Fluorescence-based assay to screen drug against Alzheimer disease

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Speeding up drug discovery

Abstract

Alzheimer disease (AD) is characterized by brain depositions of the beta amyloid (bA). The bA is the amyloid precursor protein (APP) digestion product, which is released from the cell after β-secretase and γ-secretase proteolysis.
Innoprot developed a novel fluorescence-based assay of secretases activity for new inhibitors screening. γ and β secretase commercial inhibitors have been used to perform a High content analysis of this model. The results indicated that the pharmacological inhibition of secretases implicated in AD remains a valid strategy for drug screening. Therefore, Innoprot has used this cellular model models to monitorizate the secretases activity in vivo treated with differents drugs in a automatic epifluorescent imaging system to acquire and analyze the model. Here, we presented as example a new drug denominated LP226A1 and provided from Lipopharma company which appear like new candidate to Alzheimer disease drug and it have been validated in this cellular model and animals.

Results

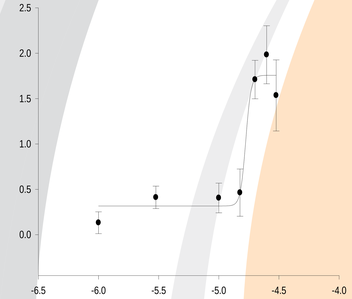

Fig.1.Determination of IC50 values for APP processing inhibitor LP226A1. Ic50 value for LP226A1was determined by treating of MDCK APP model cells with inhibitor concentrations 30uM, 25uM, 20uM, 15uM, 10uM, 3 uM and 1uM during 72h. Followed this incubation, the intracellular vesicles retented are quantified. Error bars represent the standard deviation among 5 replicae wells. Ic50 for LP226A1 was 17,8uM and zfactor for this experiment was 0,90 +/-0,01.

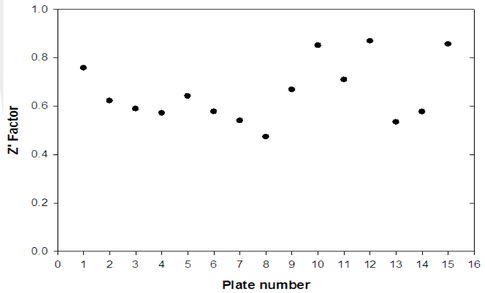

Fig.4. Screen results for library compounds inhibition. Vesicles retention ratio of 1200 compounds screened at 10uM concentration. Z´values for 15 plates screened. Z´value media obtained was 0,62+/-0,13. In this assay, 14 plates had Z´factor grater than 0.5 and only one plate had a Z´factor less than 0,5 (was 0,47).

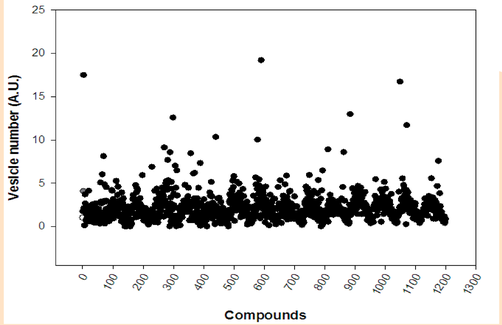

Fig.2. Screen results for library compounds inhibition. Vesicles retention ratio of 1200 compounds screened at 10uM concentration. The positive control (LP226A1) is represented in gray. The negative control is represented in white color. Of the 1200 compound screened, 57 compounds exhibiting greater inhibition than our positive control.

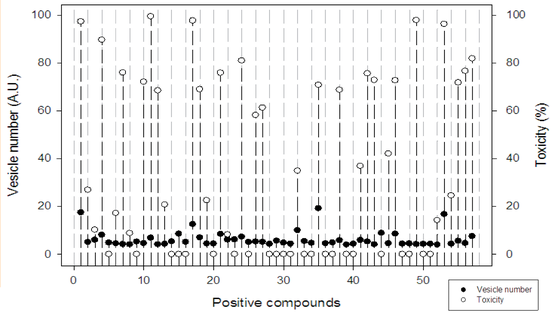

Fig.6. Toxicity results for positive compounds . Toxicity of 57 compounds screened at 10uM concentration related to their inhibitory effect. The inhibitory effect is represented in black and the toxicity percentage is represented in white. The results show that 23 compounds didn´t exhibit toxicity under experiment inhibitory conditions.

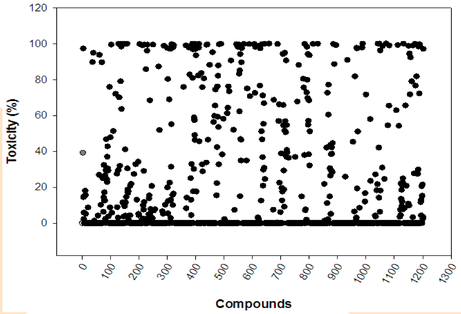

Fig.3. Screen results for library compounds toxicity. Toxicity of 1200 compounds screened at 10uM concentration. The positive control (LP226A1) is represented in gray. The negative control(DMSO)is represented in white color.

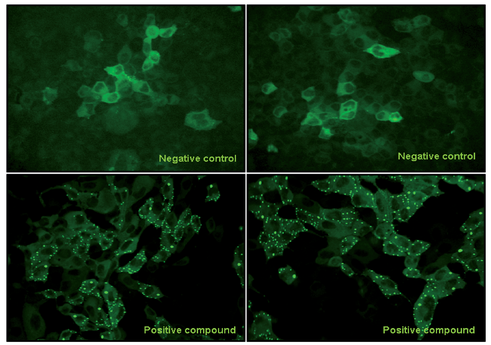

Fig.6. **Cellular fluorescence redistribution after inhibitors treatment**.The MDCK APP model culture cells were treated with DMSO as negative control (on the upper panel) or treated with an inhibitory compound at 10uM (on the lower panel). Cultured cells were observed 72h after treatment. The pictures show that the treatment leads to decreased APP processing and it produces an APP vesicles accumulation in the cell citosol.

Materials and methods

Compound stock solutions:
The library compounds are prepared in DMSO at 10mM concentration.
The positive inhibitor (LP226A1) stock solution was prepared in a concentration of 10mM in DMSO.

Cultured cells:
APP processing MDCK based model cells were cultured into 96 wells Imaging Plates BD at 0.15 cells/cm2 in 200 ul of DMEM 10% FBS and incubated at 37ºC and 5% CO2. After 12 hours, the cells were subcultured in DMEM 1%FBS for 72 hours in presence of lybrary compounds (at 10uM contration diluted in DMEM 1%FBS), positive control (at 10uM contration diluted in DMEM 1%FBS) or negative control (DMSO diluted in DMEM 1%FBS).

Image adquisicion:
APP processing MDCK based model cells were treated with library compounds during 72h. After that, the nucleus was stained with DAPI and cells with retented APP spots were detected by fluorescence using image analysis algorithms. Vesicle number was calculated relative to positive control(10mM). The retention assay was validated with an average of Z´factor media =0.62+/-0.13 for High Content Screening.

Conclusions

The Alzheimer model based in MDCK can be used in drug discovery for APP processing inhibitors o modulators.

This model have been adapted to HCS analyses based in image algorithms to test processing effects .

This model permits evaluate a lybrary of compounds, candidates to inhibitors, in living cells studying the vesicles retention.

This model allows to analyse in the space and time the compund effect in a multiparametric manner.

This model provide a strategy to evaluate drug againts secretases activity without the necessity to be permeable.