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Design, synthesis and antiproliferative activity studies of 1,2,3-triazole-chalcones†

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Abstract: A series of 1,2,3-triazole-chalcone hybrids were designed, synthesized and evaluated for their antiproliferative activity against three selected cancer cell lines (SK-N-SH, HepG-2 and MGC-803). Most of the synthesized compounds exhibited moderate to good activity against all the cancer cell lines selected. Particularly, compound **12k** showed the most excellent antiproliferative activity with an IC_{50} value of 1.53 μM against SK-N-SH cancer cells. The mechanism studies revealed that compound **12k** inhibited the proliferation of SK-N-SH cancer cells by inducing apoptosis and arresting the cell cycle at G1 phase.

Key words: 1,2,3-triazole-chalcones; antiproliferative; apoptosis; G1 phase

Cancer, being one of the leading causes of death globally, causes a great burden to both single human lives and the society as a whole. Although there have been progresses in the development of treatment and prevention of cancer, the successful treatment of cancer remains a challenge.[1] Therefore, there is still an urgent need to search for novel anticancer agents that have broader spectrum of cytotoxicity to tumor cells.[2]

Chalcones, important constituents of natural products, are abundant in edible plants where they are considered to be the precursors of flavonoids and isoflavonoids.[3] There is a growing interest in the pharmacological potential of chalcones, which constitute an important group of natural and synthetic products that have been screened for their wide range of pharmacological activities as antibacterial,[4, 5] antitumor,[6, 7] antiinflammatory,[8, 9] antifungal and antioxidant agents.[10, 11] For example, compound **(1)**, a combretastatin-like chalcone as the inhibitor of microtubule polymerization, exhibited the excellent cytotoxic activity against K562 cells with an IC_{50} of 1.1 μM.[12] A novel boronic acid chalcone **(2)** was reported by Kumar et al. as a putative MDM2 antagonist with antitumor activity against cultured tumor cells (Fig. 1).[13]

On the other hand, 1,2,3-triazole is a privileged scaffold in drug discovery with a wide array of biological activities as anti-fungal,[14] anti-

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bacterial,[15] anti-allergic,[16] anti-HIV,[17] anti-tubercular[18] and anti-inflammatory agents.[19] Recent research of its pharmacological effects became much more appealing and promising for the design of anticancer agents.[20] *N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl) arylamide was identified as a proprietary small molecule scaffold for potential antitumor agents by M.J. Miller group and compound **(3)** exhibited an IC_{50} of 46 nM against MCF-7 cancer cell line.[21] Compound **(4)**, a 1,2,3-triazol-naphthalimide hybrid, inhibited MCF-7 and SMMC-7721 cell lines with IC_{50} values of 0.348 and 0.258 μM, respectively(Fig. 1). [22]

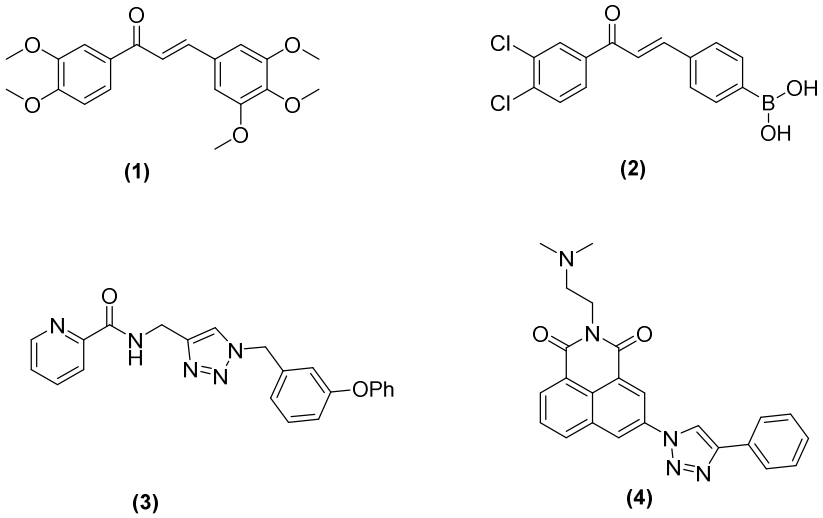

Fig. 1 Structures of chalcone and 1,2,3-triazole derivatives as antiproliferative agents previously reported

The study of new hybrid systems in which 1,2,3-triazole and chalcone are combined comprises an interesting field of research.[23-27] We have previously reported some 1,2,3-triazole-pyrimidine hybrids with good antiproliferative activity.[28] These findings have encouraged us to investigate the potential synergistic effect of 1,2,3-triazole and chalcone scaffolds. Herein, we report the hybridization of these two pharmacophores and their antiproliferative ability against the three selected tumor cell lines.

Azide intermediates **5a-e** (**Scheme 1**) and chalcones **6a-c** (**Scheme 2**) were efficiently prepared following our previous described method.[29] The general route for the synthesis of the target 1,2,3-triazole-chalcone analogues (**9a-g** and **12a-n**) was depicted in **Scheme 3**. Commercially available propargyl bromide reacted with *p*-acetylphenol or *p*-aminoacetophenone in the presence of potassium carbonate to form compound **7** or **10**, which was subjected to click reaction with appropriately azide intermediates to afford **8** or **11a-e** with good yields. **8** and **11a-e** reacted with substituted aromatic aldehyde at room temperature in a NaOH/EtOH solution to afford target compounds.

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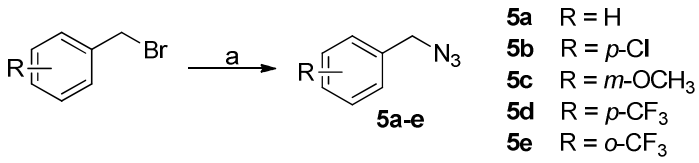
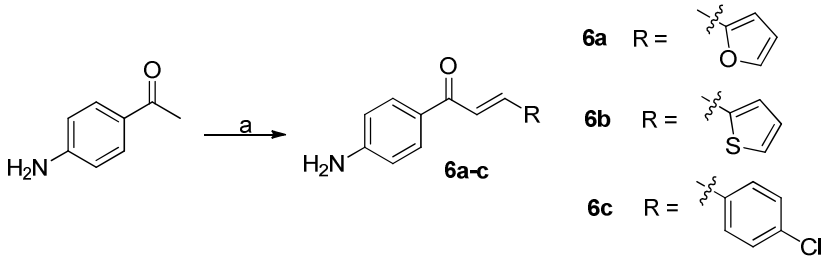
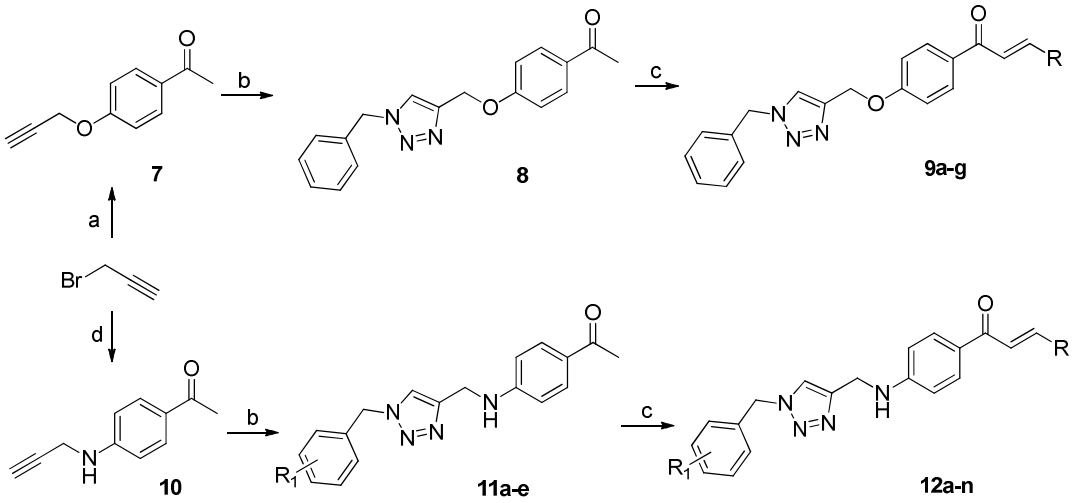


Scheme 1. Synthesis of azide intermediates **5a-e**. Reagents and conditions: (a) NaN_3, TBAB, acetonitrile, reflux.



Scheme 2. Synthesis of chalcone intermediates **6a-c**. Reagents and conditions: (a) substituted aromatic aldehyde, NaOH, EtOH, rt.



Scheme 3. Synthesis of 1,2,3-triazole-chalcone analogues. Reagents and conditions: (a) K_2CO_3, *p*-acetylphenol, acetone, reflux; (b) azide intermediates **5a-e**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, THF-H_2O (1:1), rt; (c) substituted aromatic aldehyde, NaOH, EtOH, rt; (d) K_2CO_3, *p*-aminoacetophenone, acetone, reflux.

All synthesized compounds were evaluated for their antiproliferative activity against three cancer cell lines, MGC-803 (human gastric cancer cell line), SK-N-SH (human neuroendocrine cancer cell line), and HepG-2 (human hepatocellular cancer cell line) using the MTT assay. The well-known anticancer drug 5-fluorouracil was used as the control.[30]

In order to investigate the effect of 1,2,3-trizole for inhibitory activity, non-1,2,3-triazole chalcones **6a-c** and 1,2,3-triazole chalcones **12h-j** were examined for antiproliferative activity and the results are summarized in **Table 1**. Removing the 1,2,3-triazole was clearly detrimental for the inhibitory activity against three cancer cell lines, such

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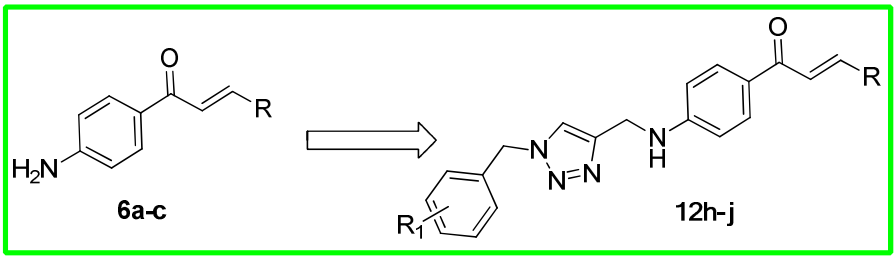
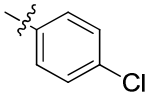
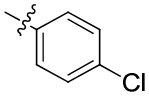
as compound **6a** vs **12h**, compound **6b** vs **12i**, compound **6c** vs **12j**. These modifications revealed that 1,2,3-triazole moiety may play a critical role in determining activity.

Table 1. Inhibitory results of preliminary evaluation against three cancer cell lines for the target compounds.



Comp.	R	R_1	IC_{50} (μM)[a]		
			SK-N-SH	HepG-2	MGC-803
6a		-	48.90 ± 1.03	> 64	> 64
12h		H	39.18 ± 0.89	42.46 ± 0.63	37.26 ± 0.75
6b		-	> 64	> 64	> 64
12i		*p*-Cl	30.88 ± 0.88	39.22 ± 0.46	29.64 ± 0.48
6c		-	58.22 ± 1.18	55.64 ± 1.07	61.23 ± 1.24
12j		*m*-OCH_3	21.22 ± 0.77	34.66 ± 0.98	38.63 ± 0.46
5-Fu	-	-	10.32 ± 0.74	10.30 ± 0.83	7.22 ± 1.04

[a]Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Data are presented as the means ± SDs of three independent experiments.

To complete the SAR study, a series of 1,2,3-triazole-chalcone hybrids were prepared and evaluated for their antiproliferative activity. The antiproliferative activity of compounds **9a−h** and **12d-g** against three cancer cells were determined initially and the IC_{50} values are shown in **Table 2**. Among them, compound **9g** showed the most potent activity with an IC_{50} of 5.47 μM against HepG-2 cells. We found that the substitution on the phenyl ring was important for the activity showing over 5-fold activity loss, when the *o, p*-diCl group was replaced with the *m*-F-*p*-(*p*-CH_3-pierazine) group (compound **9d** vs **9g**). In addition, compounds **9c** and **12f** with electron-donating trimethoxy group on the phenyl ring had more potent inhibitory effect (9.35 and 10.39 μM,

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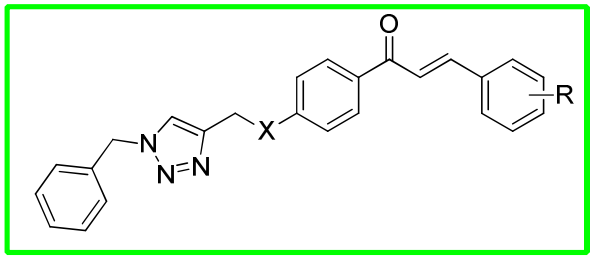
respectively) against HepG-2 cells than compounds (**9a-b**, **12d-e** and **12g**) (IC_{50} > 18 μM) with electron-withdrawing groups.

Table 2. Antiproliferative activity of the 1,2,3-triazole-chalcone derivatives



Comp.	X	R	IC_{50} (μM)[a]		
			SK-N-SH	HepG-2	MGC-803
9a	O	*o*-F	28.58 ± 1.47	18.44 ± 0.73	50.57 ± 1.20
9b	O	*p*-Cl	17.50 ± 1.24	21.01 ± 1.32	43.23 ± 0.94
9c	O	*o, m, p*-trimethoxy	14.53 ± 1.12	9.35 ± 0.73	37.25 ± 1.05
9d	O	*o,p*-diCl	36.35 ± 1.56	27.86 ± 1.45	> 64
9e	O	*o*-OCH_3	26.65 ± 1.36	24.84 ± 1.40	> 64
9f	O	*p*-NO_2	11.55 ± 0.90	24.35 ± 0.40	29.83 ± 1.19
9g	O	*m*-F-*p*-(*p*-CH_3-pierazine)	6.44 ± 0.42	5.47 ± 0.74	11.56 ± 1.38
9h	O	*m*-Cl	19.63 ± 1.29	24.02 ± 1.38	> 64
12d	N	*m, m*-diCl	20.48 ± 0.68	23.44 ± 0.54	16.78 ± 0.98
12e	N	*m, m*-diF	27.34 ± 0.93	> 64	23.48 ± 0.46
12f	N	*o, o, p*- trimethoxy	6.26 ± 0.32	10.39 ± 0.75	6.26 ± 0.32
12g	N	*p*-F	42.38 ± 0.73	38.78 ± 0.63	40.18 ± 0.95
5-Fu			10.32 ± 0. 74	10.30 ± 0.83	7.22 ± 1.04

[a]Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Data are presented as the means ± SDs of three independent experiments.

To determine whether the benzene ring and heterocycles have an effect on the activity, compounds with 4-pyridine ring (**9i** and **12b**), 3-pyridine ring (**9j** and **12c**), 2-furan ring (**12h**) were synthesized and their antiproliferative activity results were shown in **Table 3**. Replacing the trimethoxy phenyl scaffold of compound **12f** with 2-furan ring (**12h**) led to a loss of the activity. However, changing the trimethoxy phenyl ring (compound **12f**) to 3-pyridine ring (compound **12c**) led to a significant improvement of the activity against the tested cell lines. Among the pyridine-chalcones, compounds with 3-pyridine ring had better antiproliferative activity than compounds with 4-pyridine ring (compound

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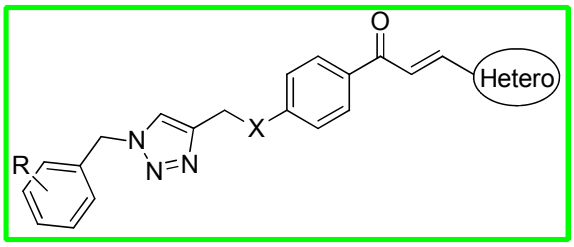
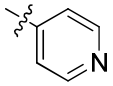
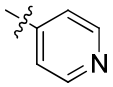
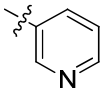
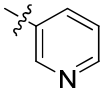
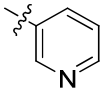
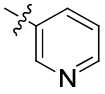
9j vs **9i**, compound **12b** vs **12c**). All the results indicated that the heterocycles may play an important role for their inhibitory activity.

Furthermore, the importance of heteroatom between chalcone and 1,2,3-trizole was investigated (**Table 3**). When the oxygen atom was replaced with a nitrogen atom (compound **9i** vs **12b**, compound **9j** vs **12c**), their antiproliferative activity were improved, indicating the significance of the nitrogen atom for their inhibitory activity.

These findings have encouraged us to synthesize more pyridine-chalcone derivatives with nitrogen atom (compound **12k-n**, **Table 3**). With all the selected three cancer cell lines, compound **12k-n** were more potent than 5-fluorouracil in the single-digit micromolar range. Especially, compound **12k** showed excellent inhibitory effect against the three tested human cancer cell lines with the IC_{50} value ranging from 1.53 to 2.73 μM. Compound **12k** with electron-donating methoxy group on the phenyl ring had more potent inhibitory effect (1.53 μM) against SK-N-SH cells than compounds (**12l-n**) (IC_{50} > 4 μM) with electron-withdrawing groups.

Table 3. Antiproliferative activity of the 1,2,3-triazole-heteroaryl chalcones



Comp.	R	X	Hetero	IC_{50} (μM)[a]		
				SK-N-SH	HepG-2	MGC-803
9i	H	O		7.43 ± 0.87	11.39 ± 1.06	26.13 ± 1.20
12b	H	NH		5.23 ± 0.68	7.02 ± 0.68	17.13 ± 0.57
9j	H	O		6.37 ± 0.15	5.22 ± 0.37	10.16 ± 1.54
12c	H	NH		5.15 ± 0.49	4.37 ± 0.38	3.43 ± 0.56
12h	H	NH		39.18 ± 0.88	42.46 ± 0.63	37.26 ± 0.75
12k	*m*-OCH_3	NH		1.53 ± 0.13	2.21 ± 0.44	2.73 ± 0.46
12l	*o*-CF_3	NH		7.29 ± 0.10	6.92 ± 0.46	3.15 ± 0.53

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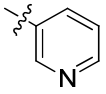
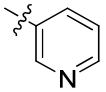
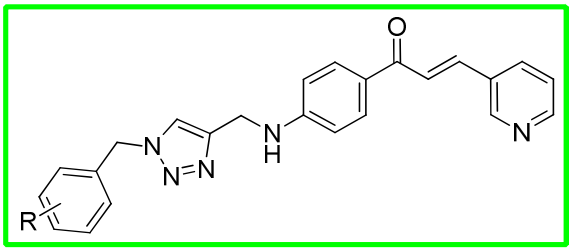
12m	*p*-CF_3	NH		7.26 ± 0.30	9.24 ± 0.36	7.19 ± 0.25
12n	*p*-Cl	NH		4.36 ± 0.43	9.32 ± 0.87	7.03 ± 0.48
5-Fu		-	-	10.32 ± 0.74	10.30 ± 0.83	7.22 ± 1.04

[a]Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Data are presented as the means ± SDs of three independent experiments.

Compounds **12k** and **12l** were further examined for possible toxicity against GES-1 (normal human gastric epithelial cell line). As can be seen in **Table 4**, we found that compounds **12k** and **12l** exhibited no significant toxicity against GES-1 (31.13 and 54.70 μM, respectively). However, compounds **12k** and **12l** exhibited remarkable toxicity against MGC-803 cancer cell line (2.73 and 3.15 μM, respectively). The results indicated that compounds **12k** and **12l** had good selectivity between cancer and normal cells.

Table 4. Inhibitory results of 1,2,3-triazole-chalcones against GES-1 cell line.



Comp.	R	IC_{50} (μM)[a]
12k	*m*-OCH_3	31.13 ± 1.56
12l	*o*-CF_3	54.70 ± 1.35
5-Fu	-	7.22 ± 1.04

[a]Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to GES-1 cell proliferation by 50% (IC_{50}). Data are presented as the means ± SDs of three independent experiments.

Apoptosis defects in cancer cells are the primary obstacle that limits the therapeutic efficacy of anticancer agents, hence the development of novel agents targeting programmed cell death has become an imperative mission for clinical application.[31] Due to the excellent cytotoxic activity against all tested cancer cell lines, compound **12k** was chosen to be further investigated regarding its mechanism of action. After 24 h incubation with **12k** at indicated concentrations, characteristic apoptotic morphological changes were observed by fluorescence microscope, including cell rounding, chromatin shrinkage, and formation of apoptotic

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bodies (Fig. 2). To characterize the mode of cell death induced by compound **12k**, the apoptotic analysis was also performed with Annexin V-FITC/PI double staining and quantitated by flow cytometry.[32] Treatment of S-KN-SH cells with compound **12k** increased, in concentration-dependent manner, the percentage of the apoptotic cells was up to 6.9%, 7.9%, 12.1%, 50.7% and 97.4%, respectively, compared to control (8.8%) (Fig. 3A and 3B).

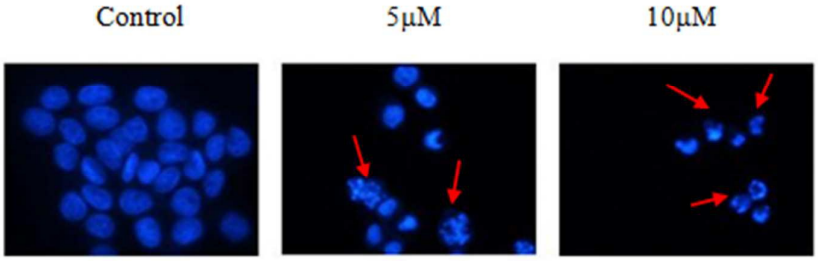
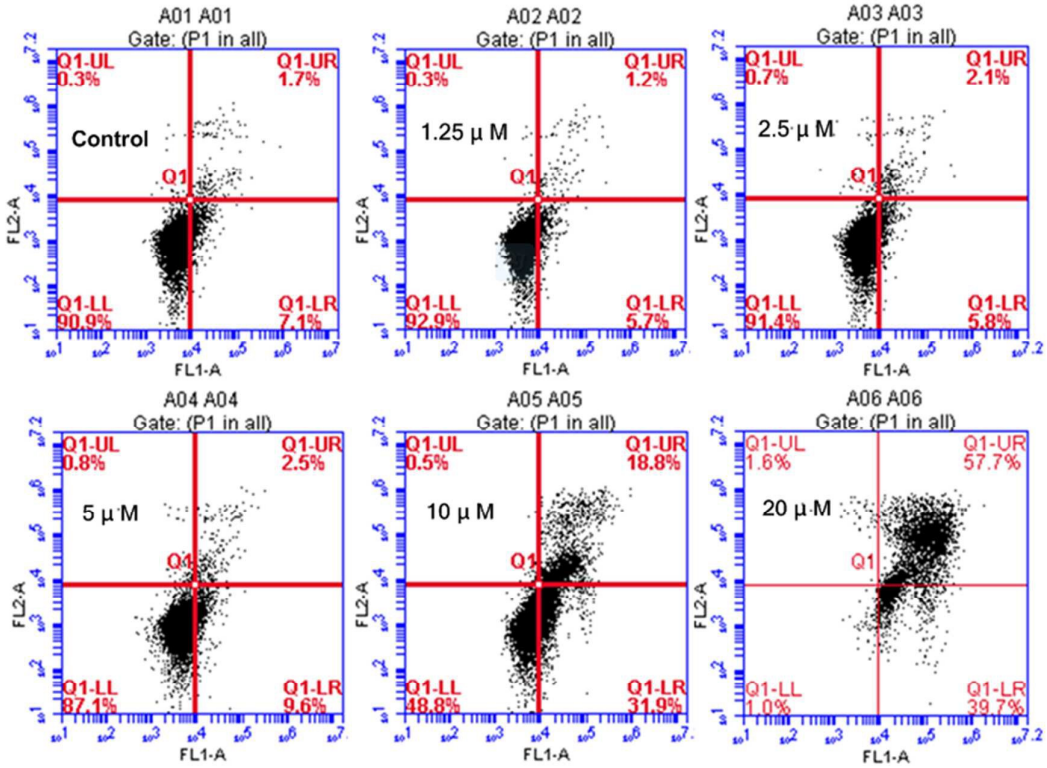


Fig. 2 Apoptosis analysis with Hoechst-33258 staining after a 24 h treatment of compound **12k** in S-KN-SH cells.



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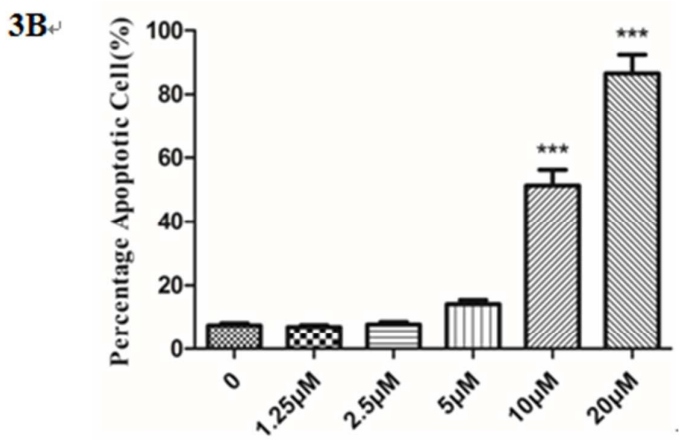

Fig. 3 Compound **12k** induced apoptosis in S-KN-SH cells (3A and 3B). Quantitative analysis of apoptotic cells using Annexin V-FITC/PI double staining and flow-cytometry calculation. ***$P < 0.01$ was considered statistically significant. Dates are mean ± SD. All experiments were carried out at least three times.

To have a better understanding of the mechanism of action of cytotoxic activity of compound **12k**, the effect of compound **12k** on the cell cycle was investigated by treating S-KN-SH cells with different concentrations (2.5, 5, 10 μM).[26] After treatment S-KN-SH cells for 24 h, it was observed that the percentage of cells in G1 phase at different concentrations were 67.06%, 71.27%, and 81.80%, respectively (Fig. 4). The results suggested that **12k** caused an obvious G1 arrest in a concentration-dependent manner with a concomitant decrease in terms of the number of cells in other phases of the cell cycle.

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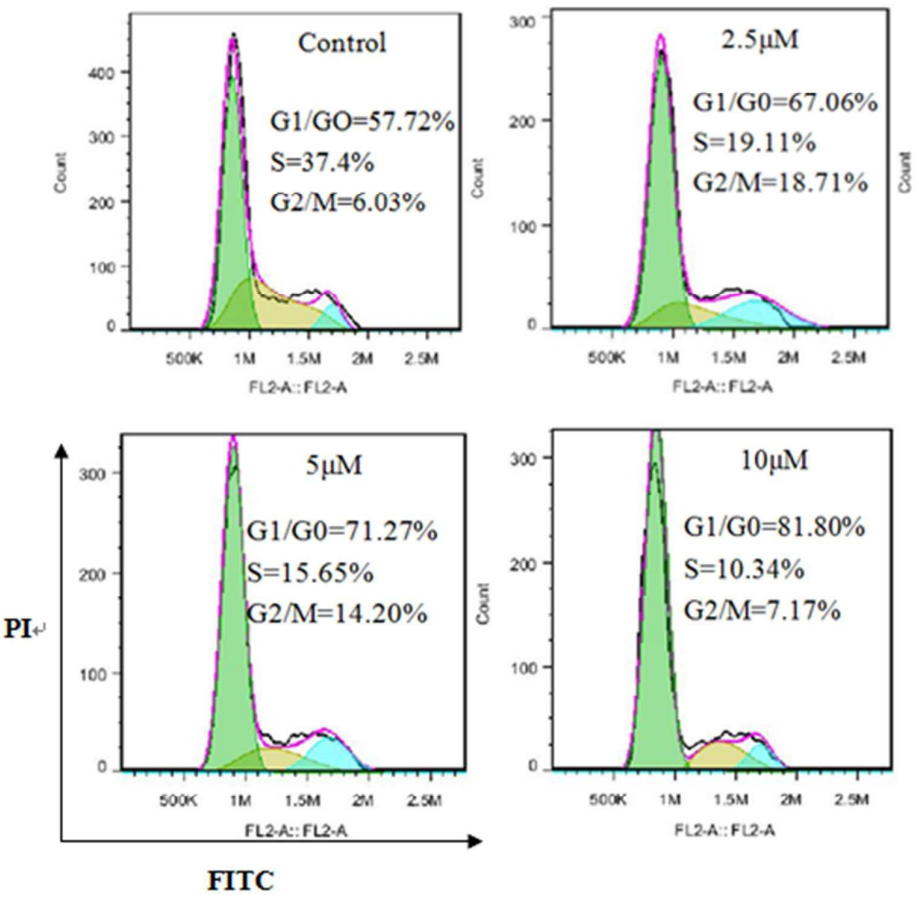

Fig. 4 Effect of compound **12k** on the cell cycle of S-KN-SH cells

At the same time, S-KN-SH cells were cultured with different concentrations of compound **12k** for 24 h, both adherent and floating cells were collected and then Western blot analysis was performed (Fig. 5). At the same time, the promising compound **12k** decreased the level of pro-caspase 3 and increased the level of active-caspase 3 and p53, which was consistent with the result of flow cytometry analysis in inducing apoptosis.

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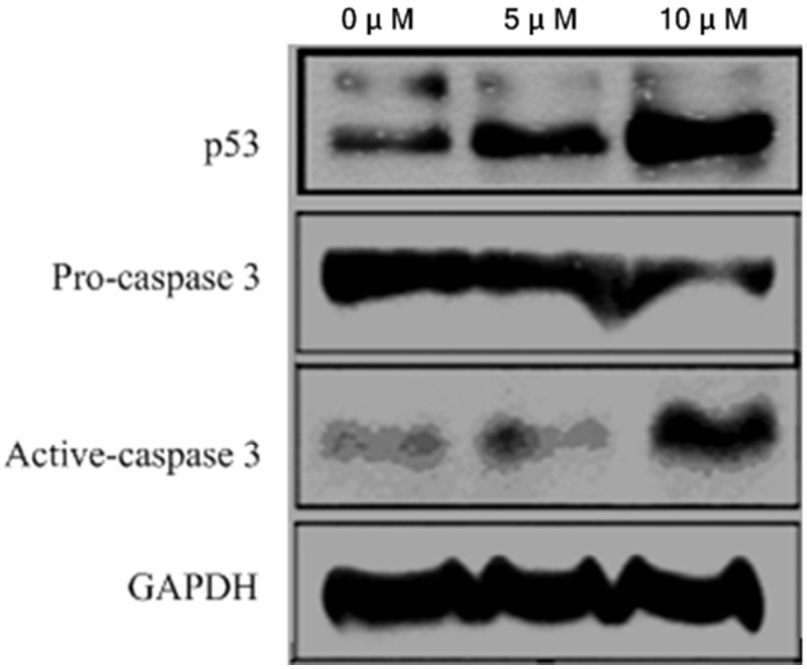

Fig. 5 Compound **12k** regulated related protein expression in S-KN-SH cells. Equal amounts of protein from whole-cell extracts were separated by SDS-PAGE.

In summary, we have discovered a new class of 1,2,3-triazole-chalcone hybrids displaying good inhibitory. Compound **12k** was more potent than 5-FU against three human cancer cell lines. Further investigation indicated that compound **12k** induced cell apoptosis and arrested cell cycle at G1 phase. Further mechanism investigations are underway and will be reported in due course.

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12k exhibited an IC_{50} value of 1.53 μM against SK-N-SH cells by inducing apoptosis and arresting the cell cycle at G1 phase.