Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Electronic Supplementary Information

Alkaloids from Rutaceae: activities of canthin-6-one alkaloids and synthetic analogues on glioblastoma stems cells

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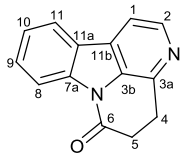
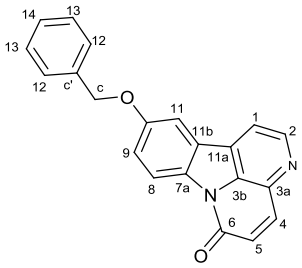
Contents:

1. Characterization of new compounds



4,5-Dihydrocanthin-6-one (8): yellow crystalline powder, mp 165−167 °C (CH_2Cl_2); IR (film, CH_2Cl_2) υ_{max} 1637, 1302, 1222, 1001, 846 cm^{-1}; **^{1}H NMR ($CDCl_3$, 400 MHz)** δ: 8.52 (2H, m, H-2,8), 8.05 (1H, d, *J* = 7.6 Hz, H-11), 7.73 (1H, d, *J* = 5.6 Hz, H-1), 7.66 (1H, t, *J* = 8.4 Hz, H-9), 7.48 (1H, t, *J* = 7.6 Hz, H-10), 3.49 (2H, t, *J* = 7.6 Hz, H-4), 3.22 (2H, t, *J* = 7.6 Hz, H-5); **^{13}C NMR ($CDCl_3$, 400 MHz)** δ: 166.86 (C=O), 144.0 (C-2), 142.74 (C-3b), 137.89 (C-7a), 135.41 (C-11b), 130.25 (C-9), 129.67 (C-3a), 124.70 (C-10), 124.52 (C-11a), 122.18 (C-11), 116.82 (C-8), 113.40 (C-1), 33.33(C-5), 27.64 (C-4); ESIMS *m/z* 223 $[M+H]^+$; HRESIMS *m/z* 223.10866 (calcd. For $C_{14}H_{11}N_2O$ 223.0869).



Benzyloxycanthin-6-one (10): yellow crystalline powder, mp 205−207 °C (CH_2Cl_2); IR (film, CH_2Cl_2) υ_{max} 1659, 1458, 1141, 1101, 743 cm^{-1}; **^{1}H NMR ($CDCl_3$, 400 MHz)** δ: 8.8 (1H, d, *J* = 5.1 Hz, H-2), 8.55 (1H, d, *J* = 8.0 Hz, H-8), 7.99 (1H, d, *J* = 9.6 Hz, H-5), 7.90 (1H, d, *J* = 5.1 Hz, H-1), 7.76 (1H, d, *J* = 2.4 Hz, H-11), 7.41-7.24 (6H, m, H-9, 12, 13, 14), 6.96 (1H, d, *J* = 9.6 Hz, H-4), 5.20 (2H, s, H-c); **^{13}C RMN (400 MHz, $CDCl_3$)** δ: 159.23 (C-6), 157.04 (C-10), 145.65 (C-2), 139.21 (C-4), 136.43 (C-3a), 136.31 (C-c'), 133.95 (C-7a), 132.45 (C-3b), 130.29 (C-11b), 129.03 (C-5), 128.72 (C-12), 128.54 (C-14), 128.25 (C-13), 125.63 (C-11a), 118.78 (C-9), 118.06 (C-8), 116.42 (C-1), 107.83(C-11), 70.81 (C-c); ESIMS *m/z* 349 $[M+Na]^+$; HRESIMS *m/z* 349.0936 (calcd. For $C_{21}H_{14}N_2NaO_2$ 349.0947).

2. Experimental data for biological assays

MGNT Stem Cell Culture. Non-adherent cultures of human MGNT stem cells (TG1 and TG10) were grown in 75 cm^2 tissue culture flasks (2500–5000 cells/cm^2) in Dulbecco's modified Eagle's medium-F12, supplemented with B27, N2 and G5 (all from Invitrogen), as described previously.[3] All cultures were maintained at 37 ^oC in a humidified atmosphere with 5% CO_2.

Isolation and Characterization of Neural Stem Cells from Human Fetal Brain. All studies with human tissue were performed under ethical approval from the University Paris-Descartes internal review board using tissue donated with informed consent after elective termination of pregnancy. Human fetal brains at embryonic day 50-55 (Carnegie stage 19-22) were carefully dissected and mechanically dissociated into single cell suspensions. Primary cells were cultured in the form of floating spheres in NeuroCult NSC Basal Medium supplemented with NeuroCult proliferation supplements (StemCell Technologies), human epidermal growth factor (EGF) (20 ng/mL), human basic fibroblast growth factor (b-FGF) (10 ng/mL) (AbCys), and heparin (20 ng/mL) (Sigma-Aldrich). The primary culture of neural stem cells (NSC24) was established and expanded with a passage every two weeks. No culture crisis, spontaneous differentiation or abnormal genetic derivation, as assessed by comparative genomic hybridization (CGH) arrays, was detected over one year of continuous culture. Clonal properties were determined after 4 months of culture (passage 12) by manual deposition of single cells in 100 µL into non-coated 96-well plates, followed by the addition of 50 µL of medium every two weeks for two months. 5.9 percent ±1.2 of CSN24 cells and 4.5% ± 1.6 of CSN25 cells yielded spheres. Immunocytochemical analysis showed that most cells expressed the NSC markers Sox2, Bmi1 and Nestin, as well as the ESC marker Nanog, while no cells immunoreactive for synaptophysin, phosphorylated neurofilaments, or NeuN, were observed.[26]

Viability Assays. The viability of cell cultures was assessed by quantifying the reduction of the cell proliferation reagent WST-1 to a water-soluble formazan. Briefly, dissociated sphere-derived TG1, TG10, and NSC24cells were plated in 96-well plates at 2 × 10^4 cells/well and treated for 24 h. Thereafter, 10 µL/well of 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate (WST-1, Roche) was added to the culture medium and incubated at 37 °C for 3 h. The negative control groups were treated with DMSO diluted in the culture medium at the higher equivalent volume used in the treated groups and showed no significant effects on the parameters analyzed compared to cells that did not receive the vehicle. The absorbance was read at 430 nm in a microplate reader (Expert Plus V1, 4 ASYS).

Western Blotting. After the treatment of TG1 and NSC24 cells with *trans*-avicennol (**1**) for 30 min, cells were washed in cold PBS and extracts were prepared by direct cell lysis in PIPES buffer (25 mM PIPES pH 6.8, 1% Triton, 0.5 mM EDTA, 0.5 mM EGTA) in the presence of protease and phosphatase inhibitors. Cell lysates were electrophoresed in NuPAGE 4-12% Bis-Tris gels (Invitrogen). The blots were then blocked at room temperature for 2 h with TBS-T buffer (50 mM Tris, pH 7.4, 150 mM NaCl, and 0.1% (v/v) Tween 20) containing 5% fat-free milk. Blots were exposed to primary and secondary antibodies following the manufacturer's instructions. Phospho-p42/p44 AKT antibody was purchased from Cell Signalling and p42/p44 AKT was from Millipore. The immunoreactivity was revealed by chemiluminescence (PerkinElmer Life and Analytical Sciences, Waltham, MA).

Statistical Analysis. The number of experimental replicates is given in the figure legends. Data obtained from independent experiments were reported as means ± SE. Student's t-test analysis was performed to determine statistical significance.