Design, Synthesis and Evaluation of Substituted Acetamides and Carbohydrazones as Dual Monoamine Oxidase and Acetylcholinesterase Inhibitors

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By

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5. Concluding remarks and future perspective

5.1. Conclusion

NDDs (neurodegenerative diseases) have a major prevalence in the elderly population and AD accounts for about 75 % of total cases. The well-established cholinergic and dopaminergic hypotheses primarily involve excessive activity of AChE and MAO-B enzymes, respectively, and are regarded as potential targets for anti-neurodegenerative drug therapy. The present study aimed to identify new small molecule MTDLs possessing dual MAO-B and AChE inhibition activity and neuroprotective properties.

In the first part (Chapter 2, **ZBTI series**), we employed pharmacophore-based virtual screening and molecular simulation methodologies to identify a novel MAO-B inhibitor (**ZINC02181408**) bearing benzothiazole thioacetamide scaffold which was further exploited chemically and pharmacologically to assess its dual MAO-AChE inhibitory and neuroprotective properties. The study produced compound **ZBTI13** as the most potent MAO-B and AChE inhibitor (MAO-B $IC_{50} = 0.015 \pm 0.007$ μM, AChE $IC_{50} = 0.114 \pm 0.003$ μM). Further lead evaluation studies revealed that compound **ZBTI13** possesses noticeable brain penetration (CNS±) and iron-chelation properties. Additionally, *in cellulo* and *in vivo* toxicity studies indicate that the compound **ZBTI13** was non-toxic in fibroblast and neuroblastoma cells up to 50 μM and up to a dose of 2000 mg/kg body weight in adult female albino mice. Moreover, the compound showed significant neuroprotective activity against dexamethasone-induced oxidative stress in SH-SY5Y cells and scopolamine-induced amnesia model in mice.

In the second part (Chapter 3, **SMA** & **SMH series**), we utilized our in-house lead MAO-B and AChE inhibitor (compound **I**, **Figure 3.1**) to design a set of 11 sesamol-derived O-acetamides (**SMA01-SMA11**) and 17 analogs of sesamol-derived extended carbohydrazones (**SMH01-SMH17**) and investigated their dual MAO-AChE inhibitory

and neuroprotective properties. Among the SMA analogs, compound **SMA09** emerged as the lead dual inhibitor (MAO-A IC_{50} = 0.160 ± 0.009 μM, MAO-B IC_{50} = 0.071 ± 0.002 μM, AChE IC_{50} = 2.611 ± 0.086 μM and BChE IC_{50} = 4.223 ± 0.162 μM). Among the SMH analogs, compound **SMH06** has emerged as a lead multifunctional molecule (MAO-A IC_{50} = 11.145 ± 0.305 μM, MAO-B IC_{50} = 0.064 ± 0.003 μM, AChE IC_{50} = 1.555 ± 0.013 μM, BChE IC_{50} = 9.936 ± 0.105 μM). Both these lead compounds **SMA09** and **SMH06** were brain penetrant and showed moderate *in vitro* antioxidant activity and iron-chelation properties. Moreover, these compounds were non-toxic to fibroblast cells up to 50 μM while compound **SMA09** showed significant *in vitro* neuroprotection against dexamethasone-induced oxidative stress in SH-SY5Y cells. These MTDLs have also displayed significant *in vivo* neuroprotection activity in the scopolamine-induced amnesia model. Additionally, compound **SMA09** was non-toxic up to a dose of 2000 mg/kg body weight in adult female albino mice.

In the third part (Chapter 4, **SEH** & **SIEH series**), a library of 22 eugenol and isoeugenol-derived carbohydrazones (**SEH01-SEH11** & **SIEH01-SIEH11**) was designed by molecular hybridization approach and evaluated for their multi-target inhibition and neuroprotective properties. Compound **SIEH10** emerged as the most selective MAO-B inhibitor with a balanced dual activity profile (MAO-A IC_{50} = 1.901 ± 0.102 μM, MAO-B IC_{50} = 0.034 ± 0.002 μM, AChE IC_{50} = 1.316 ± 0.013 μM and BChE IC_{50} = 9.827 ± 0.109 μM). Subsequent *in vitro* investigations of the isoeugenol-based lead carbohydrazone revealed the compound's brain penetration, antioxidant and iron-chelation capabilities. Further, the compound **SIEH10** was non-toxic up to 10 μM in fibroblast and toxic to neuroblastoma cells. However, the compound **SIEH10** showed no neuroprotection in the scopolamine-induced amnesia model.

In summary, we have synthesized a chemical library of 64 new compounds belonging to four different series and evaluated their MAO-AChE inhibitory potential to identify potential dual-acting lead MTDLs. Further evaluation of the most potent dual inhibitors from each series yielded brain penetrant multifunctional agents with promising *in cellulo* and *in vivo* neuroprotective profiles with an exception of compound **SIEH10**. The activity profile of the resultant new MTDLs (**ZBTI13, SMA09, SMH06 & SIEH10**) possessing significant neuroprotective properties are summarized in **Figure 5.1** and these MTDL candidates merit further investigations in *in vivo* AD/PD models.

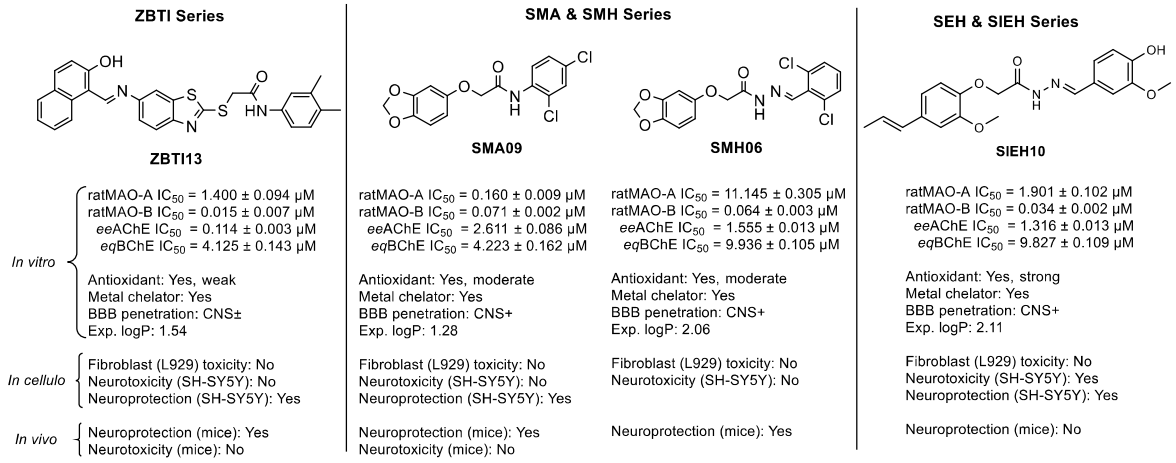

Figure 5.1. Activity profile of lead MTDLs identified from the study

One of the limitations of our study is that we have utilized the safinamide-derived pharmacophore model for the initial virtual screening study (Chapter 2) to identify new MAO-B inhibitory scaffold which was later exploited to derive new MTDLs possessing inhibitory activity against both MAO-B and AChE. Ideally, probing the chemical space virtually using a combined or hybrid pharmacophore model derived from the known MAO-B and AChE inhibitory ligands can help in the direct identification of new chemical scaffolds possessing potent dual MAO-B and AChE inhibitory properties. Such hybrid pharmacophore models upon successful experimental validation can be optimized further

and eventually help in the rapid identification of potent dual inhibitory MTDLs for NDD therapies.

5.2. Future perspective

Further work on the aforementioned research may include the following:

- Parallel lead optimization studies through rational structural modification to increase potency against AChE and selectivity.
- Mechanistic studies of lead MTDLs in 3D neuronal cell culture models using neuroblastoma cell lines and primary cortical neurons.
- Preclinical studies of lead MTDLs in specific *in vivo* AD and PD models.