Fig. S1. Activation of procaspase-7. Western blot shows cleavage of procaspase-7 after different treatments in the presence or absence of caspase-9 inhibitor Z-LEHD-fmk. PARP cleavage is shown below as activated procaspase-7 cleaves PARP. Actin was used as a loading control. Cis, cisplatin, Fis, fisetin.

Fig. S2-S3. Immunocytochemical staining of cisplatin and fisetin treated cells. Immunostaining of p53 shown by indirect immunofluorescence of treated cells stained with the mitochondrial marker Mitotracker® Red and anti-p53 antibody labeled with secondary antibody conjugated to FITC. S2, cisplatin treated cells; S3, fisetin treated cells; a, immunostained nuclear p53; b, nuclei stained with Hoechst 33342; c, mitochondria stained with Mitotracker® Red; d, co-localization of nuclear p53 and Hoechst 33342; e, co-localization of nuclear p53 and mitochondria with Mitotracker® Red; f, mask of co-localization for d; g, mask of co-localization for e; h, Nomarski image. Note significant nuclear localization of p53 in S2 and mitochondrial localization in S3.

Fig. S4-S5. Immunocytochemical staining of cisplatin and fisetin treated cells. Immunostaining of p53 shown by indirect immunofluorescence of treated cells stained with the mitochondrial marker Mitotracker® Red and anti-p53 antibody subsequently labeled with secondary antibody conjugated to FITC. S4, cisplatin + fisetin treated; S5, vehicle treated. a, immunostained nuclear p53; b, nuclei stained with Hoechst 33342; c, mitochondria stained with Mitotracker® Red; d, co-localization of nuclear p53 with Hoechst 33342; e, co-localization of nuclear p53 with mitochondria with Mitotracker® Red; f, mask of co-localization for d; g, mask of co-localization for e; h, Nomarski image. Note significant mitochondrial localization of p53 in S4.

Fig. S6. Effect of siRNA mediated downregulation of p53 on cell death. Western blots of extracts of NT2/D1 cells treated with p53 siRNA during various treatments shows downregulation of p53, p21 and upregulation of survivin levels in the presence of p53 siRNA. Negative control siRNA was used as control. Actin was used as a loading control. Bar graph shows flow cytometric analysis of the treated cell populations showing the percentages of membrane permeabilized cells with different treatments with or without p53 siRNA. Note the inhibition of cell death in the presence of p53 siRNA at 12 h. Data are representative of 3 experiments.

Fig. S7. Effect of cisplatin, fisetin and the combination at lower doses on NT2/D1 xenografts in athymic nude mice. 1. control; 2. cisplatin (1.5 mg/kg/day); 3. fisetin (1 mg/kg/day); and 4. combination of cisplatin (1.5 mg/kg/day) and fisetin (1 mg/kg/day).

Fig. S8. Drug effects on kidney. Representative sections from the kidney from treated and vehicle treated animals stained for TUNEL positive cells showing reactivity with treatments of cisplatin, fisetin and a combination of cisplatin and fisetin at lower and higher doses. Arrows indicate TUNEL positive cells.

Fig. S9. Photomicrographs of histological sections from liver spleen and kidney of treated and vehicle treated mice at indicated doses.