Synthesis of furo[3,2-c]coumarin derivatives using visible-light-promoted radical alkyne insertion with bromocoumarins

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1. **List of the substrates**

3-Bromo-4-hydroxycoumarin could be generated by treatment of commercially available 4-hydroxycoumarin with NBS using Mg(ClO₄)₂ as a Lewis acid. All alkynes were obtained from commercial suppliers and used without further purification.

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2. **Biological evaluation**

*(1) Cell Culture*

Cells were maintained in cell culture dishes in Dulbecco’s modified Eagle's medium (DMEM, WISENT), supplemented with 10% Fetal bovine serum (FBS, Gibco) 100 U/mL penicillin (Sangon Biotech, Shanghai, China), and 100 μg/mL streptomycin (Sangon Biotech, Shanghai, China). The cells were grown in a carbon dioxide incubator (37 °C, 5% CO₂).

*(2) Cytotoxicity*

The cytotoxicity was tested on the three cell lines (human lung carcinoma A549, human promyelocytic leukemia HL-60 and human colon carcinoma SW480) by MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide] (Sigma-Aldrich) assay as described. Briefly, the test cell at the exponential growth phase were collected and transferred into 96-well plates. After incubated for 24 h, compound dilutions were dispensed to the established culture plates, and doxorubicin
(Sigma-Aldrich) used as a positive control. Two days (48 h) later, the MTT solution (0.1 mg per well) was then added to each well. After further incubation for 4 h, the supernatant was removed, the crystals were fully dissolved in DMSO (150 mL), and the absorbance of each well was read at 570 nm (Sunrise, Tecan). The IC₅₀ value was determined as the concentration, at which a half of the test cell growth was inhibited. The experiment was performed in triplicate, and the data expressed as means ± SD. ³

(3) Acetylcholinesterase inhibitory activity assay

The AchE inhibitory activities were measured by a spectrophotometric method developed by Ellman et al. ⁴ The reaction was run at 25°C in a final volume of 200 μL of a 0.1M phosphate buffer (pH = 8.0), 333 μM 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB, Sigma-Aldrich), 0.035 U/mL AchE (Sigma-Aldrich), and 530 μM of acetylthiocholine iodide (Sigma-Aldrich) in 96-well microplates. Test compounds were added to the assay solution and followed at 412 nm for 5 min with a plate reader (Sunrise, Tecan). Seven different concentrations of each compound were used in order to measure the inhibition of AChE activity. IC₅₀ (concentration of the drug producing 50% of enzyme–activity inhibition) values were determined graphically from log concentration–inhibition curves.⁵

3. Reference

4. NMR Spectra for All Compounds

![NMR Spectra Image]