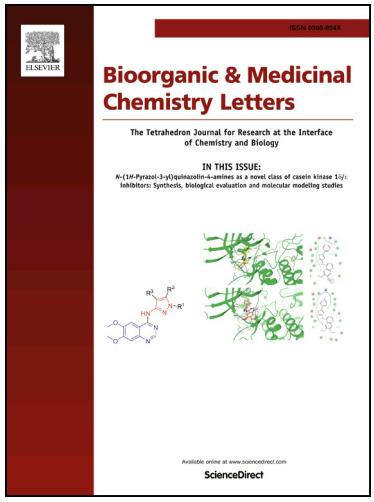

Discovery of novel 4(1H)-quinolone derivatives as potential antiproliferative and apoptosis inducing agents

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Discovery of novel 4(1H)-quinolone derivatives as potential antiproliferative and apoptosis inducing agents

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Abstract

A series of novel 4(1H)-quinolone derivatives was synthesized and evaluated for antiproliferative activity *in vitro*. The results showed that these compounds exhibited more potent antiproliferative effect against a panel of human tumor cell lines than the lead compound 7-chloro-4(1H)-quinolone **1**. Compound **7e** was found to be the most potent antiproliferative agent and to exhibit selective cytotoxic activity against HepG2 cell lines with IC_{50} value lower than 1.0 μM. Annexin V/FITC-PI assay showed that compound **7e** induced apoptosis in HepG2 cells with a dose-dependent manner. Western blotting analysis indicated that compound **7e** induced cell cycle arrest in G2/M phase by p53-depedent pathway.

Keywords: Synthesis; Quinolone; Antiproliferative Activity; Apoptosis.

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Quinolones represent a large group of naturally occurring and synthetic alkaloids. Since the discovery of nalidixic acid by Lesher in 1962, numerous quinolones have been prepared in search of more potent antibacterial agents and the structure-activity relationships of quinolones have been extensively investigated[1-3]. Quinolones were initially discovered to be specific inhibitors of the type II bacterial topoisomerase such as DNA gyrase[3-5]. Recently, topoisomerase IV[6] and penicillin-binding proteins (PBPs)[7] were also found to be the pharmacological target of this class of compounds. Due to structural and functional similarities between bacterial DNA gyrase and mammalian topoisomerase II[8], in the past decade, considerable attention was attracted to the antitumor potencies of quinolones and numerous investigations on structural modification and structure-activity relationships of quinolone derivatives as promising antitumor agents have been reported[9-14]. Consequently, quinolones have become potential lead compounds in the development of cancer chemotherapeutic agents.

An initial lead compound that interested us was the 7-chloro-4(1H)-quinolone **1** (**Figure 1**), which exerted antitumor effect by blocking DNA synthesis and damaging DNA template of tumor cell[15]. Its capsule was approved by the SFDA (State Food and Drug Administration of China) as antitumor drug for late mammary cancer and non-small cell lung cancer treatment in 2003[16]. However, **1** has limited utility for

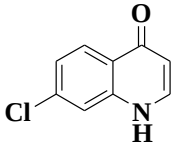

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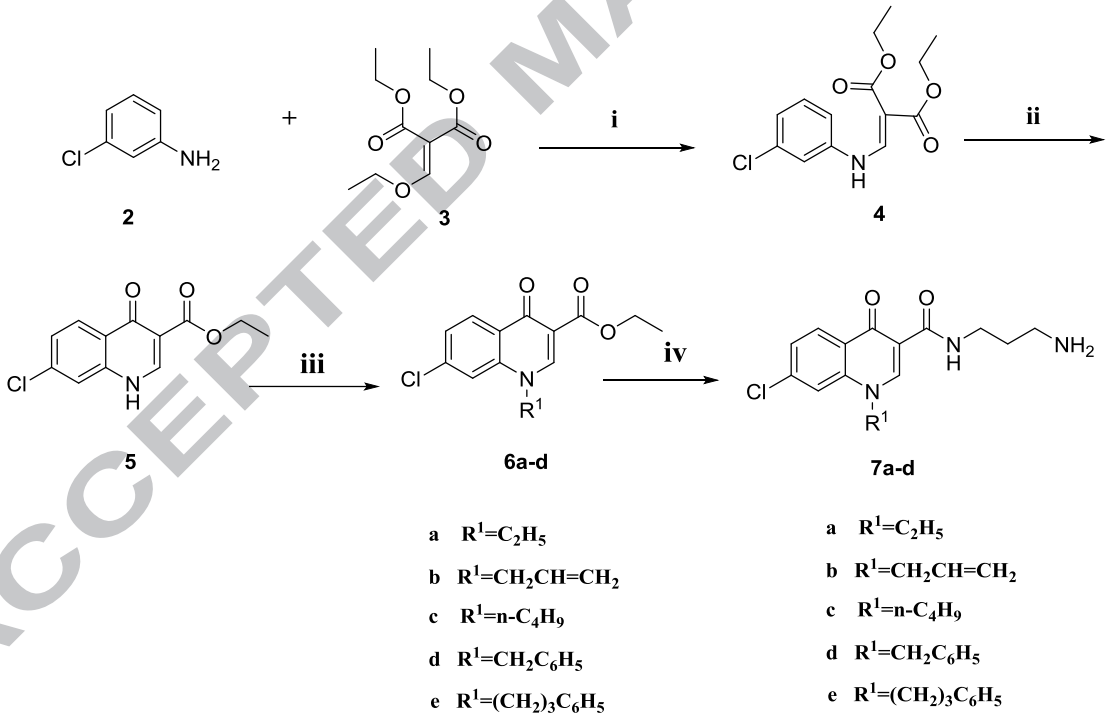
Figure 1 The chemical structure of the lead compound 7-chloro-4(1H)-quinolone

cancer therapy because of its poor solubility, which has only moderate solubility even in DMSO. To circumvent the solubility problem in the 7-chloro-4(1H)-quinolone **1** and find congeners more potent as potential antitumor agents, in the present investigation, we prepared five quinolone derivatives incorporating an alkyl substituent at position-1 as well as bearing a flexible amino side chain in position-3. We report herein the preparation of novel 7-chloro-4(1H)-quinolone derivatives, their cytotoxic activities and their underlying mechanism of action as antitumor agents.

The synthetic routes of novel 4(1H)-quinolone derivatives **7a-e** are outlined **Scheme 1**. The intermediates **4-6** were prepared according to already published



Scheme 1 Synthesis of compounds 7a-e. Reagents and conditions**:** (i) stirred at 100 ℃; （ii） diphenyl ether, reflux; (iii) DMF, NaH, alkyl halogenide, stirred at RT;（iv）$NH_2CH_2CH_2CH_2NH_2$, microwave irradiation, 150 ℃, 15 min.

methods[17]. Amination of carboxylates **6a-e** with an excess of diamines without solvent by subjecting to microwave irradiation (150℃, 15 min) provided the targeted compounds **7a-e** in 45-70% yield. The chemical structure of all the newly synthesized compounds was characterized by MS, HRMS, ^{1}H NMR and ^{13}C NMR spectra.

The antiproliferative effects of novel quinolone derivatives **7a-e** were investigated and compared with the reference drugs adriamycin, cisplatin and 7-chloro-4(1H)-quinolone **1** by MTT assay against a panel of tumor cell lines. In order to enhance water solubility, all compounds were prepared in the form of hydrochloride salt before use. The tumor cell line panel was consisted of human laryngeal carcinoma (Hep-2), breast carcinoma (MCF-7), gastric carcinoma (BGC-823), liver carcinoma (HepG2), colorectal carcinoma (HCT-8), cervical carcinoma (Hela) and prostate carcinoma (PC-3). The results were summarized in **Table 1**. As shown in **Table 1**, 7-chloro-4(1H)-quinolone **1** failed to display cytotoxic activities against seven human tumor cell lines at the concentration of 50.0 μM, and the poor water-soluble property might be contributed to its weak activity. While compounds **7a-e** bearing a flexible amino side chain displayed potent cytotoxic potencies against seven human tumor cell lines with IC_{50} values lower than 50.0 μM. Interestingly, compound **7e** bearing a (3-phenyl) propyl group in N1 of quinolone was found to be the most potent cytotoxic agent with IC_{50} values lower than 10.0 μM against seven human tumor cell lines investigated. In addition, compound **7e** exhibited selective cytotoxic activity with IC_{50} values of lower than 1.0 μM against HepG2 cell lines. These results indicated that the bulky substituent at position-1 might facilitate

Table 1 Cytotoxic activities of quinolones derivatives **7a-e** *in vitro*

Compds	IC_{50} (μM)±SD [a]							ClogP [c]
	Hep-2 [b]	MCF-7	BGC-823	HCT-8	HepG2	HeLa	PC-3	
7a	28.2±2.6	35.5±4.6	30.2±2.8	31.6±4.2	13.8±2.0	20.0±1.8	12.6±1.6	1.148
7b	36.5±4.8	31.6±3.2	20.0±3.2	34.7±3.5	17.0±2.8	22.4±3.4	13.2±1.4	1.393
7c	21.7±2.4	12.6±2.1	16.6±1.8	22.3±3.2	5.23±0.48	15.0±2.0	12.0±2.2	2.206
7d	8.31±1.2	5.02±0.68	6.71±1.0	14.5±1.8	5.42±0.82	4.54±0.36	9.12±1.0	3.177
7e	3.24±0.42	3.12±0.44	3.20±0.65	6.03±0.58	<1.0	2.31±0.42	6.92±0.82	3.095
1	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	1.053
Adriamycin	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	-
Cisplatin	10.8±1.8	9.62±1.2	8.88±0.72	10.2±1.6	6.68±0.58	12.4±1.4	8.92±0.61	-

[a] Cytotoxicity as IC_{50} for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay. Values with standard deviations (SD) are averages of at least three independent determinations.

[b] Cell lines include human laryngeal carcinoma (Hep-2), breast carcinoma (MCF-7), gastric carcinoma (BGC-823), liver carcinoma (HepG2), colon carcinoma (HCT-8), cervical carcinoma (Hela), prostate carcinoma (PC-3).

[c] ClogP represent the calculated n-octanol/water partition coefficient (log Pow), and the values produced by Chemdraw software.

antiproliferative potency.

It is noted that compound **1** having ClogP values of 1.05 failed to exhibit antiproliferative activity at the concentration of 50 μM, and compound **7a, 7b** and **7c** with low ClogP values also exhibited weaker antiproliferative potency, while compound **7d** and **7e** having slightly high ClogP values showed more potent antiproliferative effect. These results suggested that the ClogP values of the whole molecule seemed to be a very important determinant factor of the antiproliferative properties of this class of compounds.

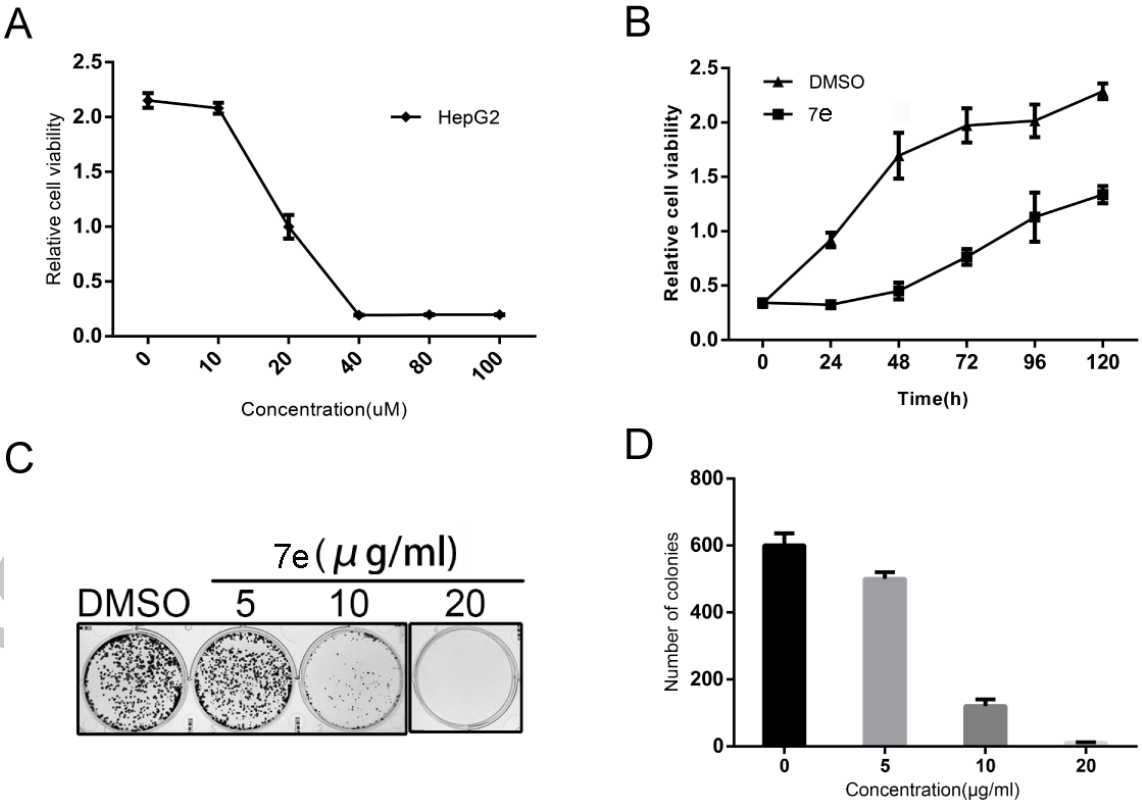


Figure 2 Compound 7e inhibited the viability of HepG2 cell lines. (**A)** HepG2 cells were seeded in 96 well plate, after 24 h, the cells were treated with indicated concentration of compound **7e** for 24 h. The cell viability was measured with CCK-8 assay. (**B)**1000 HepG2 cells were seeded in 96 well plate for 24 h, treated indicated time with 10 μM compound **7e**. The cell viability was measured with CCK-8 assay. (**C** and **D)** The number of colonies of HepG2 cells was counted after the treatment with indicated concentration of compound **7e** for 24 h.

We also examined the inhibitory effect of the selected compound **7e** on growth of HepG2 using the Cell Counting Kit-8(CCK-8) assay. Compared with the vehicle treated cells, compound **7e** inhibited efficiently the proliferation of HepG2 in the time-dependent manner (**Figure 2A**). To investigate whether compound **7e** affected tumorigenicity-correlated responses *in vitro*， then plate colony formation assay was performed. The data indicated that the single HepG2 cell were more sensitive to compound **7e** at the concentration of 10 μM (**Figure 2 and 2C)**. These results suggested that compound **7e** not only inhibited proliferation of HepG2, but also induced cell death.

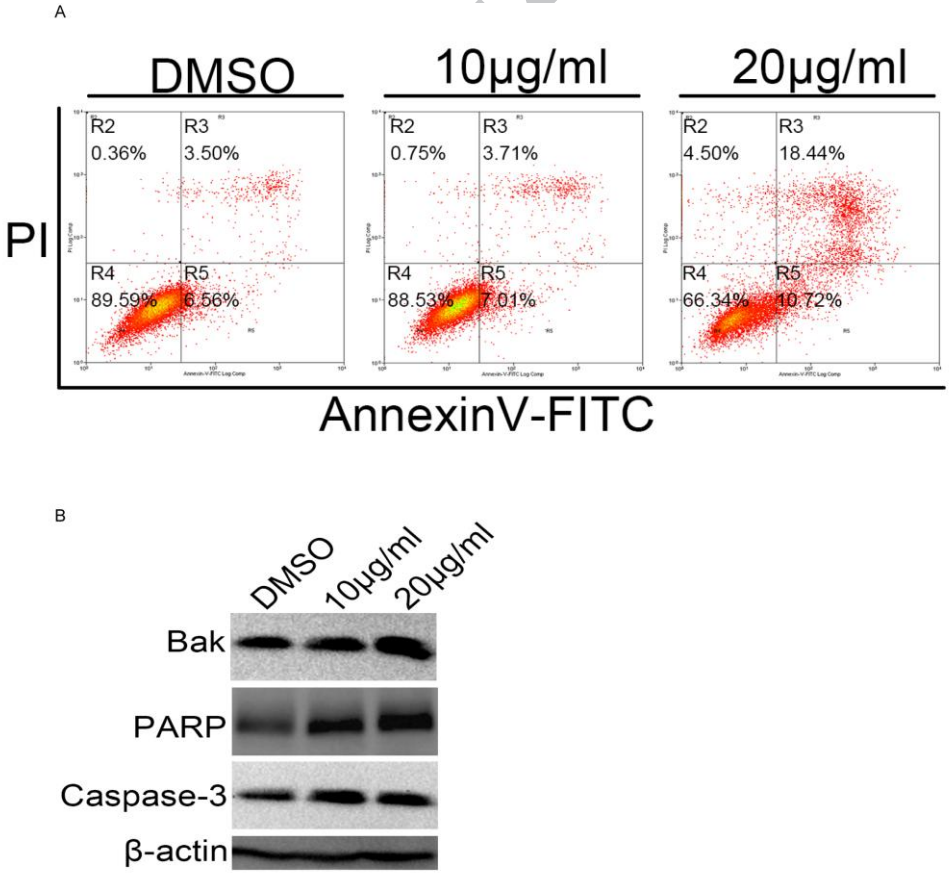


Figure 3 Compound 7e induced apoptosis in HepG2 cells. (**A**) HepG2 cells were treated with indicated concentration of compound **7e** for 24 h and then binding with Annexin V/FITC-PI. The results were analyzed with flow cytometry. (**B**) The protein levels of Bak, PARP and Caspase-3 were detected by western blot, β-actin was used as an internal control.

To evaluate which type of cell death induced by compound **7e**, we performed Annexin V/FITC-PI assay. Obviously, compound **7e** induced apoptosis in HepG2 cell lines with a dose-dependent manner (**Figure 3A**). We detected the level of maker protein about apoptosis, the level of Bak, a pro-apoptotic protein, increased by stimulating of compound **7e**, However, the quantity of PARP (poly ADP-ribose polymerase) and Caspase-3 increased instead of decreasing in a dose-dependent manner (**Figure 3B**). Possibly, PARP is thought to be a receptor for DNA damage, which is activated by identifying structural damage of DNA. Thus, PARP plays a important role in repairing the DNA which is suffered damage from compound **7e**.

Stimulated with different concentrations of compound **7e**, the cell cycle of HepG2 was detected by PI staining, the results demonstrated that the phrase of G2/M was arrested dramatically with increasing concentration of compound **7e** (**Figure 4A**). Subsequently, we screened the 10 signaling pathways purchased from Qiagen using luciferase assay in HepG2 cell lines after compound **7e** treatment. The results indicated that the luciferase activities of several pathways were altered with different levels. Particularly, the activity of p53 increased by about 1.5 times (**Figure 4B**). p53 plays an important regulation role in phase of G2/M. Western blotting analysis was used to evaluate the activity of p53 and G2/M phase related protein kinases after compound **7e** treatment, p53 and p21, a downstream gene of p53, increased dramatically in protein level, and Cyclin B1 and CDK1 decreased in a dose-dependent manner (**Figure 4C**). These results suggested that compound **7e** induced cell cycle arrest in G2/M phase by p53-depedent pathway possibly.

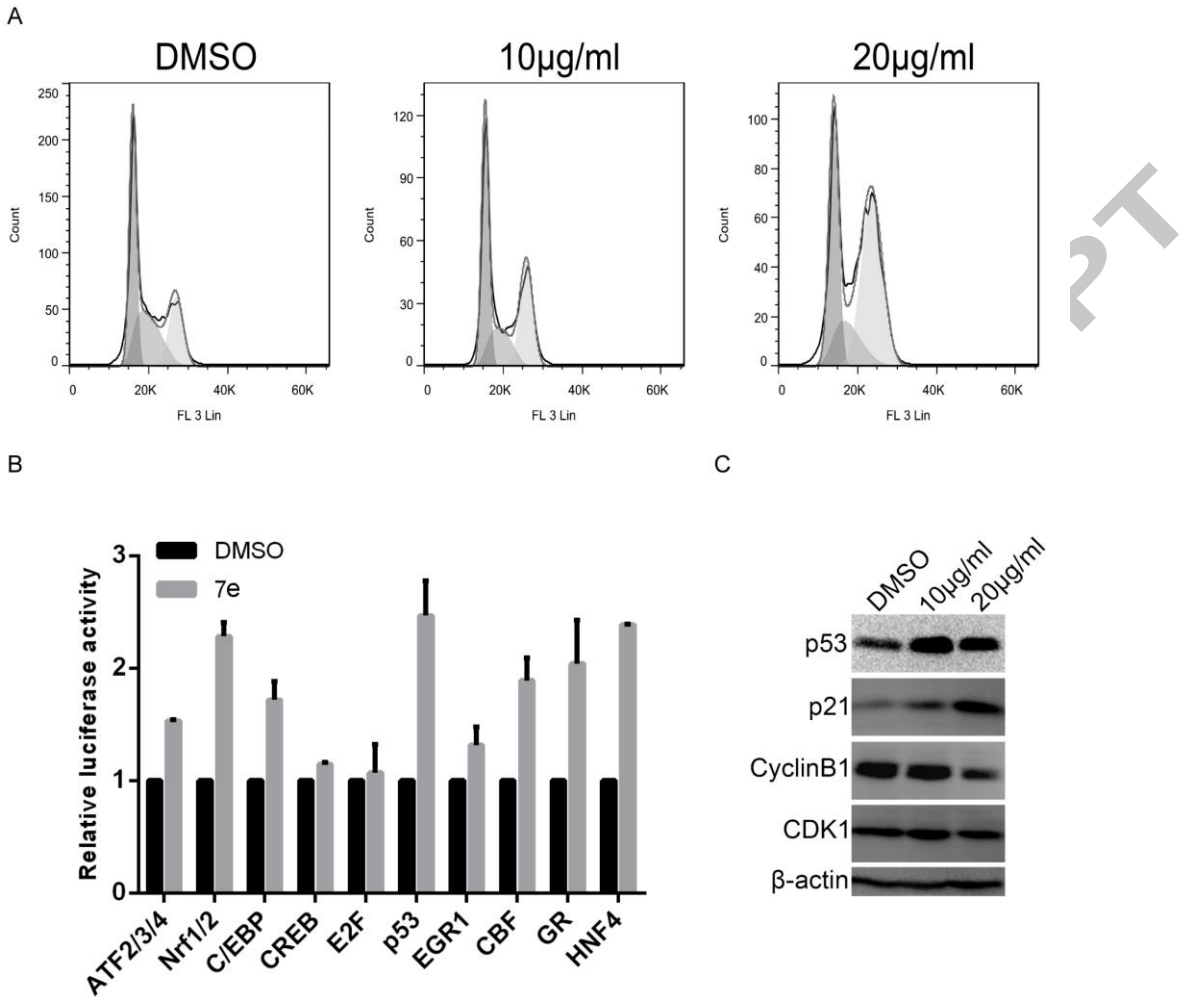


Figure 4 Compound 7e induced cell cycle arrest in G2/M phase. (**A**) HepG2 cells were treated with indicated concentration of compound **7e** for 24 h and cell cycle was assayed with PI staining. The results were analyzed with flow cytometry. (**B**) Luciferase activity of different signaling pathway reporters after treatment with compound **7e**. HepG2 cells were transfected with the indicated pathway reporter for 24 h, treated with 4 μM of compound **7e** for another 24 h and then performed the dual-luciferase assay. **(C)** The protein levels of p53, p21, Cyclin B1 and CDK1 were detected by western blot, β-actin was used as an internal control.

In conclusion, we have synthesized a series of novel quinolones incorporating an alkyl substituent at position-1 as well as a flexible amino side chain in position-3. These compounds exhibited more potent antitumor activities against a panel of human tumor cell lines than the lead compound 7-chloro-4(1H)-quinolone **1**. And compound **7e** was found to be the most potent antitumor agent and exhibit selective cytotoxic

activity against HepG2 cell lines with IC_{50} value lower than 1.0 μM. Compound **7e** induced apoptosis with a dose-dependent manner in HepG2 cells. Western blotting analysis indicated that compound **7e** induced cell cycle arrest in G2/M phase by p53-depedent pathway. Undoubtedly, to acquire more information about the structural requirements for improving cytotoxic activities, the synthesis of more new quinolones with different substituent at other positions is needed.

Acknowledgments

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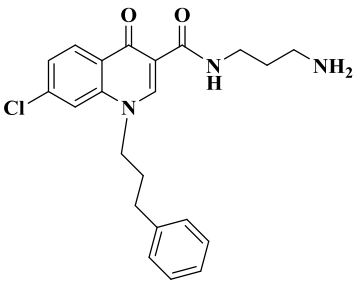
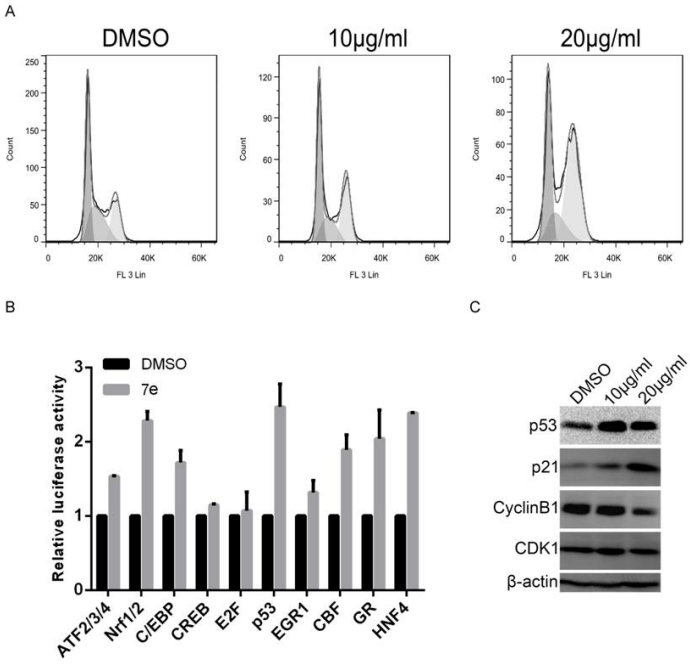
Graphic abstract



7e



The representative compound **7e** could induce apoptosis in HepG2 cells with a dose-dependent manner and cell cycle arrest in G2/M phase by p53-depedent pathway. .