Supplementary Methods

Cell cuture

IGROV-1 and JEG-3 tumour cells were routinely passaged as adherent monolayers in $75cm^2$ (T80) vented flasks (Corning Inc., supplied by Merck Eurolabs, Lutterworth, UK) in a 5% CO_2 humidified atmosphere. The medium was RPMI 1640 without FA (Gibco Life Technologies, Paisley, Scotland). This was supplemented with 10% dialysed fetal calf serum (PAA laboratories, Yeovil, Somerset, UK), 0.02mg/ml gentamicin, 2mM L-glutamine and 20nM RS-5-formyl-tetrahydrofolate (leucovorin; LV; David Bull Laboratories, Warwick, UK or Faulding, UK).

Growth inhibition studies – attached cell counting

Cells were seeded at a density of 1×10^5 cells in duplicate T25 tissue culture flasks (Corning, UK) in 8mls of medium. After ~24h, 1ml of 10 x desired compound concentration and 1ml of medium was added. For FA protection studies, 1ml of 10μM FA was added to the culture 30min prior to the compounds. At 0h (time of compound addition) and 120h the attached cells were harvested with trypsin-EDTA, centrifuged at ~900 x g for 5mins, and the pellets resuspended in an appropriate volume of ice-cold 70% EtOH (0.1ml to 15ml) to yield a haemocytometer count of ~50 cells per field. Fixed cells were stored at 4^oC for a maximum of 1 week prior to being counted.

Pharmacokinetic measurements in mouse tissues

Tissues were homogenised in PBS (1ml/g for liver and 3ml/g for kidney, spleen and tumour). One hundred μl homogenate was used for standard curve quality controls and analysis. A 10μl aliquot of internal standard (CB300899; 22) together with 0.3ml of ice-cold acetonitrile was added to each sample, vortexed and left to stand at room temperature for 10mins. Following centrifugation at 13,000 rpm for 10mins, the supernatant was transferred to LC-MS/MS autoinjection vials and stored at 4^oC prior to analysis. The LC-MS/MS analytical method for the determination of BGC 945 in plasma is described in Wood *et al* (26).