the presence of [^{3}H]tamoxifen (84 Ci/mmol; Amersham). Following such treatment, the cells were washed with PBS, collected by trypsinisation and counted for radioactivity in ready Emulsifer-safe scintillant (PerkinElmer Boston, MA 02118, USA).

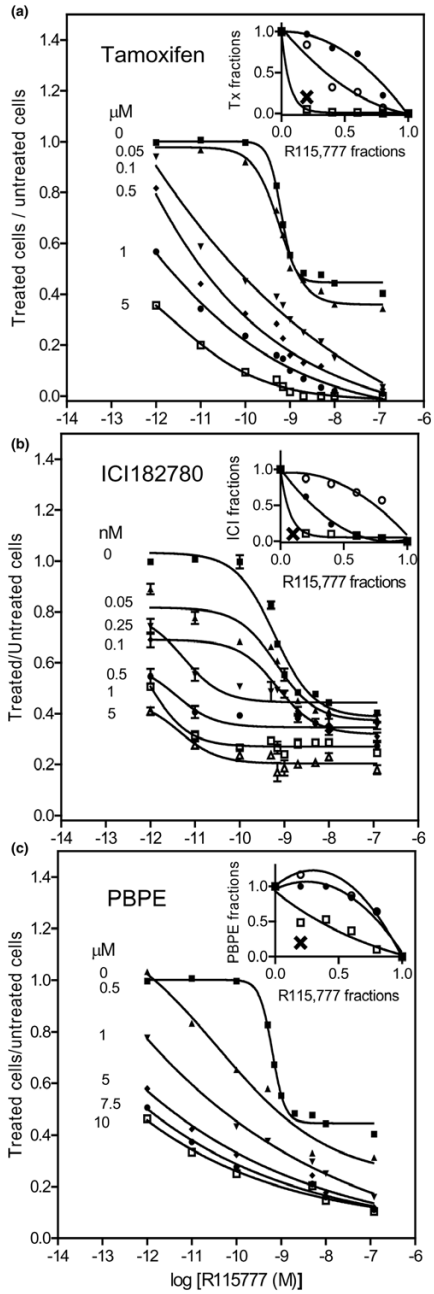
Results and discussion

Effects of R115,777 when combined with different anti-estrogens on MCF-7 cell proliferation

We assessed the ability of R115,777 alone and in combination with anti-estrogens to inhibit the proliferation of MCF-7 cells. Cells were incubated for 5 days with incrementally increasing concentrations of R115,777 together with either Tam, ICI182,780 (pure anti-estrogen) or PBPE (selective AEBS ligand). For each compound, we first plotted individual dose response curves from which the following IC_{50} values were derived: 5.9 nM (R115,777), 4.4 μM (Tam), 0.52 nM (ICI182,780) and 8.8 μM (PBPE). Analyses of cell proliferation curves (Fig. 1a–c) showed strong inhibitory effects at low concentrations of R115,777 when associated with each of the three anti-estrogens and there was a suggestion of synergy for each of the combined pairs. To construct isobolograms according to the method described by Steel and Peckham [18] we carried out another set of experiments using combinations of the two drugs at concentrations resulting in 50% cell growth inhibition. Additive effects close to synergistic were observed between Tam and R115,777 (Fig. 1a, insert), confirming our earlier results using another FTI (FTI-277) from a different chemical class in association with Tam [14]. Although it can be argued that there is only a tenuous difference between additivity and synergy for this combination of an FTI with Tam, and it is accepted that this type of analysis is not really precise enough to definitively establish additivity between two agents, the methodology does identify clear additivity with two different FTIs with diverse molecular structures. To extend this observation to the evaluation of other anti-estrogens further, isobolograms were constructed. Isobologram analyses (Fig. 1b,c, inserts) revealed a synergistic inhibition of MCF-7 growth with combinations of R115,777 with both ICI182,780 or PBPE. Because the main high affinity targets of Tam are ERs and AEBS [5,6,8,19,20] and because of the synergistic effects between R115,777 and the ER ligand, results in agreement with data from Ellis *et al*. [21], together with the additive or synergistic effects observed with Tam, we had expected a negative effect with the combination that includes the selective AEBS ligand. Surprisingly though (Fig. 1c), PBPE also synergizes with R115,777, suggesting that cross-talk between FTIs and Tam is likely to occur via at least two different pathways.

To determine at what level this cross-talk occurs, we analysed the combined effects of R115,777 and Tam on various markers of cellular apoptosis. The rationale for this study was provided by an earlier publication by Ellis *et al*. [21] who proposed that hydroxy-tamoxifen and ICI182,780 (two selective ligands)

Figure 1



Effects of combining anti-estrogens and R115,777 on the inhibition of MCF-7 cell proliferation. Studies were performed using 1.5×10^5 cells in 60 mm dishes incubated for 5 days with increasing concentrations of **(a)** R115,777 and tamoxifen, **(b)** R115,777 and ICI182,780, or **(c)** R115,777 and PBPE. Dose response interactions between these anti-estrogens and R115,777 were evaluated using the isobologram method. The isoeffect curves were constructed, at the IC_{50} point, according to the Materials and methods [18]. X symbolizes the data point obtained with a combination of the two agents giving 50% inhibition. **(a)** When X falls within the envelope of additivity, the combination is considered as having an additive effect. **(b,c)** When X falls to the left of the envelope the combination is considered as having a supra-additive effect (synergism). PBPE, N-pyrrolidine(-phenylmethyl-phenoxy)-ethanamine-HCl.