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8-Hydroxyquinoline derivatives induce the proliferation of rat mesenchymal stem cells (rMSCs)

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Abstract—A series of 8-hydroxyquinoline derivatives with different substituted groups at 2- or 5-position have been synthesized and characterized. Their effects on the proliferation of the rat marrow-derived mesenchymal stem cells (rMSCs) have been evaluated by MTT assay and flow cytometry. We also analyzed the ability of these compounds to regulate the proliferation of rMSCs and the relationship with the structures of 8-hydroxyquinoline. Compounds **8**–**11**, in which, the vinyl-substituents are on the 2-position of 8-hydroxyquinoline, appear to be able to induce the proliferation of rMSCs. These results show that compounds **8**–**11** provide a kind of new substances for regulating the proliferation of rMSCs.

1. Introduction

The mesenchymal stem cells (MSCs) have been demonstrated as an attracted cell source for tissue-engineering applications, offering significant advantages over other stem cell, because of their ability to be easily isolated and expanded from bone marrow aspirates and their versatility for differentiation into organs and tissues[1–3]. They have recently been used clinically to treat osteogenesis imperfecta and some neurological diseases. But the amount of MSCs in the adult bone marrow is small and the ability of proliferation is weak,[4] so it is crucial to develop new substances to grow and expand the rMSCs in vitro on a large scale.

Recently, several small heterocyclic molecules have been demonstrated to be capable of inducing stem cell proliferation and differentiation in vitro.[5,6] 8-Hydroxyquinoline derivatives are important constituents in a variety of pharmaceutically important compound classes. They have become of interest as a new class of potent HIV-1 integrase inhibitors,[7] in modeling of the inhibition of retroviral integrases,[8] protein tyrosine kinase inhibitors,[9] protozoal and retroviral co-infections,[10] and anti-HIV-1 agents.[11] In our previous papers,[12] we reported that carbazole connected to the fullerene moiety played an important role to assist the electron-donating ability and hole delocalization, and generated a long-lived charge-separated state. Now we are surprised to find out that the carbazole covalently linked to 8-hydroxyquinoline has the ability to induce the proliferation of rat mesenchymal stem cells (Table 1). The structures of these compounds were characterized by EI-MS, ^{1}H NMR spectroscopy, elemental analysis, and IR spectroscopy. To the best of our knowledge, reports about 8-hydroxyquinoline containing a triphenylamine unit, an 8-hydroxyquinoline unit, and a carbazole unit are novel. MTT assay and flow cytometric analysis were performed to evaluate the effects of these compounds on proliferation of rMSCs.

2. Results and discussion

A series of 8-hydroxyquinoline derivatives as shown in Table 1 have been synthesized successfully. The effects of these compounds on the proliferation of rMSCs were measured by MTT assay. When the experimental groups were compared with the control group, it was found that most of the compounds could induce proliferation of the rat mesenchymal stem cells.

Keywords: Rat mesenchymal stem cells (rMSCs); Proliferation; 8-Hydroxyquinoline derivatives.
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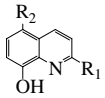
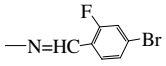
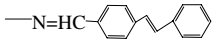
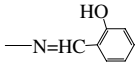
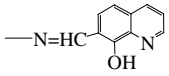
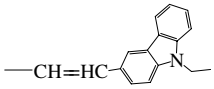
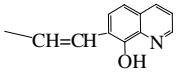
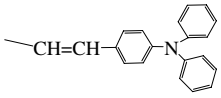
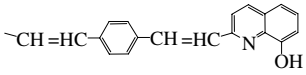
Table 1. Chemical structure of 8-hydroxyquinoline derivatives

	R_1	R_2
		
1	–H	$-NH_2$
2	$-CH_3$	–H
3	–H	–H
4	–H	
5	–H	
6	–H	
7	–H	
8		–H
9		–H
10		–H
11		–H

8-Hydroxyquinoline derivatives, proposed as chemiluminescent probes, were highly fluorescent. In order to exclude the possibility that the 8-hydroxyquinoline derivatives may influence the absorbance, contrast groups were added and compared with the experimental groups before control group. We found that the experimental groups for compounds **1**, **4**–**7** produced false-positive results. Therefore, only compounds **8**–**11** have effects on the proliferation of rMSCs. Figure 1 shows the comparison of proliferation activities of rMSCs influenced by compounds **8**–**11** with different concentration grades.

The optical densities (OD) increased with the increasing concentration in the experimental groups added compounds **8**, **10**, and **11**, respectively. The optical densities of rMSCs treated with compound **8** at the concentration of 0.7132×10^{-3} mol/L and compound **10** at the concentration of 0.8140×10^{-3} mol/L were lower than that of control group; while the others were higher. However, no significant differences were found in the optical densities of compound **10** at the concentration of 3.984×10^{-3} mol/L. The optical densities of compound **9** at the concentration of 0.7389×10^{-3} mol/L and 7.34×10^{-3} mol/L were almost the same, which concentration maybe exceeded the certain range. In our experiments, the optical densities of rMSCs treated with compound **11** were the highest. MTT assay revealed a significant increase of rMSCs treated with compounds **8**–**11**, especially **8**, **9**, and **11**.

In order to demonstrate the ability of the proliferation of the rMSCs induced by these compounds further, compounds **8**, **9**, and **11** were selected to conduct the flow cytometric analysis at two concentrations. Figure 2 shows the percent proliferation index (PI) of the control group, solvent group, positive contrast group added bFGF (basic fibroblast growth factor), and experimental groups added compounds **8**, **9**, and **11**.

Compared with the control group, the PI increased in the experimental groups added compounds **8**, **9**, and **11**. However, the values of adding compound **9** at 3.182×10^{-4} mol/L and compound **11** at 2.401×10^{-4} mol/L were not significant. bFGF, known to cause cell proliferation, has been used as positive contrast group to compare the ability of proliferation with these compounds. It is found that the PI in the presence of compounds **8** and **9** is also statistically significantly higher than that of the bFGF, while compound **11** shows no significant difference at the concentration of

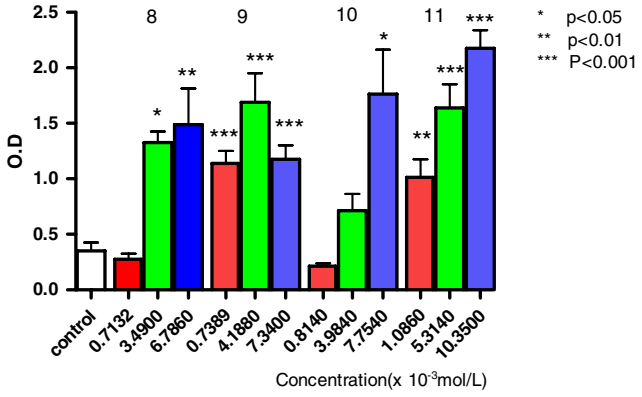


Figure 1. Effect of compounds **8**–**11** on the proliferation of rMSCs by MTT assay. Each bar represents means ± SD from five independent experiments. Values are statistically significantly higher than control group (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

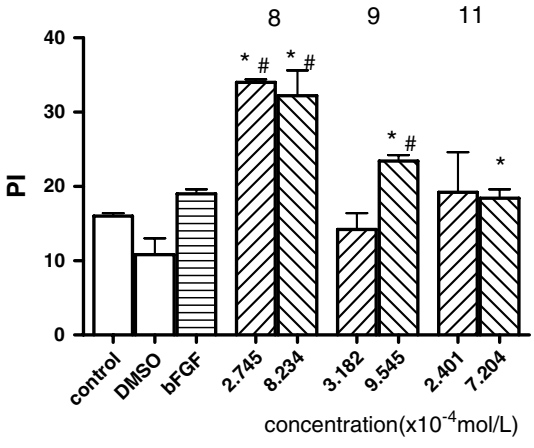


Figure 2. Effects of compounds **8**, **9**, **11** on the PI of rMSCs by cell cycle analysis. Each bar represents means ± SD. * and # denote values that are statistically significantly higher than control group and contrast group, respectively ($p < 0.05$).

7.204×10^{-4} mol/L. The results indicate that compounds **8**, **9**, and **11** are good stimulators for inducing proliferation of the rMSCs, and especially compounds **8** and **9** are powerful.

The results suggest that the structures of the derivatives are very important for the proliferation activities of rMSCs. 8-Hydroxyquinoline derivatives with vinyl-substituent at 2-position can induce the proliferation of rMSCs. On the other hand, 5-imino-substituted 8-hydroxyquinoline derivatives have no effect on the proliferation of rMSCs.

3. Summary

A series of 8-hydroxyquinoline derivatives have been synthesized successfully, and MTT assay and flow cytometric analysis were used as proliferation index to evaluate the effects of these compounds. The proliferation activities of rMSCs are found to depend on the nature of the substituents present on the 8-hydroxyquinolines. Compounds **1**–**7** are inactive in promoting the proliferation of rMSCs due to the imino-substituents on the 5-position of 8-hydroxyquinolines; while compounds **8**–**11** are active since the vinyl-substituents are on the 2-position. The results of flow cytometric analysis were compatible with the MTT assay, suggesting that 8-hydroxyquinoline derivatives **8**–**11** stimulated DNA content synthesis as well as increased cell numbers in rMSCs. The results indicate that compounds **8**–**11** are the powerful stimulators for the proliferation of rMSCs, provide small organic molecules as new stimulators to induce the proliferation of stem cells, and imply the importance of rMSCs in tissue repair. The cell proliferation has a close relationship with the growth, regeneration, repair after trauma, and process of programmed cell death, and it is also the basis for forming tissue, organ, and system by cell differentiation.

4. Materials and methods

4.1. Isolation and in vitro expansion[13] of rMSCs

The rat bone marrow was washed out with low-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco Life Science) containing 10% fetal bovine serum (FBS), fully mixed, and centrifuged at 300*g* for 10 min, and then the supernatant was removed. The remainder was re-suspended in low-glucose DMEM and after centrifugation to remove the supernatant, 4 mL low-glucose DMEM was added and thoroughly mixed. Four milliliters of Percoll (Sigma) preservative solution diluted at 0.56:0.44 was added into a 10-mL centrifuge tube, before the bone marrow was gently added from 2 cm above the surface of the former solution, centrifuged at 900*g* for 30 min. The mononuclear cell layer was collected and washed with DMEM twice, then the cell number was counted and adjusted to the concentration of 1×10^9 L^{-1}, followed by cell culture in low-glucose DMEM supplemented with 10% fetal bovine serum in the presence of 5% CO_2 with saturated humidity at 37 °C. Five days later, the culture medium was replaced and the unattached cells were removed. The culture medium was subsequently changed every 3–4 days and the rMSCs growing to nearly confluence were digested at room temperature with 0.25% pancreatin for 2–3 min. The rMSCs 1×10^4 in number were then passaged to the fifth generation, reaching a total cell number of 1×10^7. The cultured cells were identified by marking of Bromodeoxyuridine (Brdu, Sigma. Chemical, St. Louis, MO), staining of CD44 (PharMingen; San Diego, CA, USA), and CD44/brdu double labeled staining.

4.2. Synthesis of the 8-hydroxyquinoline derivatives

Melting points were determined using an Electro-thermal IA 900 apparatus and the thermometer was uncorrected. IR spectra were recorded on a Perkin-Elmer Fourier transform infrared spectrometer and measured as KBr pellet. ^{1}H NMR spectra were determined in DMSO, $CDCl_3$ or $(CD_3)_2CO$ with a Bruker DRX 400 MHz spectrometer. Chemical shifts (δ) were given relative to tetramethylsilane (TMS). The coupling constants (J) were reported in Hz. Elemental analyses were recorded with a Perkin-Elmer 2400 analyzer. EI-Ms spectra were performed with a FINNIGAN Trace DSQ mass spectrometer at 70 eV using a direct inlet system. UV visible spectra were measured with a Shimadzu UV-2550 spectrophotometer. Experiment course was monitored by TLC. Column chromatography was carried out on silica gel.

4.2.1. 2-[2-(Ethyl-9*H*-carbazol-3-yl)-vinyl]-quinolin-8-ol. A mixture of 3.97 g of 2-methyl-8-hydroxyquinoline (25 mmol), 5.57 g of *N*-ethylcarbazole-3-carboxaldehyde (25 mmol), and 25 mL of acetic anhydride was stirred and heated at 125 °C for 40 h under nitrogen atmosphere. After cooled, it was subsequently poured into 100 mL of icy water and stirred for 4–5 h. The icy water was filtered off and a brown black solid was obtained. The solid was then dissolved in 35 mL DMF by heating to 120 °C, 50 mL of aqueous hydrochloric acid (36–38%) was added to the solution, and the mixture was heated at 125–135 °C for 2 h. The precipitated orange solid was filtered off, washed with water, and then mixed with 30 mL DMF and poured into a 100 mL flask. 2.50 g (20 mmol) of triethylamine was added to the mixture at boiling point and a red solution was obtained. It was subsequently poured into icy water (300 mL). The yellow solid obtained was filtered off, washed with water, and dried to afford compound **8**. Then it was purified by column chromatography on silica gel (100–200 mesh) using ethyl acetate/petroleum ether as an eluent (3.35 g, 35.10%). $R_f = 0.52$ (ethyl acetate/petroleum ether = 1:4). mp 141 °C. FT-IR (KBr) ν (cm^{-1}): 3392.59, 3037.03, 2972.83, 2923.45, 1618.04, 1593.40, 1561.36, 1507.15, 1489.90, 1477.58, 1332.19,1245.94, 1231.16, 962.55, 748.17, 587.99. ^{1}H NMR (DMSO) δ: 9.48 (s, 1H), 8.53 (s, 1H), 8.30 (d, 1H, $J = 16.08$ Hz), 8.27 (d, 1H, $J = 8.53$ Hz), 8.21 (d, 1H, $J = 7.66$ Hz), 7.87 (d, 1H, $J = 8.55$ Hz), 7.79 (d, 1H, $J = 8.58$), 7.68 (d, 1H, $J = 8.56$ Hz), 7.63 (d, 1H, $J = 8.56$ Hz), 7.50 (d, 1H, $J = 16.11$ Hz), 7.49 (t, 1H, $J = 8.14$ Hz), 7.37 (q, 2H, $J = 7.18$ Hz), 7.26 (t, 1H, $J = 7.60$ Hz), 7.10 (d,

1H,J = 6.48 Hz), 4.47 (q, 2H, J = 7.20 Hz), 1.35 (t, 3H, J = 7.15 Hz). EI-Ms m/z (M^+): 364. Anal. Calcd for $C_{25}H_{20}N_2O$: C, 82.42; H, 5.49; N, 7.69. Found: C, 82.31; H, 5.81; N, 7.55.

4.2.2. 2,7-Vinyl-bi-quinolin-8-ol. 1.64 g of 2-methyl-8-hydroxyquinoline (10 mmol) and 1.80 g of 8-hydroxyquinoline-7-carbaldehyde (10 mmol) were dissolved in 15 mL of acetic anhydride under nitrogen atmosphere. After they were stirred and heated to 125 °C for 40 h, the black solution was poured into icy water (100 mL) and stirred for 3–5 h after cooled. The yellow solid obtained was filtered off and dried with calcium chloride anhydrous under reduced pressure. The crude product was purified by column chromatography on silica gel (100–200 mesh) using ethyl acetate/petroleum ether as an eluent. A pure yellow solid was obtained (0.66 g, 19.2%). mp 231–233 °C. FT-IR (KBr) v (cm^{-1}): 3412.68, 2956.49, 2924.59, 2853.85, ^1H NMR ($CDCl_3$) δ: 7.19 (d, 1H, J = 7.21 Hz), 7.31 (t, 2H, J = 8.0 Hz), 7.41 (q, 2H, J = 7.6 Hz, 8.0 Hz), 7.57 (q, 1H, J = 3.2 Hz, 4.4 Hz), 7.66 (d, 1H, J = 8.4 Hz), 7.94 (d, 1H, J = 7.6 Hz), 8.15 (d, 1H, J = 8.4 Hz), 8.39 (d, 1H, J = 15.6 Hz), 8.67 (d, 1H, J = 8.4 Hz), 8.83 (d, 1H, J = 3.2 Hz). EI-Ms m/z (M^+): 314. Anal. Calcd for $C_{20}H_{14}N_2O_2$: C, 76.43; H, 4.46; N, 8.92. Found: C, 76.30; H, 4.50; N, 8.82.

4.2.3. 2-[2-(4-Diphenylamino-phenyl)-vinyl]-quinolin-8-ol. A mixture of 1.59 g of 2-methyl-8-hydroxyquinoline (10 mmol), 2.73 g of 4-diphenylamino-benzaldehyde (10 mmol), and 35 mL of acetic anhydride was stirred and heated at 125 °C for 40 h under nitrogen atmosphere. After cooled, it was subsequently poured into 150 mL of icy water and stirred for 3–5 h. The black solid obtained was filtered and washed with water and dried with calcium chloride anhydrous under reduced pressure. The crude product was purified by column chromatography on silica gel (100–200 mesh) using ethyl acetate/petroleum ether as an eluent. A pure yellow solid was obtained (1.11 g, 25.69%). mp 152–153 °C. FT-IR: v (cm^{-1}): 3447.17, 3034.92, 1637.02, 1587.72, 1565.93, 1509.27, 1491.83, 1462.84, 1327.16, 1280, 830, 749, 694, 620. ^1H NMR [$(CD_3)_2CO$] δ: 7.03 (d, 2H, J = 6.74 Hz), 7.09–7.13 (m, 7H), 7.32–7.42 (m, 6H), 7.36 (d, 1H, J = 15.84 Hz), 7.62 (d, 2H,J = 8.61 Hz), 7.77 (d, 1H, J = 8.60 Hz), 8.01 (d, 1H, J = 16.18 Hz), 8.25 (d, 1H, J = 8.32 Hz). ESI-Ms m/z (M+H): 415. Anal. Calcd for $C_{29}H_{22}N_2O$: C, 84.06; H, 5.31; N, 6.76. Found: C, 83.82; H, 5.46; N, 6.66.

4.2.4. 2,2′-(1,4-Phenylenedvinylene)bis-quinolin-8-ol. A mixture of 2-methyl-8-hydroxyquinoline (5.0 mmol, 0.795 g), terephthalaldehyde (2.5 mmol, 0.335 g), and acetic anhydride (10 mL) was stirred and heated at 125 °C for 40 h under nitrogen. After cooled, it was subsequently poured into icy water (50 mL) and stirred overnight. The yellow solid obtained was filtered and washed with water and acetone. The solid was dissolved in 15 mL DMF by heating to 120 °C. Aqueous hydrochloric acid (36–38%, 10 mL) was added to the solution and the mixture was heated at 120–130 °C for 2 h. The precipitated orange solid was filtered off and washed with water. And then the orange solid was dissolved in 10 mL DMF. Triethylamine (5.0 mol, 0.505 g) was added to the solution at the boiling point and a red solution was obtained. It was subsequently poured into icy water (100 mL). The yellow solid obtained was filtered off, washed with water, and dried to afford compound **11** (0.72 g, 87%). A purified compound **11** was obtained by recrystallization from toluene and had a melting point of 226–227 °C. ^1H NMR ($CDCl_3$) δ: 7.12 (d, 2H, J = 1.3 Hz), 7.40 (m, 4H), 7.56 (d, 2H, J = 16.2 Hz), 7.79 (d, 2H, J = 7.7 Hz), 7.80 (s, 4H), 8.19 (d, 2H, J = 16.2 Hz), 8.31 (d, 2H, J = 8.6 Hz).

4.3. The MTT cell proliferation assay

The effects of 8-hydroxyquinoline derivatives on the proliferation of rMSCs were measured by MTT assay–NADP related dehydrogenase existing in live mitochondria could reduce yellow MTT to insoluble formazan. Formazan was dissolved in DMSO, and the levels of absorbance were then detected by using Bio-kinetics Reader. The rMSCs subculture for five generations was cultured with complete culture medium in 96-well culture plate at the density 1×10^8/L. rMSCs were divided into several groups: serum-free culture medium was used for the control group; 8-hydroxyquinoline derivatives were used for contrast groups; for the experimental groups, the 8-hydroxyquinoline derivatives at low, middle, and high final concentrations were added to the control group. Each group had 5 wells. The total volume was 200 μL after the cells were continuously cultured for 3 days. After 72 h incubation, 20 μl MTT (5 mg/ml Sigma) was added and incubated at 37 °C for 4 h. The supernatant was then eliminated carefully before adding 150 μL dimethylsulfoxide and vibrated for 10 min. The values of absorbance (A) at 490 nm were detected by bio-kinetics reader (PE-1420, USA).

4.4. Cell cycle analysis

DNA content was measured by flow cytometry, a technique used to number and analyze particles suspended in a liquid environment. rMSCs were sealed in 6-well plates followed by stimulation in DMEM supplemented with 10% FBS and 8,9,11 (100 μg/mg, 300 μg/mg) for 1 day, respectively. After treatment, cells were washed twice with PBS (phosphate-buffered saline), trypsinized, and fixed in methanol for 20 min. Subsequently, cells were precipitated (5 min of centrifugation at 500g), washed with PBS, and re-suspended in 1 mL PBS containing 40 μg RNase A per mL and 40 μg of propidium iodide (PI) per mL. After incubation for 30 min at 37 °C, flow cytometric analysis was performed with an EPICS XL-MCL flow cytometry (Coulter), then the proliferative index (PI) were assayed. PI = [(S + G2/M)/G0/G + S + G2/M] × 100%.

4.5. Statistical analysis

The results were presented as means ± SD and analyzed statistically using a one-way analysis of variance as well as a Student's t test. The statistical analyses were

performed with SPSS software, and the differences in mean values resulting in $P < 0.05$ were considered statistically significant.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.05.004.

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