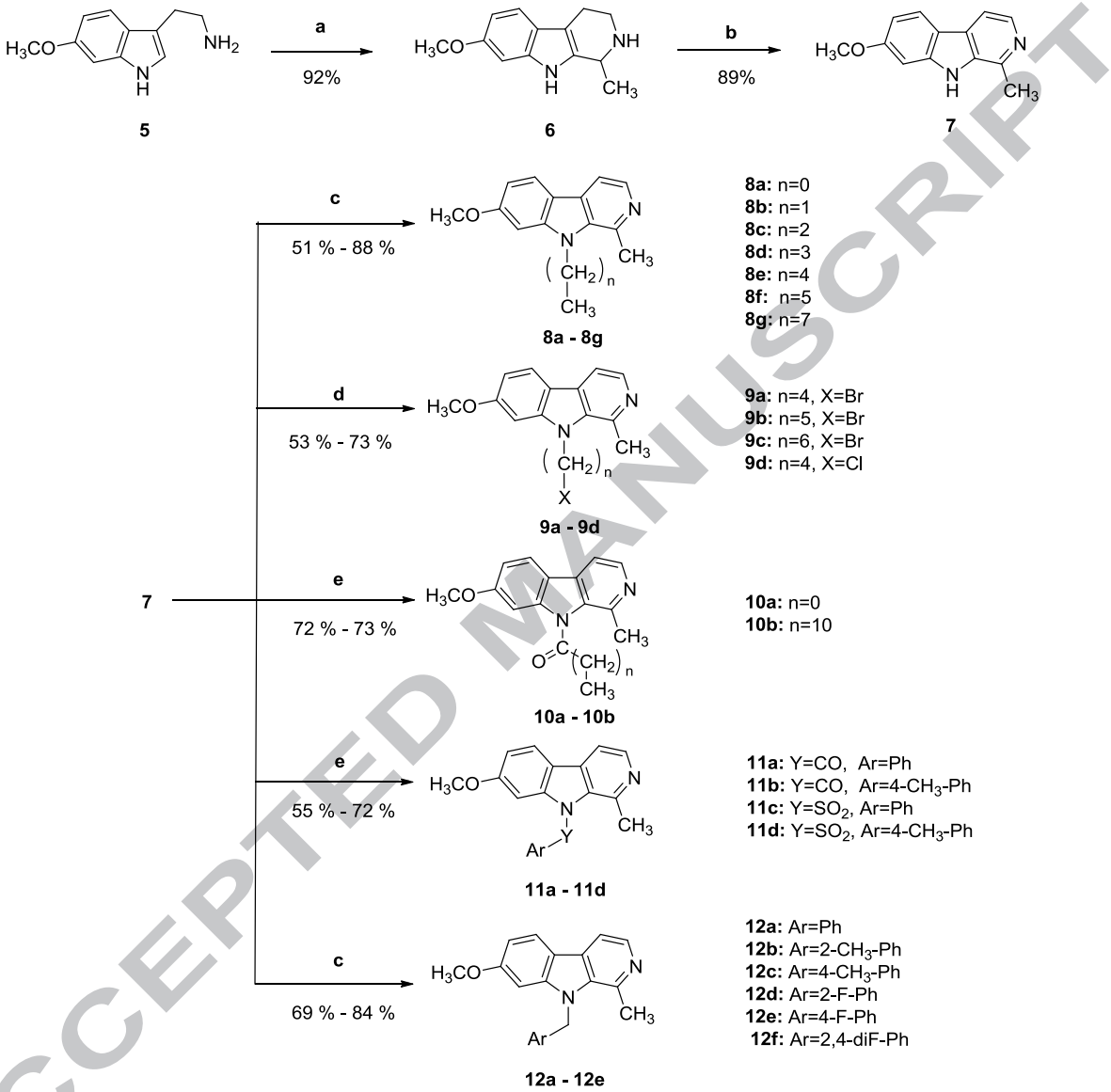


Fig. 1 Previous studies and design strategy of 9-substituted harmine derivatives

The synthetic route for obtaining harmine and its N^9-substituted derivatives is depicted in **Scheme 1**. 1,2,3,4-Tetrahydro-harmine (**6**) was prepared from 6-methoxy-tryptamine (**5**) via a Pictet–Spengler reaction according to a previously described method.[19] Compound **6** was then oxidized to produce the harmine (**7**) using Pd/C (5%) in refluxing toluene. Harmine (**7**) was *N*-alkylated by treatment with sodium hydride (NaH) and an alkyl or benzyl halide in dimethylformamide (DMF) at room temperature, to yield compounds **8a**–**8g**, **9a**–**9d** and **12a**–**12f**. The N^9-acyl harmine derivatives **10a**, **10b** and **11a**–**11d** were synthesized by reaction with an acyl chloride, such as acetyl chloride, benzoyl chloride or benzenesulfonyl chloride, in a mixture of

pyridine and DMF at room temperature. The chemical structures of all synthetic products were confirmed by mass spectroscopy and ^{1}H and ^{13}C NMR spectroscopy.



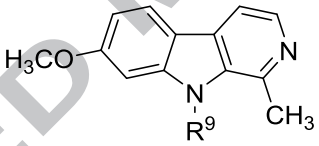
Scheme 1. *Synthesis* of 9-substituted harmine derivatives. Reagents and conditions: (a) HCl (aq. 0.1N), 35% acetaldehyde, 40 °C, 6 h; (b) 5% Pb/C, dry toluene, reflux, 24 h; (c) NaH, dry DMF, RBr or RI, rt, 12 h; (d) NaH, dry DMF, RBr_2 or RCl_2, rt, 8 h; (e) RCOCl or $ArSO_2Cl$, pyridine, DMF, rt, 2 h.

The *in vitro* anticancer activity of the synthesized compounds was evaluated against four human cancer cell lines (MCF-7, SGC-7901, SMMC-7721 and A-549) by means of an MTT assay[20] with doxorubicin as a positive control. The IC_{50} values (the concentration of causing 50% inhibition of

cancer cell growth) are listed in **Table 1**. The results show that most N^9-substituted harmine derivatives have moderate to excellent anticancer activity for the four human cancer lines tested. Furthermore, all the synthesized compounds displayed much stronger activity against SGC-7901 and A-549 cells than did the parent compound (**7**), which suggests that N^9-substituted harmine derivatives could have a significantly improved the anticancer effect.

The N^9-alkyl derivatives **8a**–**8g** were found to be more potent than compound **7** in the inhibition of cancer cell growth. Of these compounds, **8g** exhibited good activity against SGC-7901, SMMC-7721 and A-549 with IC_{50} values of 6.68, 6.95 and 4.77 μM, respectively. Unfortunately, there was no obvious pattern of the impact of alkyl chain length on inhibitory activity.

Table 1. *In vitro* cytotoxic activities of 9-substituted harmine derivatives against four human cancer cell lines



Compounds	R^9	IC_{50} (μM) [a]			
		MCF-7	SGC-7901	SMMC-7721	A-549
8a	Methyl	> 50	9.67±0.06	43.62±0.82	4.97±0.30
8b	Ethyl	> 50	29.62±0.62	23.98±0.17	26.35±1.04
8c	Propyl	> 50	19.35±0.08	48.80±0.75	9.66±0.51
8d	Butyl	> 50	11.35±0.08	28.64±0.85	6.09±0.47
8e	Pentyl	> 50	20.50±0.75	17.20±1.01	6.37±0.35
8f	Hexyl	> 50	13.29±0.36	14.39±0.93	4.89±0.22
8g	Octyl	> 50	6.68±0.20	6.95±0.14	4.77±0.17
9a	4-Bromobutyl	0.85±0.07	10.62±0.20	8.34±0.31	12.19±0.06
9b	5-Bromopentyl	0.76±0.03	9.34±0.12	6.49±0.22	4.80±0.05
9c	6-Bromohexyl	0.45±0.02	6.94±0.24	3.79±0.09	0.87±0.01
9d	4-Chlorobutyl	2.94±0.28	7.21±0.10	17.94±0.58	8.73±0.35
10a	Acetyl	> 50	6.39±0.06	> 50	1.27±0.01
10b	Dodecanoyl	> 50	8.76±0.17	49.06	14.61±0.39
11a	Benzoyl	> 50	7.69±0.34	> 50	2.81±0.13
11b	4-Methylbenzoyl	> 50	2.88±0.09	> 50	1.33±0.08
11c	Benzenesulfonyl	> 50	5.95±0.10	> 50	0.83±0.04
11d	4-Methylbenzenesulfonyl	> 50	1.56±0.01	> 50	0.48±0.01

12a	Benzyl	>50	38.20±0.86	45.62±1.89	7.49±0.11
12b	2-Methylbenzyl	>50	17.21±0.25	39.46±1.09	8.34±0.16
12c	4-Methylbenzyl	>50	5.29±0.08	21.34±0.84	3.89±0.02
12d	2-Fluorobenzyl	>50	43.63±2.51	>50	5.67±0.06
12e	4-Fluorobenzyl	>50	7.21±0.10	>50	8.73±0.35
12f	3,5-Difluorobenzyl	>50	6.47±0.07	>50	18.39±0.12
Harmine	H	68.33±2.82	40.82±1.22	59.44±1.98	42.25±2.17
Dox.	-	0.83±0.02	1.20±0.03	1.39±0.05	0.76±0.02

[a] Human tumor cells were treated with six concentrations each for 48 h. IC_{50} values (the concentration of 50% proliferation-inhibitory effect) were calculated by the Logit method from the results of at least three independent tests and expressed as the mean ± SD.

The N^9-haloalkyl derivatives **9a**–**9g** showed moderate or potent anticancer activity against all tested cells, and the N^9-bromobutyl derivative **9a** (IC_{50}=0.85–12.89 μM) was more potent than the N^9-chlorobutyl compound **9d** (IC_{50}=2.94–17.94 μM). Among the N^9-bromoalkyl harmines **9a**−**9c**, compounds **9b** and **9c** were the most promising compounds against all tested cell lines, with IC_{50} values of 0.76–9.34 and 0.45–6.94 μM, respectively. Interestingly, all of these N^9-haloalkyl molecules were more potent in inhibiting cancer cell lines growth than the corresponding N^9-alkyl derivatives (the following pairs of compounds should be compared: **8d** *vs* **9a**, **8e** *vs* **9b**, and **8f** *vs* **9c**) or harmine. The results indicate that introducing a haloalkyl group in the N^9-position is beneficial in improving anticancer activity.

The dodecanoyl derivative (**10b)** was found to be less potent than the N^9-acetyl derivative (**10a**). In the series of compounds having a benzyl, benzoyl or benzenesulfonyl substituent at the N^9-position of the indole ring, the order of potency was found to be N^9-benzenesulfonyl > N^9-benzoyl > N^9-benzyl (**11a** > **11c** > **12a**, and **11b** > **11d** > **12b**). Compounds with a 4′-aryl substituent at N^9 showed lower anticancer activity than compounds having a 4′-methylaryl substituent (**11a** < **11b, 11c** < **11d,** and **12a** < **12b**). Promisingly, compounds **11c** and **11d** were seen to selectively inhibit the growth of A-549 cells, with IC_{50} values of 0.83 and 0.48 μM, respectively,

and were far more potent than the parent compound (**7**) (IC_{50} = 42.25 μM) and of equal potency to doxorubicin (IC_{50} = 0.76 μM).

Based on the above analysis, some valuable SARs can be summarized (**Fig. 2**): (1) N^9-alkyl and N^9-aryl groups are beneficial to anticancer activity; and (2) N^9-bromo alkyl derivatives are more potent than chloro-substituted and N^9-alkyl derivatives, and for N^9-bromoalkyl derivatives the inhibitory activity increases with increasing alkyl chain length; (3) in the series of N^9-aryl derivatives, the activity sequence was N^9-benzenesulfonyl > N^9-benzoyl > N^9-benzyl. In addition, the introduction of a methyl group in position $C^{4'}$ could significantly improve the anticancer activity.

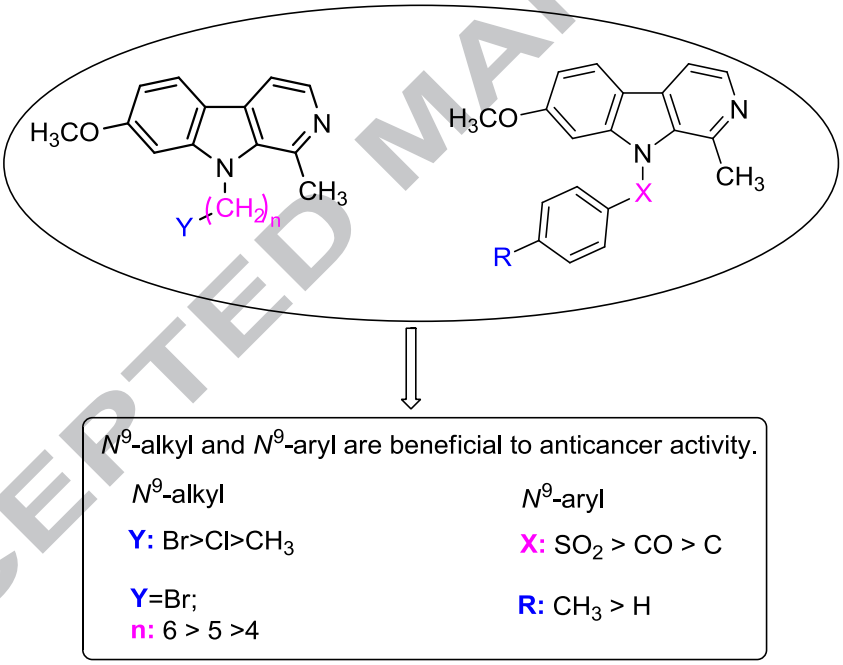


Fig. 2 Structure-activity relationships (SARs) of 9-substituted harmine derivatives.

To clarify the effect of 9-substituted harmine derivatives on cell growth, the effect of compound **11d** on cell-cycle progression in A-549 cells was investigated. A-549 cells were treated with **11d** at concentrations of 0.1, 0.5 and 1 μM for 48 h, and the cell-cycle distributions analyzed by flow cytometry. As can be seen in **Fig. 3**, compound **11d** at a concentration of 0.1 μM induced a slight accumulation of cells in the G2/M phase (16.42%) compared with the control group (14.62%). When

the concentration of compound **11d** was 0.5 μM, the percentage of cells in the G2/M phase was markedly increased, from 14.62% to 31.09%. Meanwhile, the percentage of cells in the G0/G1 phase was significantly decreased from 53.54% to 36.99%. When the A-549 cells were treated with compound **11d** at a concentration of 1 μM, the percentage of cells in the G2/M phase was significantly increased to 36.64%. These results suggested that compound **11d** could arrest A-549 cells in the G2/M phase in a dose-dependent manner. It can also be seen from **Fig. 3** that compound **11d** induced a significant and dose-dependent increase in the percentage of A-549 cells in the sub-G1 phase, which indicates that the A-549 cells treated with compound **11d** had undergone apoptosis.

Therefore, the induction of apoptosis in A-549 cells by compound **11d** was determined using an Annexin V-FITC/PI double staining assay[21, 22]. As shown in **Fig. 4**, after treatment of the A-549 cells with **11d** at concentrations of 0.1, 0.5 and 1 μM for 48 h, the rate of apoptosis of the cells was 21.44%, 54.29% and 71.70%, respectively, all of which are significantly higher than the rate achieved with blank control group (3.27%, DMSO). This finding indicates that 9-substituted harmine derivatives could induce apoptosis of A-549 cells in a dose-dependent manner.

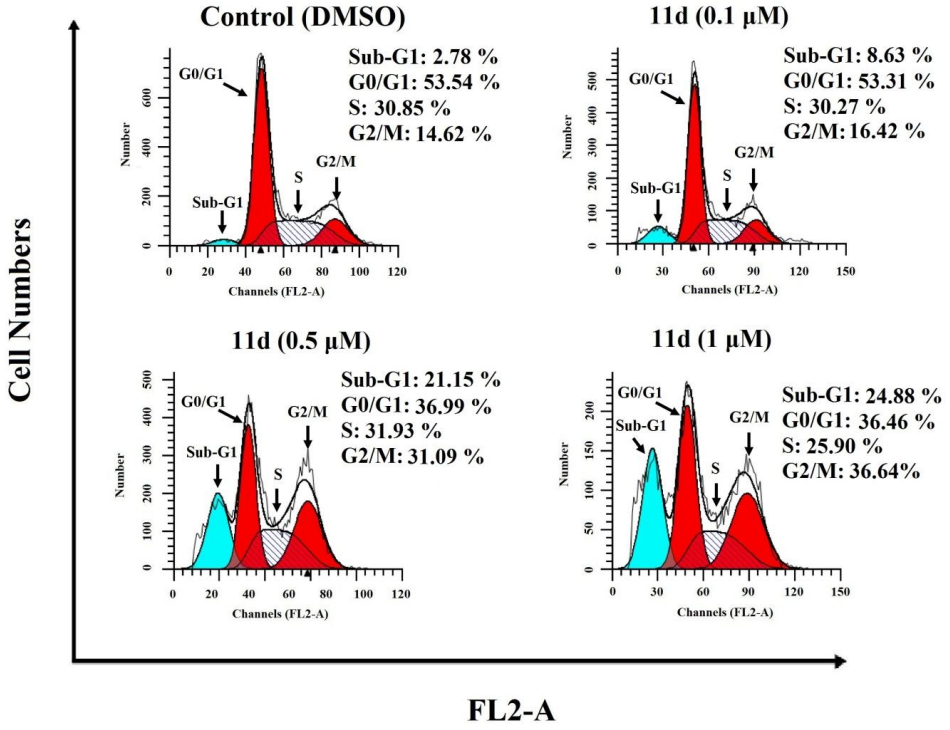


Fig. 3 Effects of 11d on the cell cycle in A-549 cells. The cells were harvested and analyzed for apoptosis by flow cytometric analysis after treatment with 11d (0, 0.1, 0.5 and 1 µM) for 48 h.

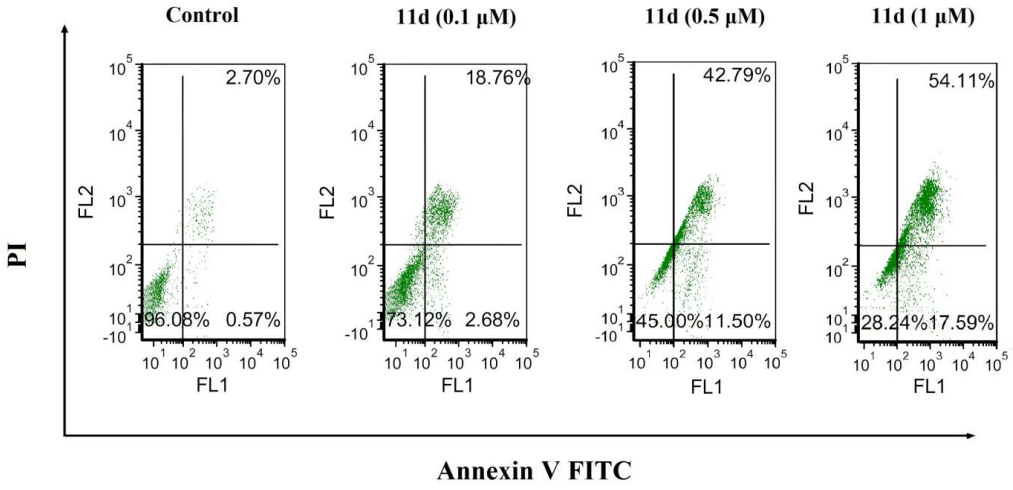


Fig. 4 Effects of compound 11d on the induction of apoptosis in A-549 cells. The cells were treated with 11d (0, 0.1, 0.5 and 1 µM) for 48 h. The cells were then harvested and analyzed for cell-cycle progression by flow cytometry.

In summary, a series of N^9-substituted harmine derivatives were synthesized and their anticancer activity against four human tumor cell lines *in vitro* evaluated. The majority of the derivatives exhibited potent anticancer activity. SARs studies indicated that the N^9-position plays an important role in modulating the anticancer activity, and that introducing a haloalkyl or benzenesulfonyl group in the N^9-position is beneficial to anticancer activity. The preliminary study of the mechanism revealed that compound **11d** could cause cell cycle arrest in the G2/M phase and induce cell apoptosis in a dose-dependent manner. In conclusion, compounds **9a**–**9c**, **11c** and **11d** are good candidates for further study and development as anticancer agents.

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Supplementary data

Supplementary data (experimental procedure and spectroscopic characterizations of the compounds) associated with this article can be found at XXXXXXXX.

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Graphical Abstract