EC50 values of cell viability compared to EC50 values of neurite area

Cells were replated on d2 and compounds were added to the culture medium in at least 5 different concentrations. After 23 h, resazurin was added to the medium and the fluorescence signal was determined 30 min later. Subsequently, H-33342 and calcein-AM were added and cells were imaged 30 min later to quantify neurite area and number of viable cells per field. The concentration-response-curves were used to determine the median effective concentrations (EC50 values). (A) Scatter plot of EC50 for reduction of viability (assessed by resazurin measurement) vs EC50 for reduction of neurite area. For compounds printed in italics, EC50 values were determined by extrapolation or, in cases of low toxicity of the compound, the highest concentration measured was taken. The solid line marks points of equal EC50 for effects on neurite area and viability. The area left of the dashed line contains compounds that act at least four times more potent on neurites than on overall viability (green dots). All data points are based on means ± SEM of EC50 values from 3 independent experiments. Abbreviations: methylmercury (MeHg), methamphetamine (METH), cycloheximide (CHX), bisindolylmaleimide I (Bis I), brefeldin A (BFA), cadmium chloride (CdCl2), sodium dodecyl sulphate (SDS), sodium orthovanadate (Na3VO4). (B) Ratios of EC50 of viable cells (number of calcein positive cells; as in figure 9) vs EC50 of neurite area are displayed for general (unspecific) toxicants (orange), and compounds assumed to affect neurites specifically (green); compounds with insufficient background information are displayed in black. (C) The means ± SEM of the group of general toxicants and of putative neurite outgrowth inhibitors are shown. The two groups were statistically different (p < 0.01). More importantly, the 95% confidence band of the unspecific group was stretched from a ratio of 0.3 to 4.7. Compounds outside this band are likely to be useful as test compounds to study specific neurite outgrowth inhibition. This applied to all putatively-specific (green) compounds in B.