PLEIOTROPIC AGENTS AGAINST ALZHEIMER DISEASE: *IN VITRO* TOXICOLOGICAL SCREENING ON RECENTLY DEVELOPED SG-COMPOUNDS

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Introduction: The Alzheimer's Disease (AD) etiopathology involves alterations of several physiological pathways, both in peripheral and central nervous system regions, such as decreased lipid metabolism, lowered tau phosphorylation, increased neuro-inflammation, and unbalanced autophagy. This multi-faceted impairment leads to aberrant protein aggregation and uncontrolled neuronal cell death, resulting in the well-known decline of cognitive functions. To date, different mono-targeting approaches have been investigated to treat AD, but none of them seems to achieve the desired effect of reversing it. There is growing evidence implicating the role of autophagic flux (ATG) as a crosslinking effector in neurodegenerative disorders. Moreover, a marked impairment of this process has been observed in several AD models, suggesting that it could play a pivotal role in the development and progression of the pathology. We have designed and characterized a new class of synthetic small molecules with a biphenylmethane scaffold, namely SG compounds, to act as pleiotropic agents against AD. Among them, SG-2was identified as a promising hit-compound able to promote lipid metabolism and neuroprotective effects in several in vitro models. Moreover, when systemically administered to CD-1mice at sub-micromolar doses, SG-2induced an improvement in learning and memory. Finally, SG-2also showed the ability to promote autophagy in in vitro experiments. Recently, several SG-2analogues have been designed and synthetized with the aim to increase the chemical diversity of SG-2.

Material and methods: In order to evaluate the phenotypic profile of new SG2analogues, we performed in vitro screening on 30 compounds. This included a comprehensive panel of absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) assays in order to identify the most promising compound for progression in the drug discovery value chain. These assays included cytotoxicity in four different cell lines (MCF-7, HEK293, hTERT, and U2-OS), hERG liability, CYP450 inhibition (2C9, 2C19, 2D6, and 3A4) and off-target liability against HDAC6, HDAC8, SIRT7, PDE4C1, and Aurora B kinase. All screening experiments were performed in triplicate at a concentration of 10μM. The results for each compound were normalized using the respective raw data to high and low controls. The data were collected and analyzed in the form of a traffic-light system using specific and validated criteria.

Results: Overall, tested compounds showed no cytotoxic effects on all four cell lines, with a percentage of inhibition of cellular growth lower than 15% in most cases. Similarly, no relevant behavior was observed - except for a few cases - when compounds were tested against off-targets (HDAC6, HDAC8, SIRT7, PDE4C1, and Aurora B kinase). Most of tested compounds showed high values of inhibition against hERG channel and some isoforms of CytochromeP450. Ten of the SG2analogues showed a completely clean and safe profile with a percentage of inhibition lower than 30%.

Discussion and conclusions: More than 30 compounds were tested. Notably, ten of the tested SG analogues showed a completely clean and safe toxicological, pharmacological and pharmacokinetic profile. Interestingly, some of the clean compounds were designed as SG2prodrugs; however, further studies will be necessary to confirm both their activity and metabolic pathway. Future in vitro and *in vivo* studies will be planned to investigate the safety and pharmacological potential of these novel biphenylmethane scaffold-based molecules in neurodegenerative disorders such as AD. In conclusion, our study will help us to carry out the MedChem optimization of the pleiotropic agent SG2, which could provide an innovative approach to AD.