Supplementary information

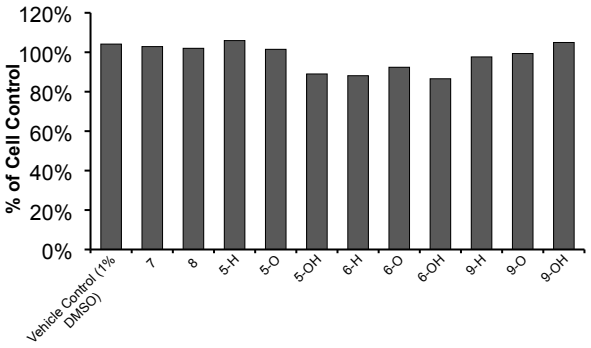

Figure 4. Cell viability of compounds at 100 μM. Cell viability was measured using the MTS assay and expressed as a percent of cell control (i.e. untreated cells). n=2.

Lucigenin-enhanced chemiluminescence

The antioxidant capacity of mexiletine and analogues was also examined using lucigenin-enhanced chemiluminescence. Segments of abdominal aorta (3 mm long) taken from SD rats were dissected out in Krebs-Hepes solution(in mM: NaCl 99.0; KCl 4.7; $CaCl_2$ 1.9; $MgSO_4$ 1.2; K_2HPO_4 1.0; $NaHCO_3$ 25.0; HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) 20.0; and glucose11.1; pH 7.4) and incubated at 37°C in 6 well plates containing Krebs-HEPES buffer solution, mexiletine or analogues at 10 μM, NADPH (nicotinamide adenine dinucleotide phosphate, 100 μM) and DETCA (diethylthiocarbamate, 300 μM) for 45 min in a 37°C incubator (Forma Scientific, Marietta, OH, USA). After a 45 min incubation period, aorta segments were placed in a 96 well Optiplate (Perkin Elmer, Downers Grove, IL, USA) containing NADPH (100 μM), test compounds (10 μM) and lucigenin (5 μM). Photon emission was detected by a luminescence counter (Perkin Elmer) which read each well 12 times over 25 min. Background photon emission counts of the 96 well Optiplate were also taken prior to addition of the tissue segments. Vessel segments were then dried in a 65°C oven (Daihan Scientific, Seoul, Korea) overnight in order to determine the dry weight of the tissue. Luminescent counts were expressed as average counts per mg of dry tissue weight[1].

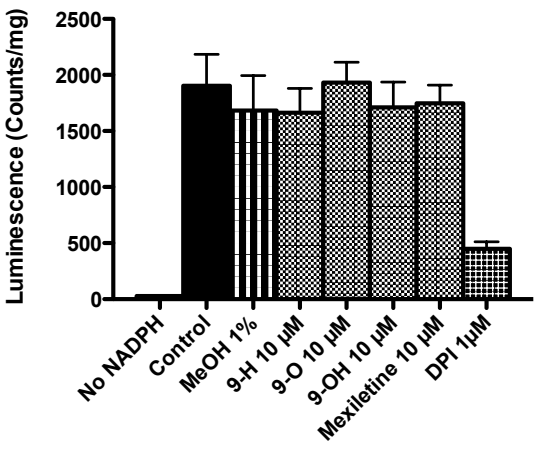

Figure 5. The effects of mexilitene and the isoindoles on lucigenin-enhanced chemiluminescence in rat aorta (n=3-4).

AAPH-induced cell lysis

C57Bl/6 mice were deeply anaesthetised (halothane 5% in O_2/room air) and blood (0.5 ml) was obtained via cardiac puncture. Erythrocytes were separated from plasma by centrifugation at 2600 rpm for 10 min at 4°C. Packed erythrocytes were resuspended in ice cold phosphate-buffered saline (PBS in mM, NaCl 137; KCl 2.7; Na_2HPO_4 10 and NaH_2PO_4 2) and centrifuged again for 5 min at 1500 rpm and stored at 4°C for a maximum of 3 days. Erythrocyte suspensions were incubated in a 96 well plate with glutathione (1 mM) and test compounds for 30 min. Following this incubation period, the water-soluble free radical generator 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH, 100 mM) was added to each of the wells and lysis immediately measured using a spectrophotometer (Thermo Electron Corporation, Vantaa, Finland) at 690 nm wavelength and 37°C after gentle vortexing. Absorbance readings of the wells were taken every 5 min for 5 h[1].

References

1. N.V. Jani, J. Ziogas, J.A. Angus, C.H. Schiesser, P.E. Macdougall, R.L. Grange, C.E. Wright, *Eur. J. Pharmacol*. 2012**, 695**, 96-103.