Mechanisms of action and reduced cardiotoxicity of pixantrone; a topoisomerase II targeting agent with cellular selectivity for the topoisomerase IIα isoform

Supplemental Figures S1 and S2
**Fig. S1.** Effect of pixantrone on the topoisomerase IIα- and topoisomerase IIβ-mediated relaxation and cleavage of supercoiled pBR322 plasmid DNA to produce linear DNA. These fluorescent images of the ethidium bromide-stained gels show that topoisomerase IIα and topoisomerase IIβ converted supercoiled (SC) pBR322 DNA to relaxed (RLX) DNA. Under the separation conditions the supercoiled DNA ran ahead of the linear DNA because the gel was run with ethidium bromide in the gel only. Topoisomerase IIα or topoisomerase IIβ was present in the reaction mixture, as indicated, in all but the lane marked pBR322. The 20 µl reaction mixture contained 40 ng of pBR322 DNA and 60 ng of topoisomerase IIα or 6 ng of topoisomerase IIβ, as indicated. The electrophoresis was carried out on a 1.2% agarose gel containing ethidium bromide only in the gel at 80 V for 1 h and then at 10 V for 15 h. The etoposide positive control produced significant amounts of linear DNA (LIN). Pixantrone treatment detectably increased the formation of linear DNA for both topoisomerase IIα and topoisomerase IIβ. At pixantrone concentrations above 0.2 µM there was significant inhibition of the relaxation reaction. A small amount of nicked circular DNA (NC), which may arise from strand breakage during isolation, is normally present in pBR322 DNA.

**Fig. S2.** Effect of pixantrone on the topoisomerase IIα- and topoisomerase IIβ-mediated relaxation and cleavage of supercoiled pBR322 plasmid DNA to produce linear DNA. These fluorescent images of the ethidium bromide-stained gels show that topoisomerase IIα and topoisomerase IIβ converted supercoiled (SC) pBR322 DNA to relaxed (RLX) DNA. Topoisomerase IIα or topoisomerase IIβ was present in the reaction mixture, as indicated, in all but the lane marked pBR322. Left figure: The 20 µl reaction mixture carried out for 15 min at 37 °C contained 150 ng of pBR322 DNA and 250 ng of topoisomerase IIα or 75 ng of topoisomerase IIβ, as indicated. The electrophoresis was carried out at 40 V for 10 min and then at 20 V for 18 h. Right figure: For etoposide controls the 20 µl reaction mixture contained 75 ng of pBR322 DNA and 125 ng of topoisomerase IIα or 40 ng of topoisomerase IIβ, as indicated. The electrophoresis was carried out at 40 V for 10 min and then for 20 V for 18 h on a 1.6% agarose gel containing ethidium bromide in the gel and in the running buffer. The etoposide positive controls produced significant amounts of linear DNA (LIN). Pixantrone treatment detectably increased the formation of linear DNA for topoisomerase IIα. At pixantrone concentrations above 0.2 µM there was significant inhibition of the relaxation reaction.