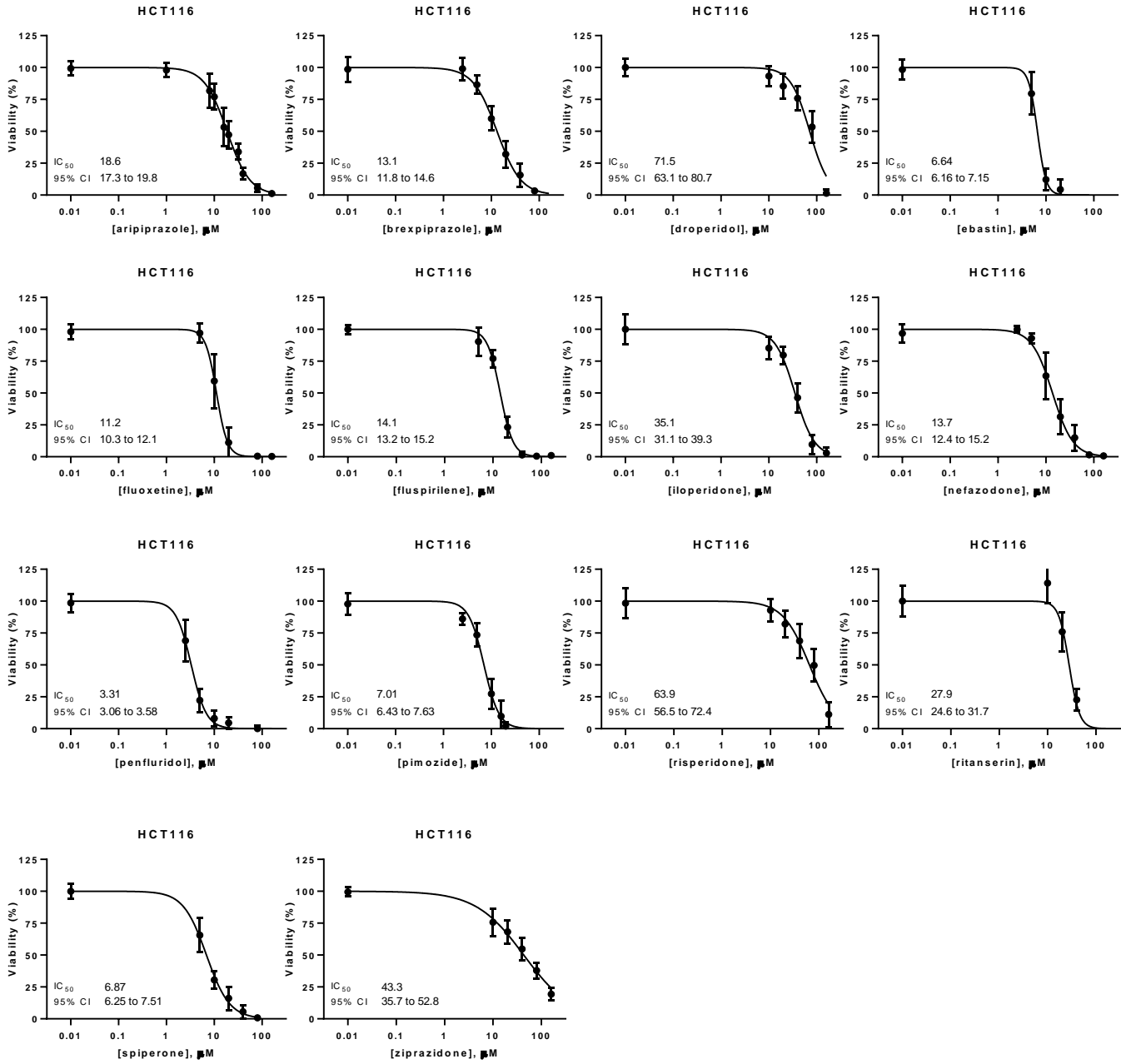
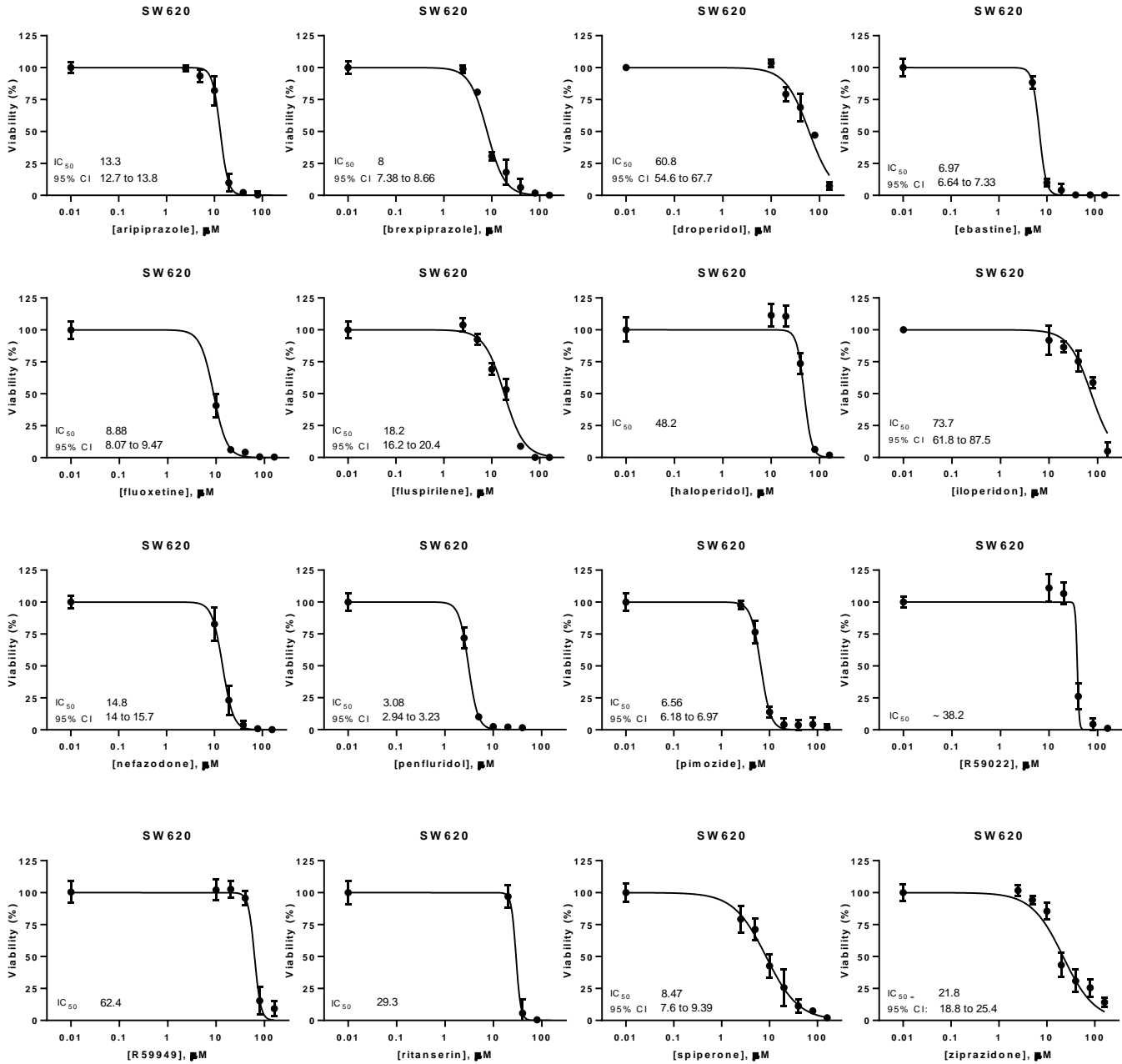
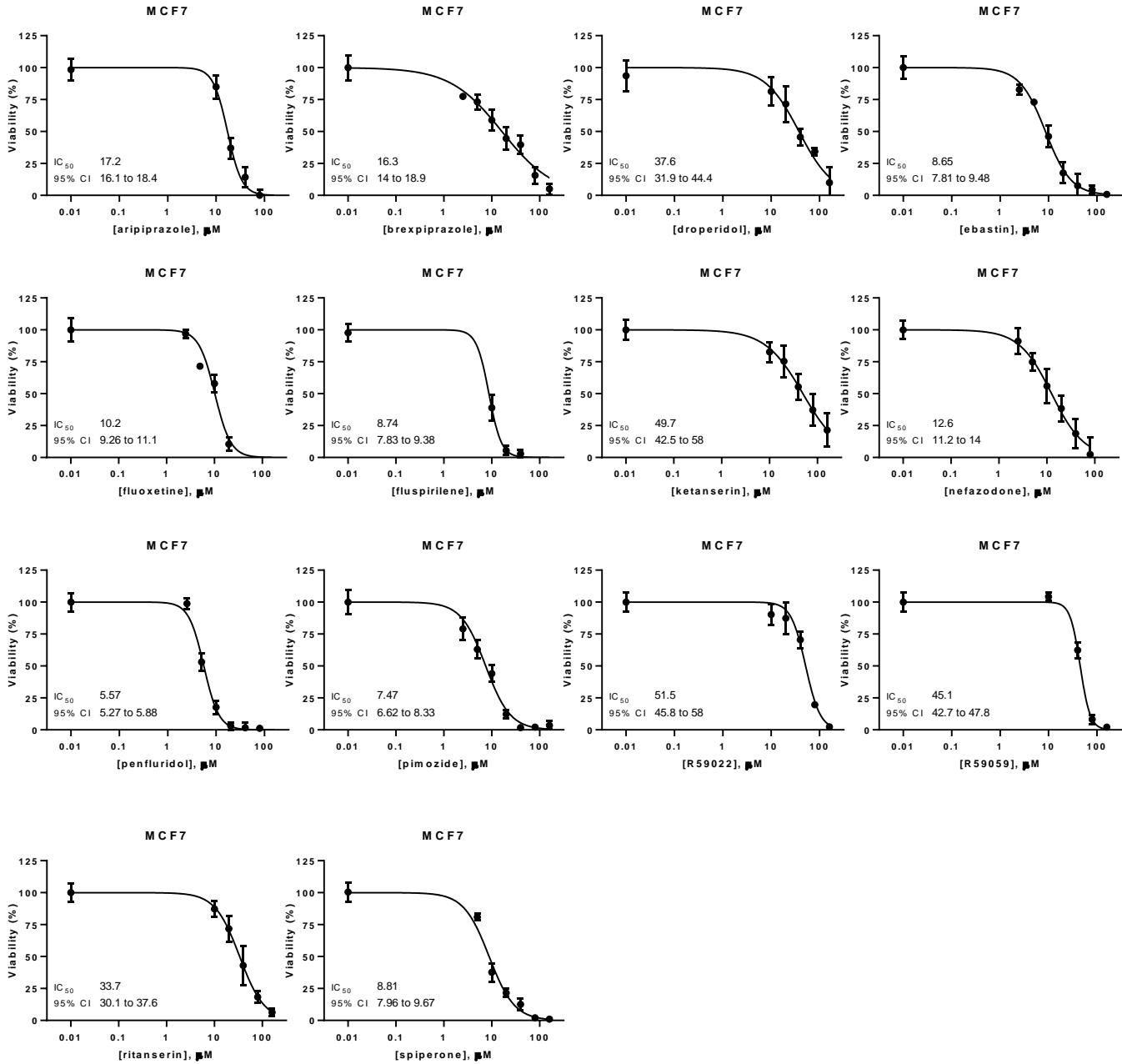
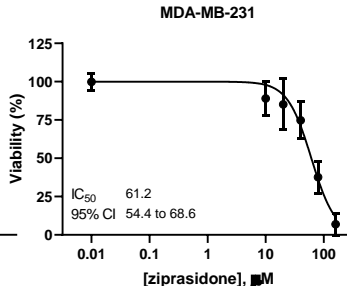
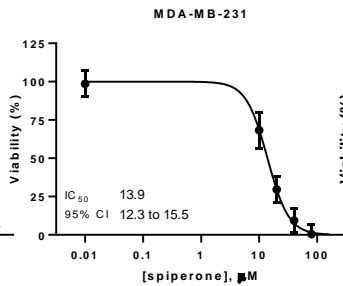
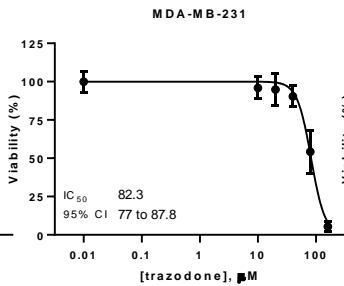
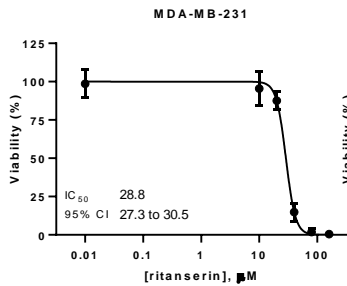
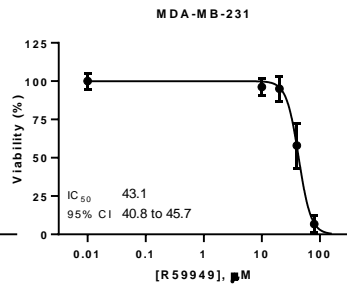
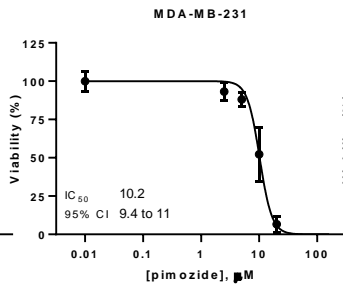
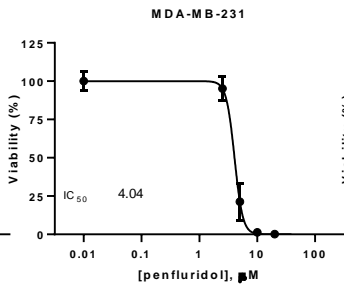
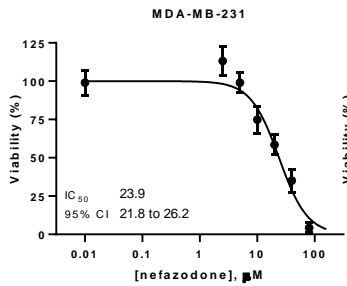
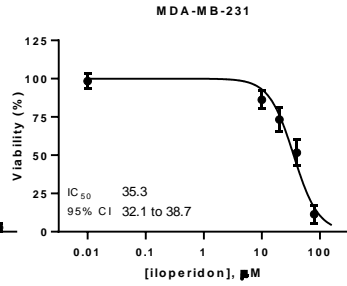
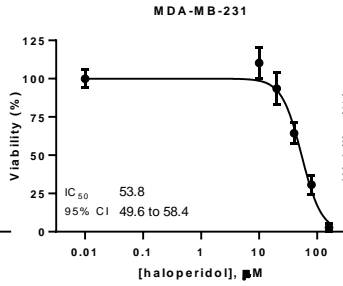
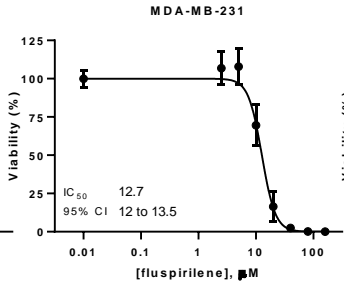
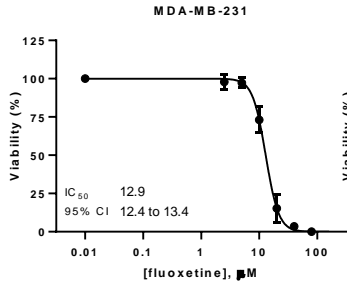
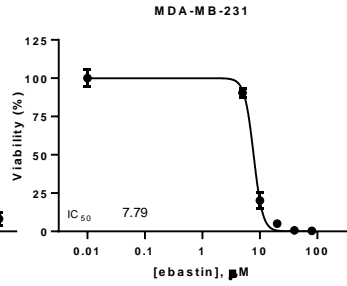
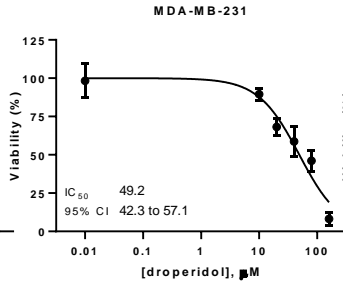
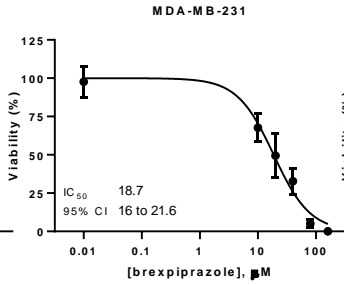
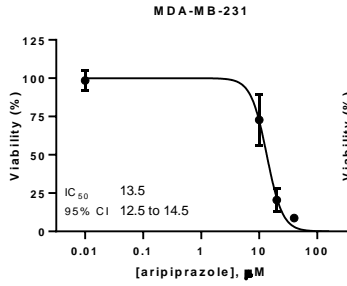


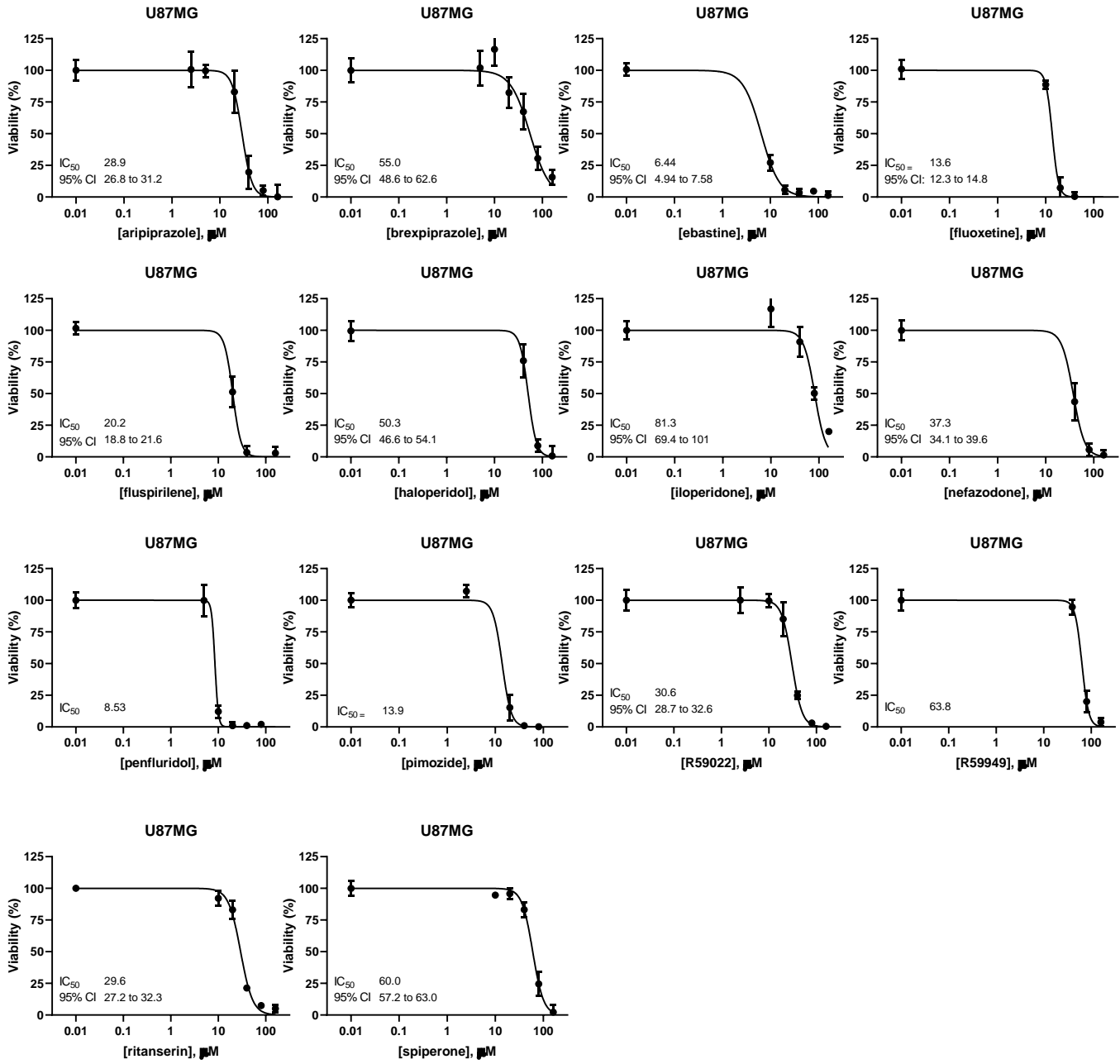
Supplementary figure 1. Effect of psychotropic drugs on cancer cell lines viability. Six cancer cell lines were treated for 72 h with scalar doses of drugs ranging from 10 to 160 µmol/L. The screened drugs included antipsychotics, antidepressant, antihistamines and three compounds used in scientific research with reported serotonin receptors antagonistic activity (R59949, R59022; WAY-100135). Cell viability is shown as percentage of viable cells versus control. Data are presented as mean ± standard deviation (SD) from three independent experiments, each performed in quadruplicate

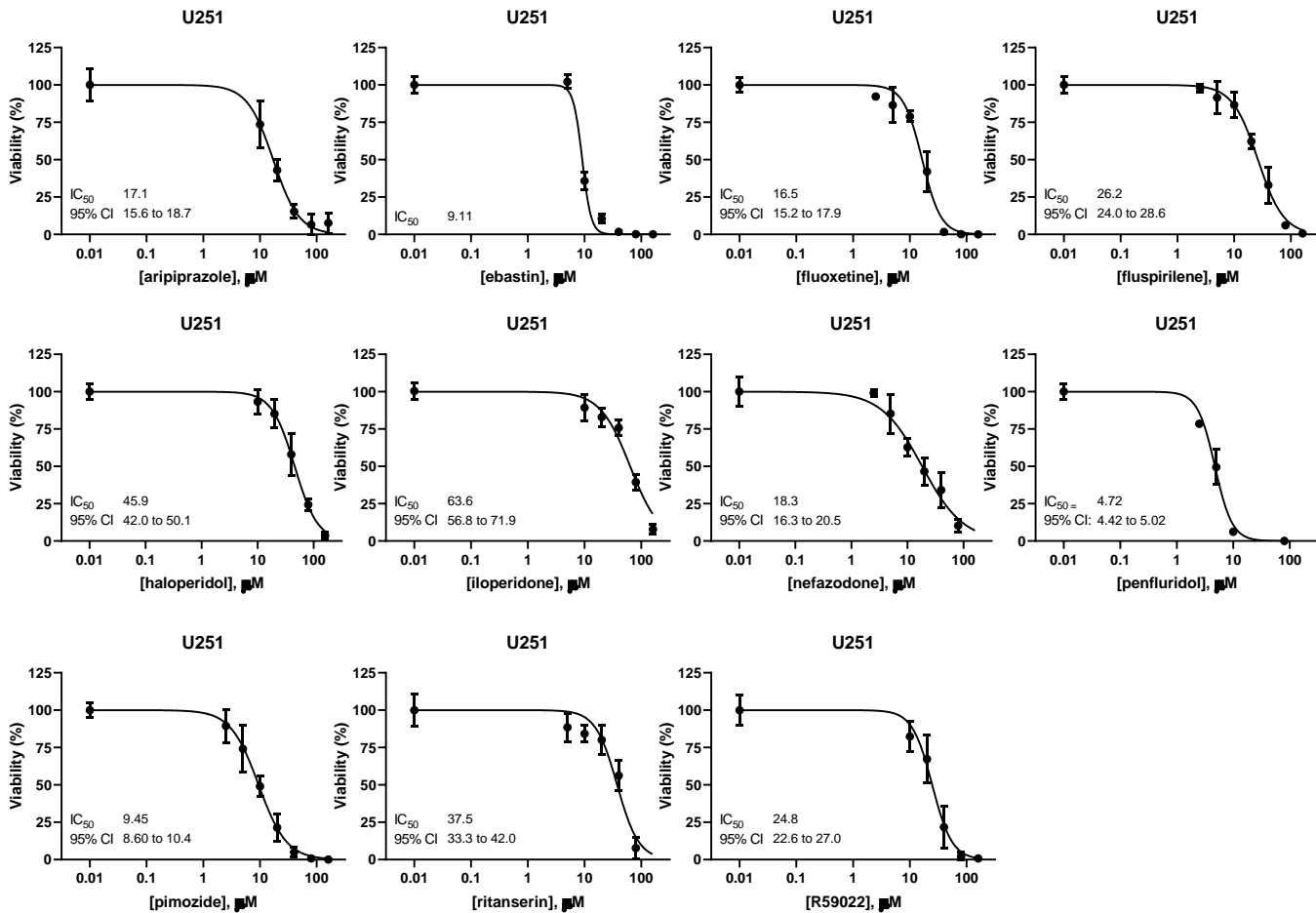




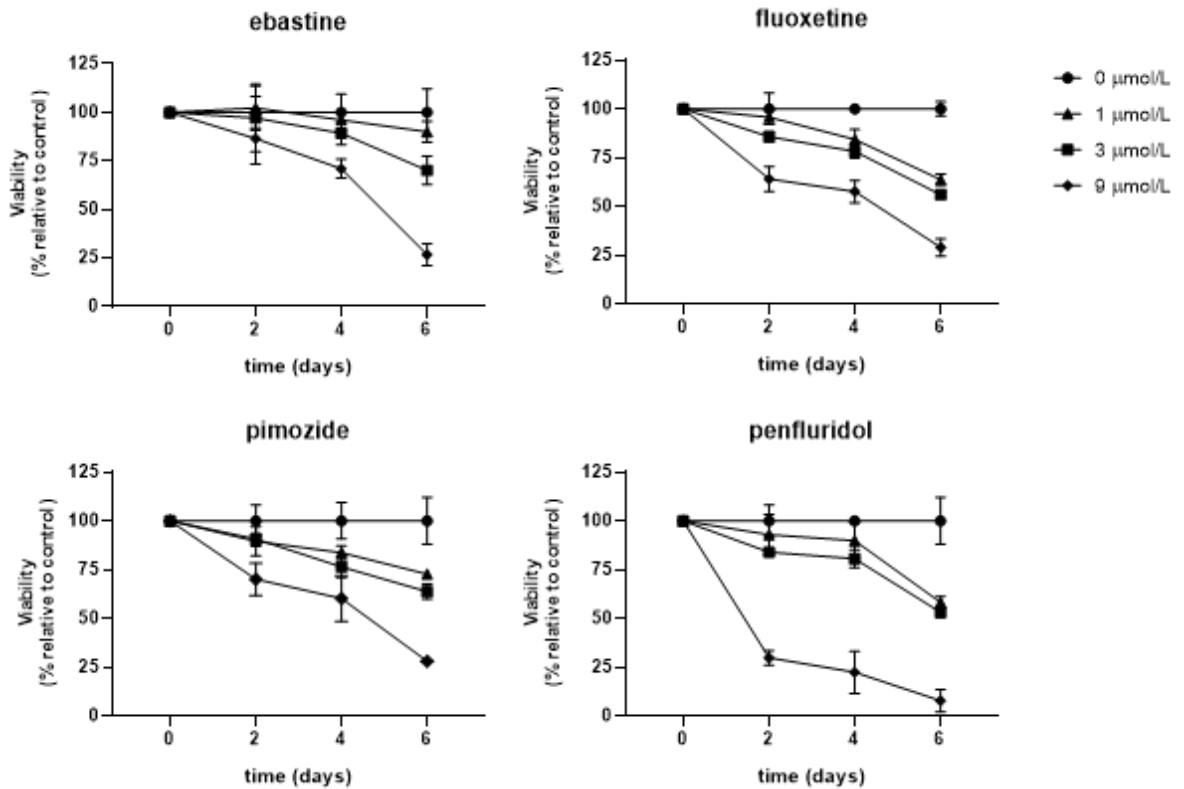




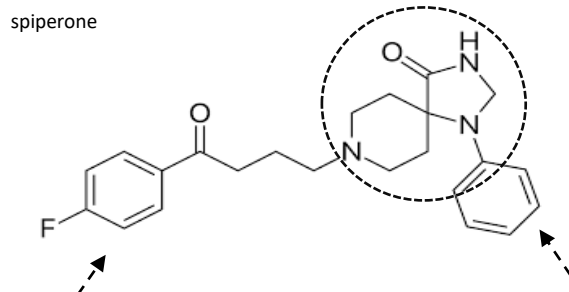
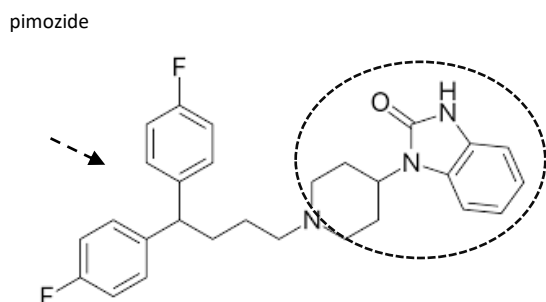
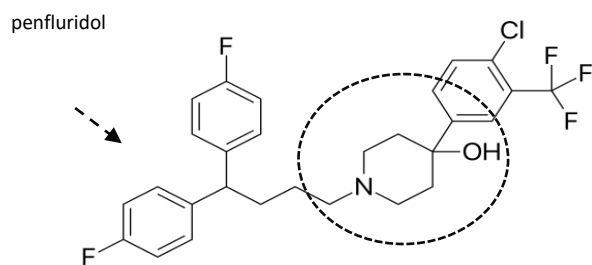
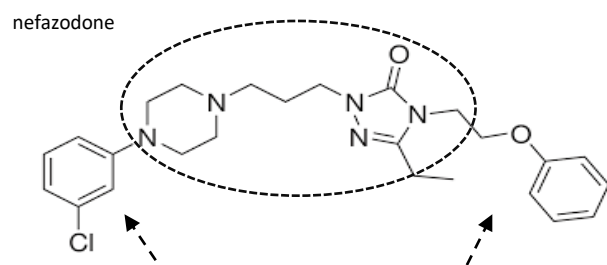
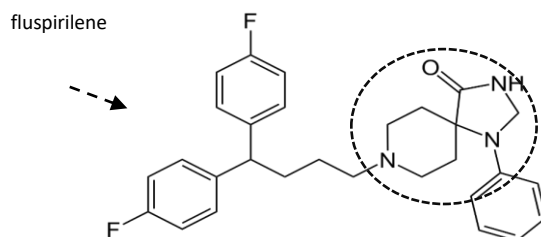
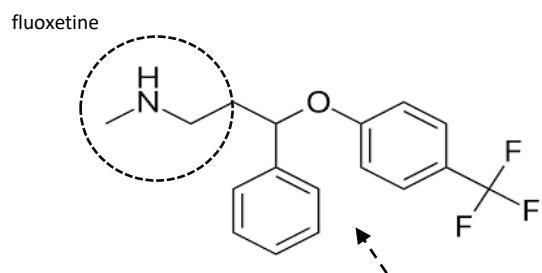
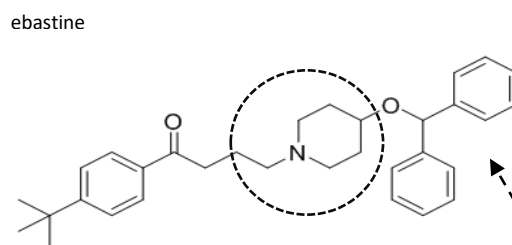
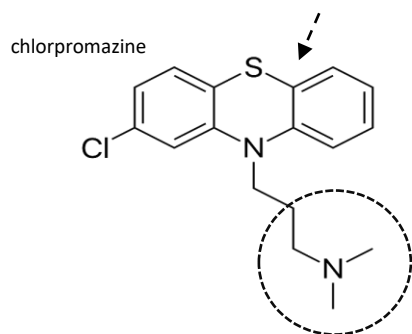
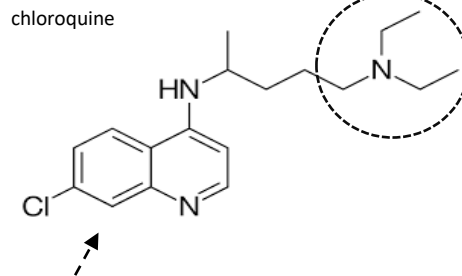
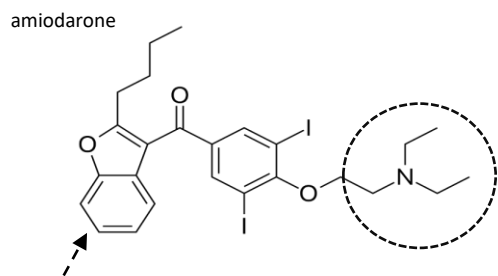




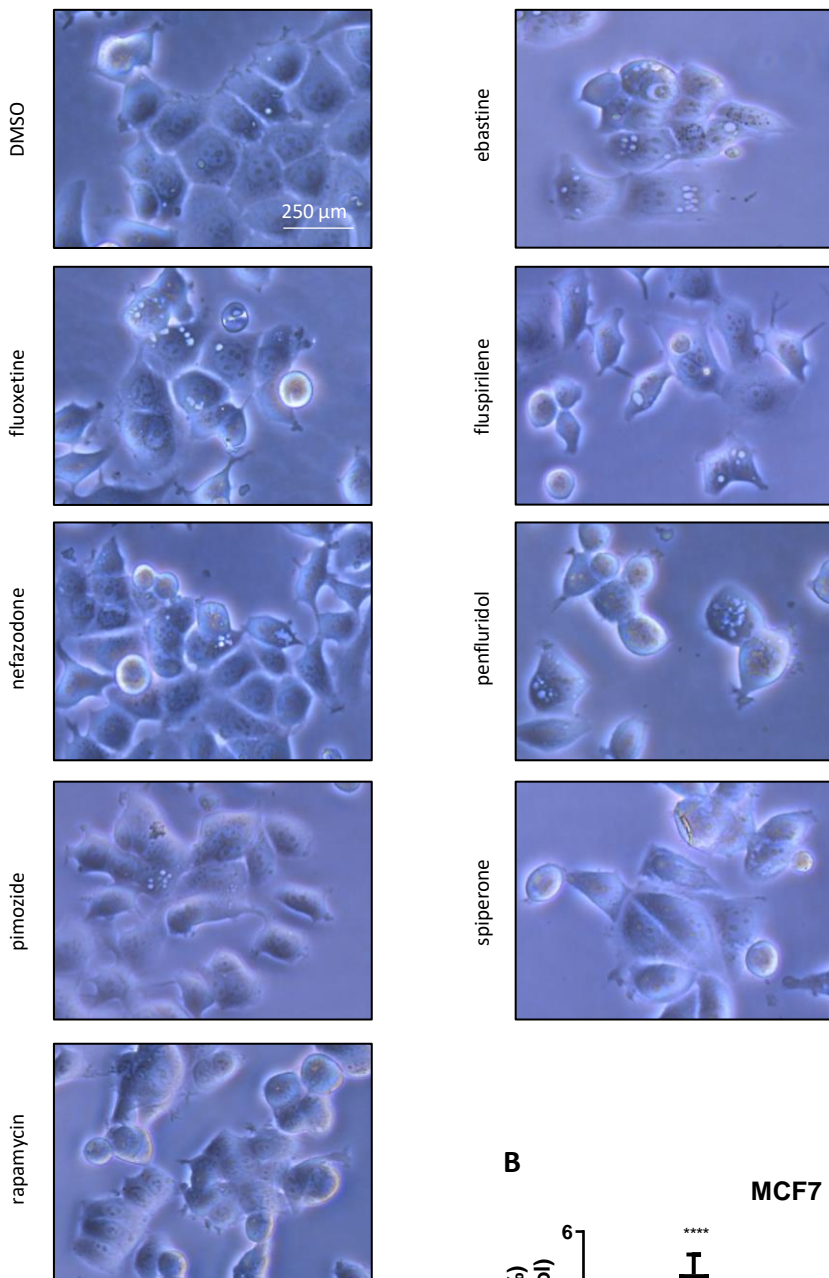
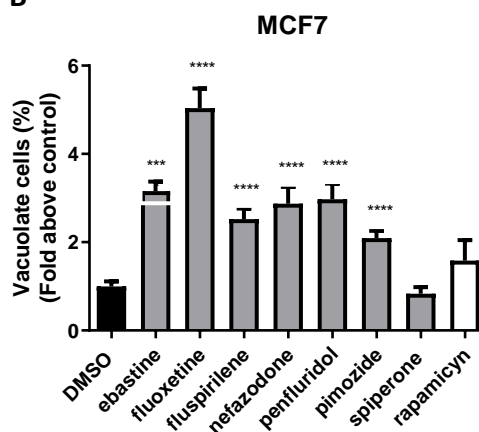
Supplementary figure 2. Dose response curves showing the cytotoxic effect of psychotropic drugs on neoplastic cells. Cells were treated for 72 h with scalar doses of drugs ranging from 10 to 160 µmol/L. Viabilities were assessed by MTT assay, normalized for cells treated with vehicle only and expressed as percentage. The best fit values of IC₅₀ values were calculated the by using a variable slope model (GraphPad Prism 7). Each data point represents the mean of at least three independent experiments. IC₅₀, drug concentration reducing by 50% viability compared to control; 95% CI, 95% confidence interval.



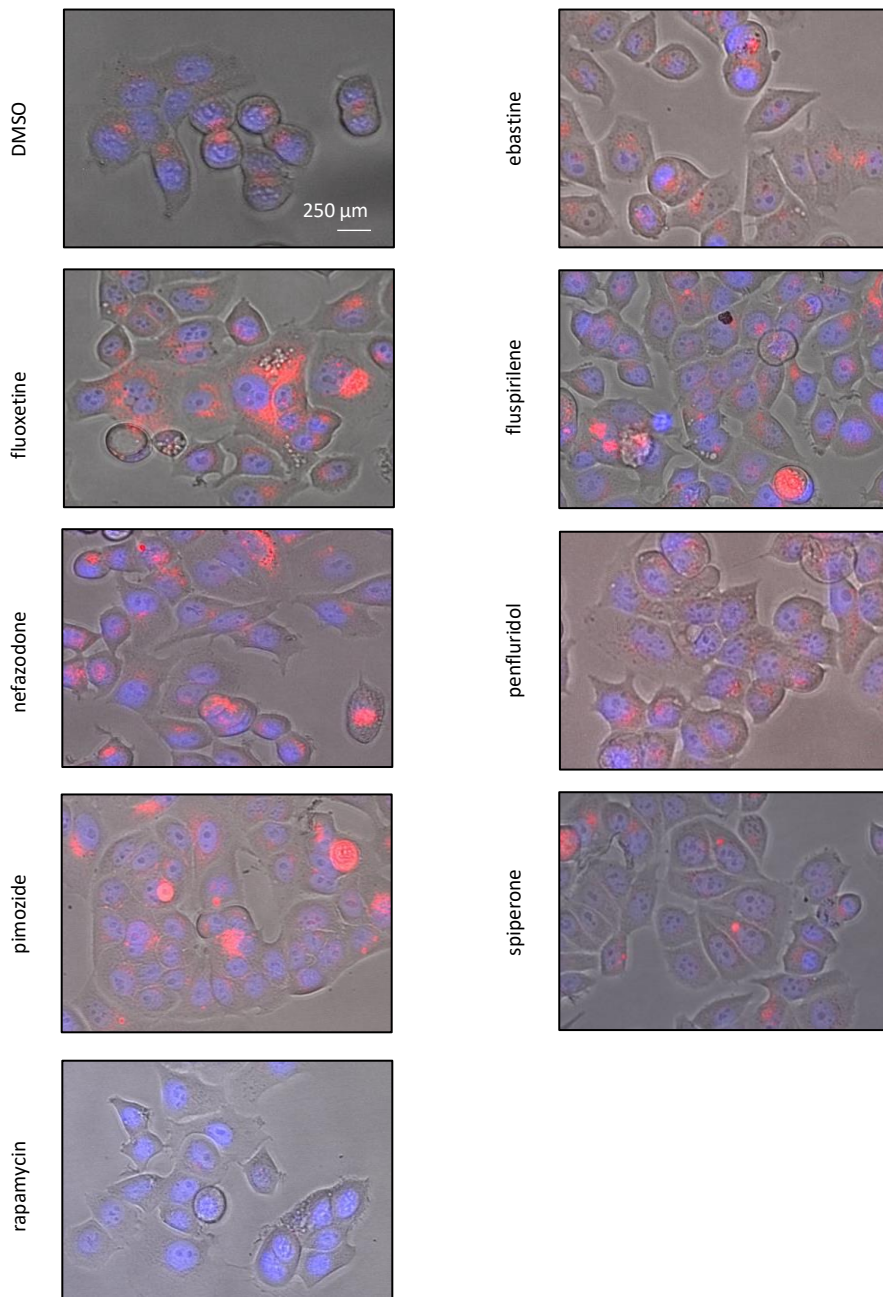
Supplementary Figure 3. Dose and time response curves showing the cytotoxic effect of ebastine, fluoxetine, pimoziide and penfluridol on MCF7 cells. Cell were treated for 6 days with increasing doses of drugs. Viabilities were assessed by MTT assay and normalized relative to controls treated with vehicle only. Data are expressed as the mean \pm SD of one representative out of three independent experiments performed in quintuplicate.³



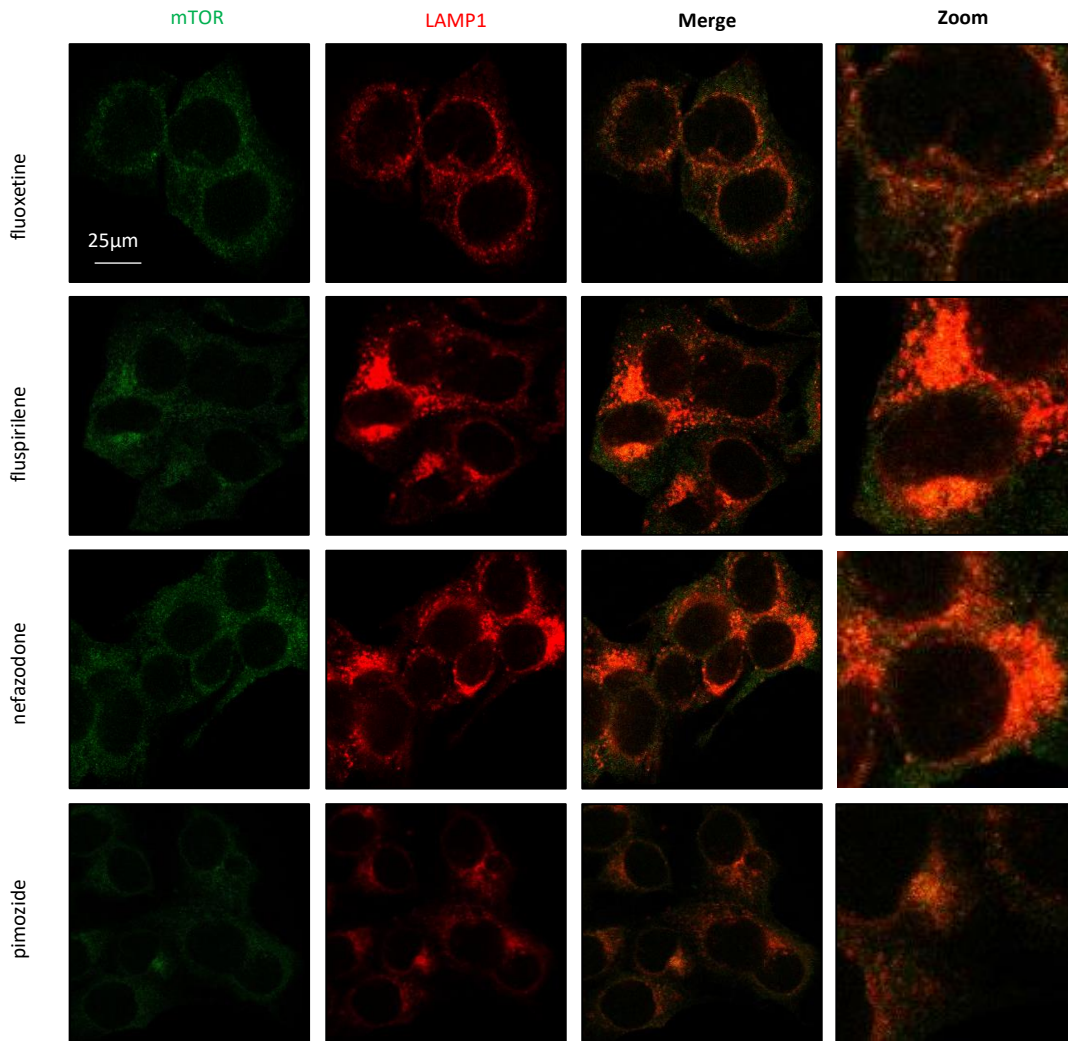
Supplementary Figure 4. CADs features of psychotropic drugs with antitumoral activity. Structure of cationic amphiphilic drugs and psychotropic drugs are shown. Circles indicate the hydrophilic region containing an amine group protonated at physiological pH, arrows indicate the hydrophobic region containing aromatic groups.

A**B**

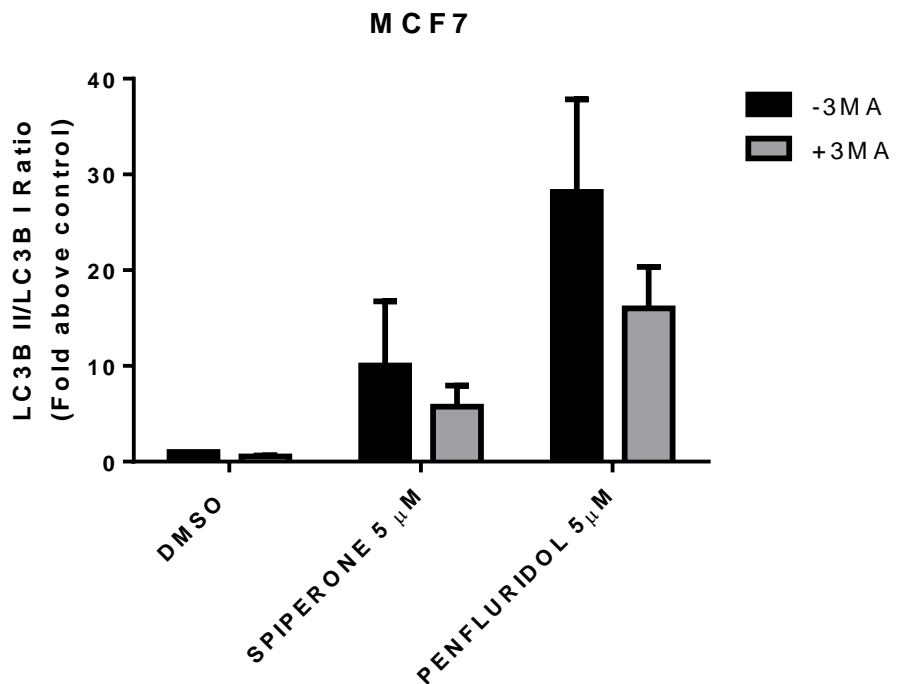
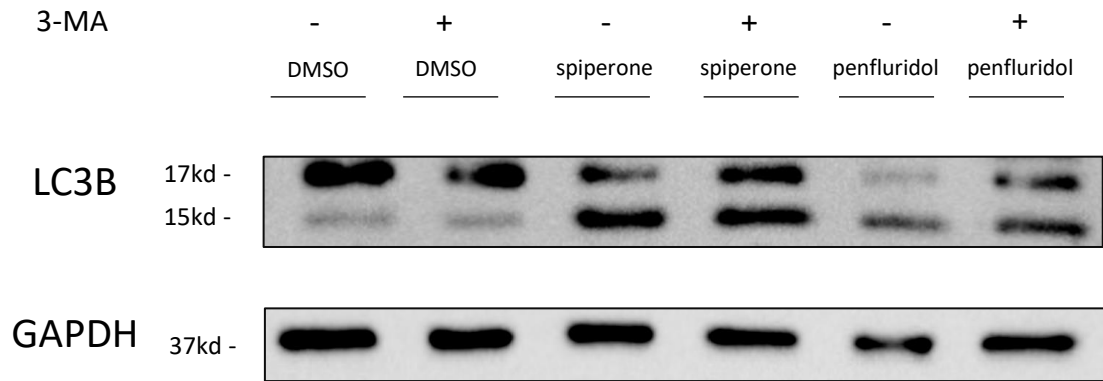
Supplementary Figure 5. Vacuolar structures formation after treatment of MCF7 cells with psychotropic drugs. Morphological alterations associated with the treatment of MCF7 cells with psychotropic drugs (5 $\mu\text{mol/L}$) were investigated after 6 h of exposure by phase contrast microscopy (original magnification 20x). Representative images of cells treated with DMSO, positive control, ebastine, fluoxetine, fluspirilene, nefazodone, penfluridol, pimoziide, spiperone and rapamycin, positive control (A). Histogram showing quantification of vacuoles as fold change relative to control (B). Data are expressed as the mean \pm SD of a representative experiment out of three independent experiments performed in triplicate. **, Student's T-test $p < 0.01$; ***, Student's T-test $p < 0.001$.



Supplementary Figure 6. Psychotropic drugs induce acidic compartment formation perturbing lysosomal and autophagic functioning. Vacuolar structures associated to treatment with psychotropic drugs in MCF7 were analyzed, after 6h exposure, staining cells with LysoTracker Deep Red. Pictures were acquired at fluorescence microscopy (magnification: 20x). representative images of cells treated with DMSO, negative control, ebastine, fluoxetine, fluspirilene, nefazodone, penfluridol, pimozone, spiperone and rapamycin



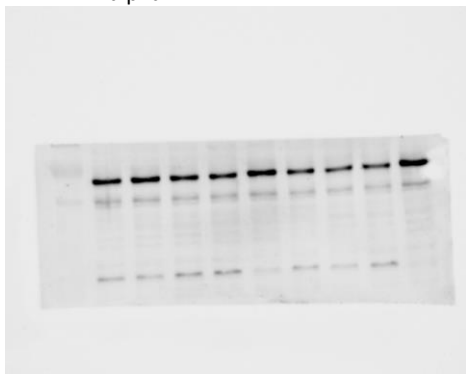
Supplementary Figure 7. Psychotropic drugs alter autophagy flux through mTOR pathway inhibition. Delocalization of mTOR from lysosomal membrane was evaluated in MCF7 after 16h treatment with psychoactive drugs. mTOR was stained using mTOR primary antibody and Alexa Fluor 488 secondary antibody (green). Lysosomes were stained using LAMP1 primary antibody and Alexa Fluor 536 secondary antibody (red). Representative images of fluoxetine, fluspirilene, nefazodone, pimozide



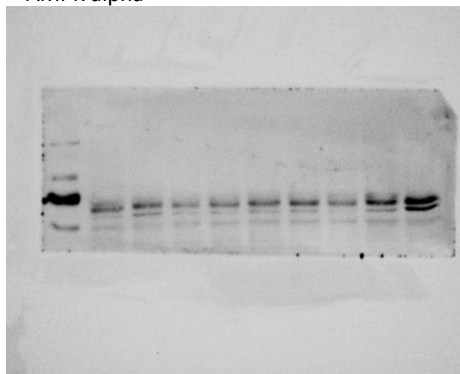
Supplementary Figure 9. 3-MA does not induce LC3B expression upon psychotropic drugs stimulation. Western blot analysis showing effects on autophagic pathway after 16 hours treatment with siperone and penfluridol plus 3-MA on MCF7. Lysates analyzed LC3B and GAPDH (A). Histogram showing quantification of the relative expression of LC3B II/I. Densitometry are expressed as the mean \pm SD of three independent experiments.

Raw data of western blot for review

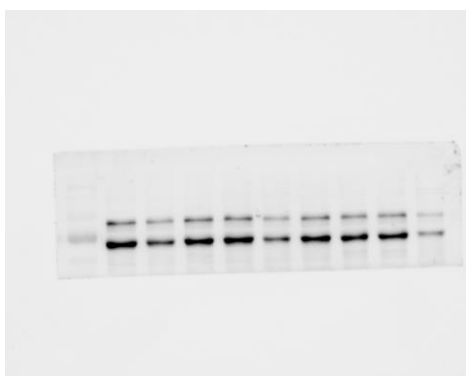
P-AMPK alpha



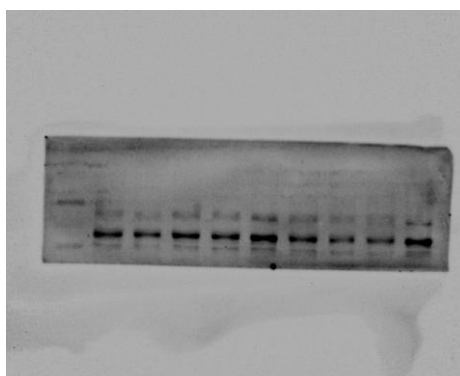
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P-P70S6K



P70S6K



P-S6



S6



GAPDH

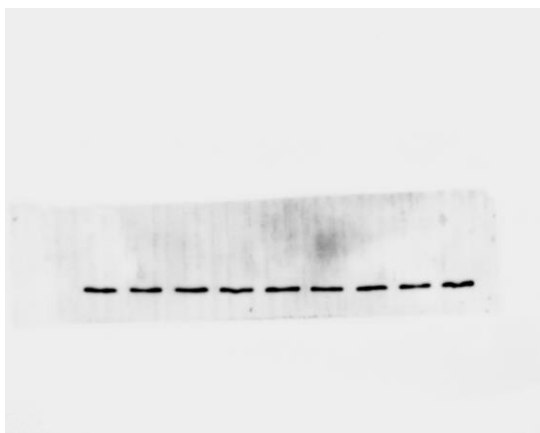
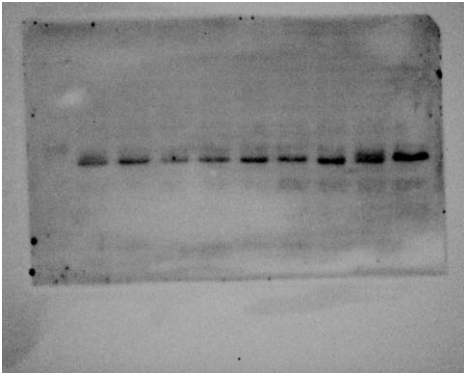
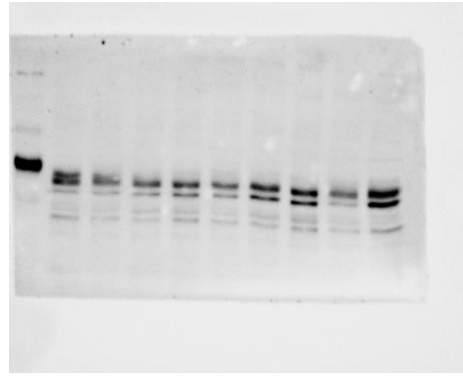


Figure 8. Psychotropic drugs alter autophagy flux through mTOR pathway inhibition. Western blot analysis showing effects on mTOR pathway after 16h treatment with psychotropic drugs on MCF7. Lysates analysed for p-AMPK α T172, AMPK, p-P70S6K T389, P70S6K, P-S6 S235/236, S6, LC3B and GAPDH (A). Histogram showing quantification of P70S6K (B), S6 (C) and AMPK (D) phosphorylation normalized on total protein P70S6K and S6. **Raw data of experiment 1**

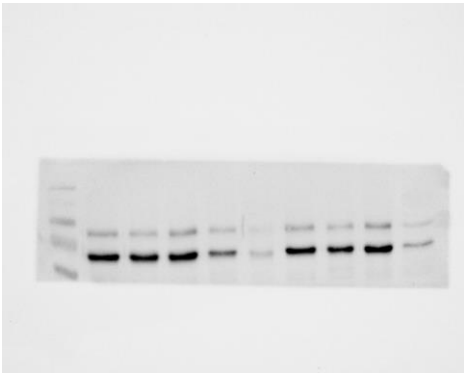
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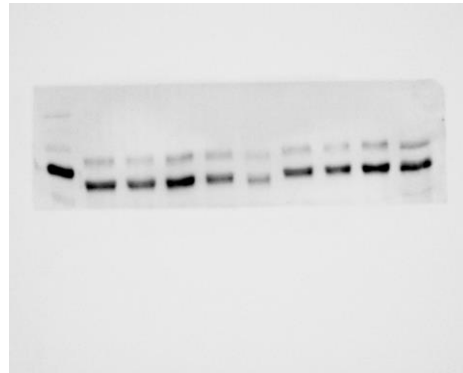
AMPK alpha



P-P70S6K



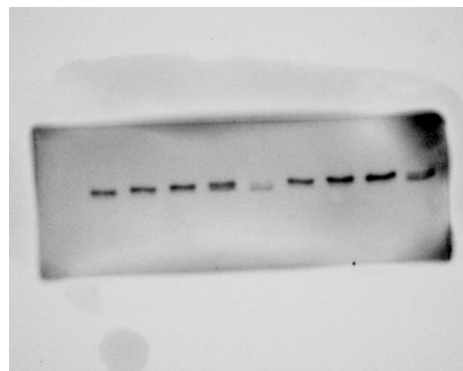
P70S6K



P-S6



S6



GAPDH

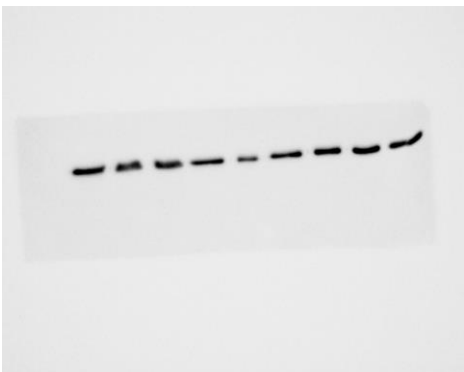
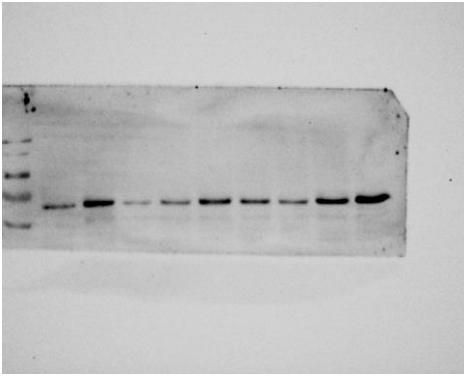
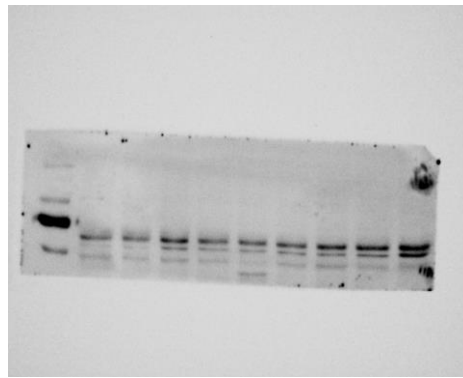


Figure 8. Psychotropic drugs alter autophagy flux through mTOR pathway inhibition. Western blot analysis showing effects on mTOR pathway after 16h treatment with psychotropic drugs on MCF7. Lysates analysed for p-AMPK α T172, AMPK, p-P70S6K T389, P70S6K, P-S6 S235/236, S6, LC3B and GAPDH (A). Histogram showing quantification of P70S6K (B), S6 (C) and AMPK (D) phosphorylation normalized on total protein P70S6K and S6. **Raw data of experiment 2**

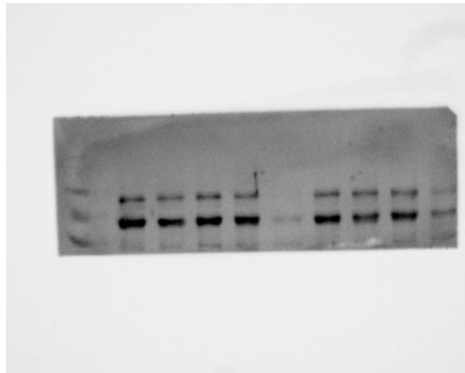
P-AMPK alpha



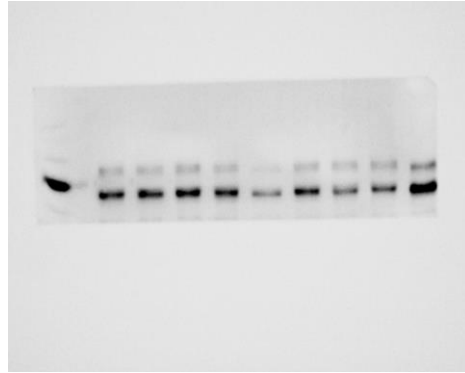
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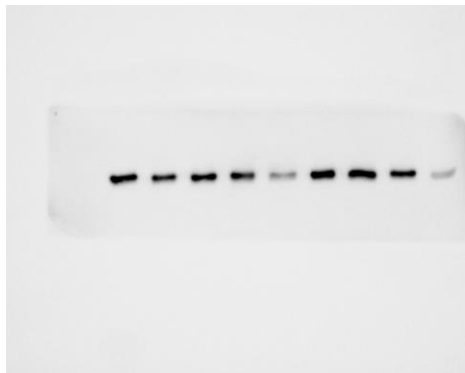
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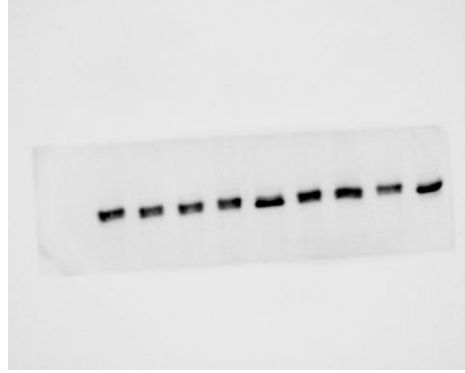
P70S6K



P-S6



S6

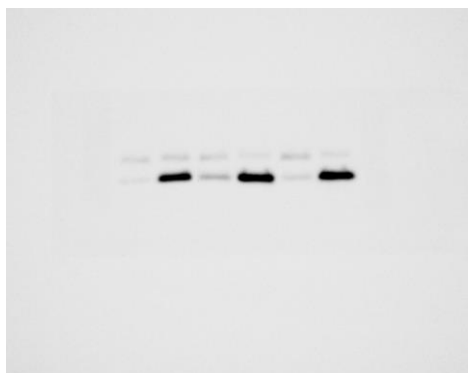


GAPDH



Figure 8. Psychotropic drugs alter autophagy flux through mTOR pathway inhibition. Western blot analysis showing effects on mTOR pathway after 16h treatment with psychotropic drugs on MCF7. Lysates analysed for p-AMPK α T172, AMPK, p-P70S6K T389, P70S6K, P-S6 S235/236, S6 and GAPDH (A). Histogram showing quantification of P70S6K (B), S6 (C) and AMPK (D) phosphorylation normalized on total protein P70S6K and S6. **Raw data of experiment 3**

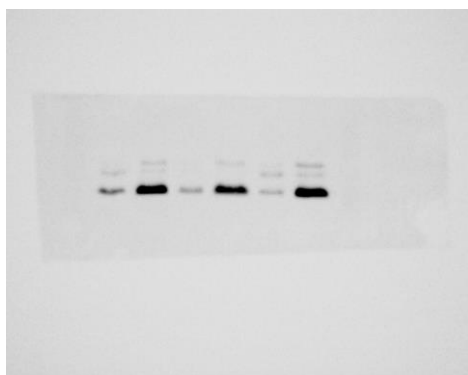
LC3B- DMSO/spiperone/ebastine



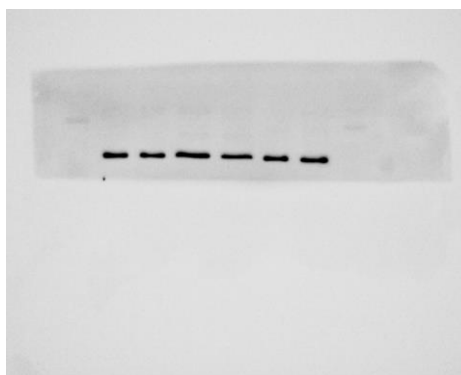
GAPDH- DMSO/spiperone/ebastine



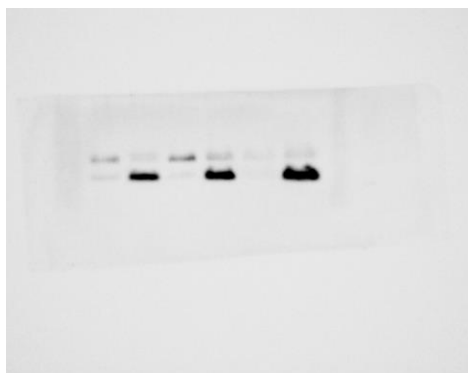
LC3B- pimozone/penfluridol/fluspirilene



GAPDH- pimozone/penfluridol/fluspirilene



LC3B- fluoxetine/nefazodone/serum starved

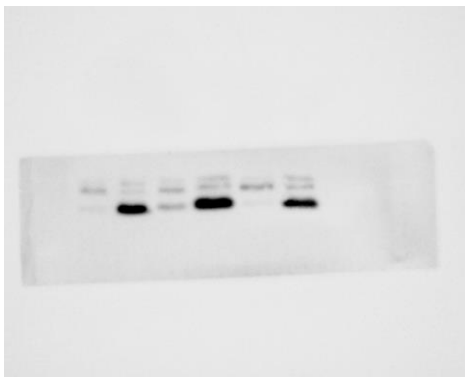


GAPDH- fluoxetine/nefazodone/serum starved



Figure 8. Psychotropic drugs alter autophagy flux through mTOR pathway inhibition. Western blot analysis showing effects on mTOR pathway after 16h treatment with psychotropic drugs on MCF7. WB showing the relative expression of LC3B II/I upon chloroquine treatment **Raw data of experiment 1**

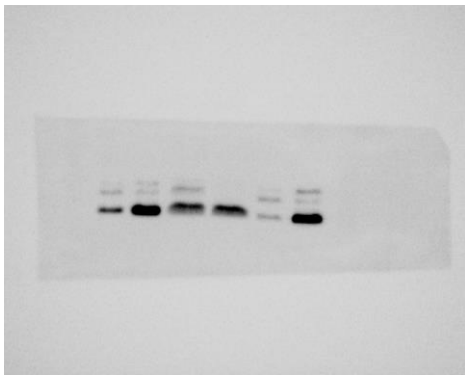
LC3B- DMSO/spiperone/ebastine



GAPDH- DMSO/spiperone/ebastine



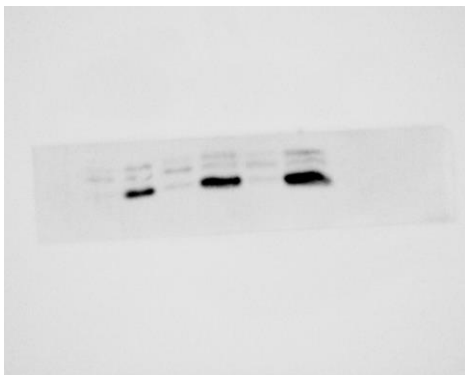
LC3B- pimoziide/penfluridol/fluspirilene



GAPDH- pimoziide/penfluridol/fluspirilene



LC3B- fluoxetine/nefazodone/serum starved

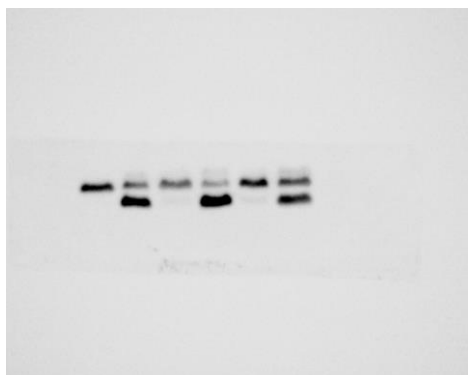


GAPDH- fluoxetine/nefazodone/serum starved

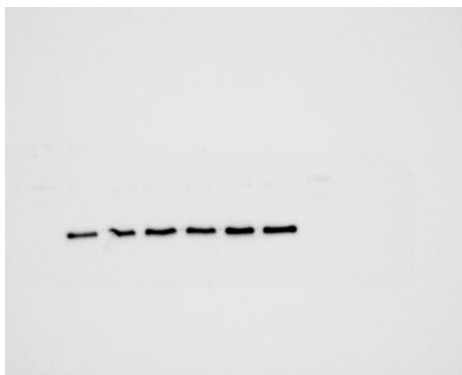


Figure 8. Psychotropic drugs alter autophagy flux through mTOR pathway inhibition. Western blot analysis showing effects on mTOR pathway after 16h treatment with psychotropic drugs on MCF7. WB showing the relative expression of LC3B II/I upon chloroquine treatment **Raw data of experiment 2**

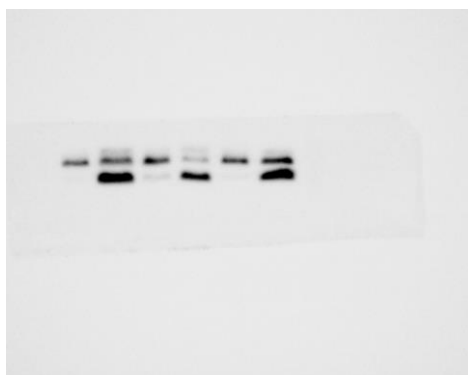
LC3B- DMSO/spiperone/ebastine



GAPDH- DMSO/spiperone/ebastine



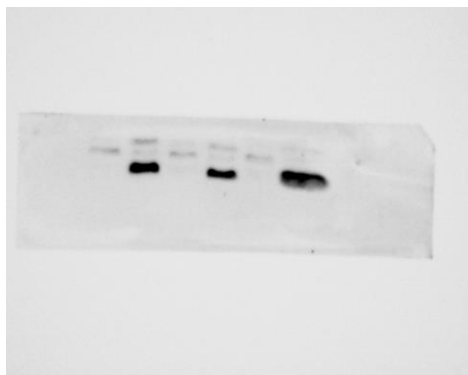
LC3B- pimozone/penfluridol/fluspirilene



GAPDH- pimozone/penfluridol/fluspirilene



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GAPDH- fluoxetine/nefazodone/serum starved

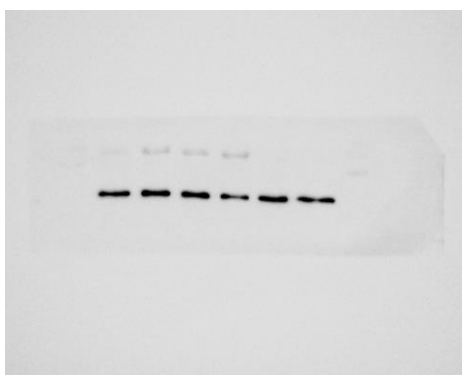
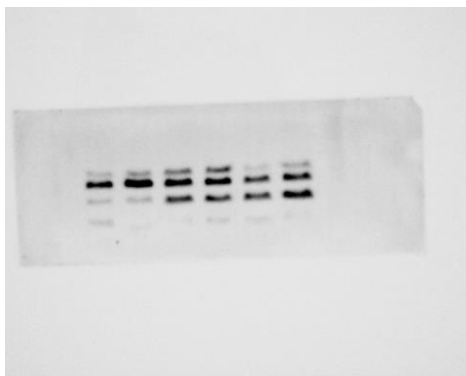


Figure 8. Psychotropic drugs alter autophagy flux through mTOR pathway inhibition. Western blot analysis showing effects on mTOR pathway after 16h treatment with psychotropic drugs on MCF7. WB showing the relative expression of LC3B II/I upon chloroquine treatment **Raw data of experiment 3**

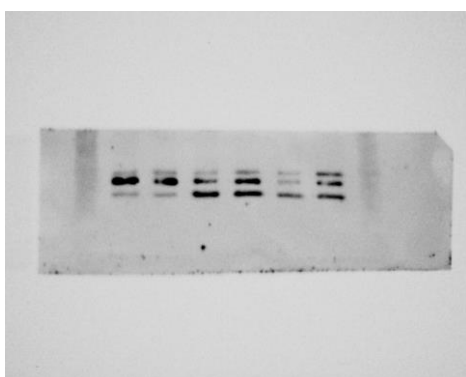
LC3B- experiment 1



GAPDH- experiment 1



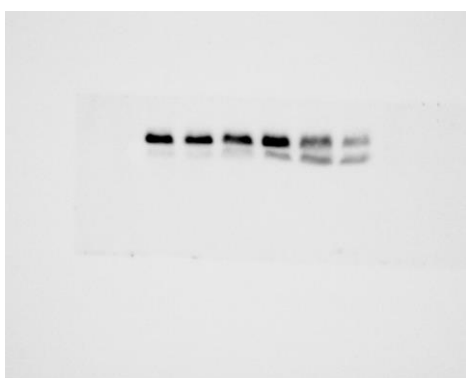
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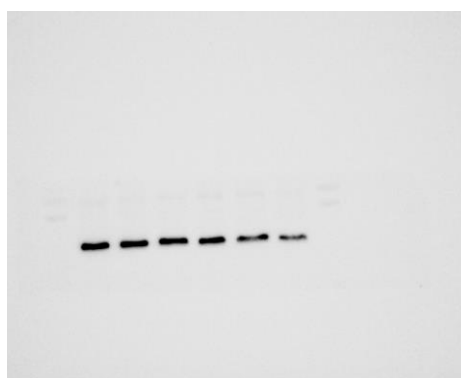
GAPDH- experiment 2



LC3B- experiment 3



GAPDH- experiment 3



Supplementary Figure 9. 3-MA does not induce LC3B expression upon psychotropic drugs stimulation. Western blot analysis showing effects on autophagic pathway after 16h treatment with spiperone and penfluridol plus 3-MA on MCF7. Raw data of experiment 1