New Brain Penetrant Multitarget Compounds against Parkinson´s Disease

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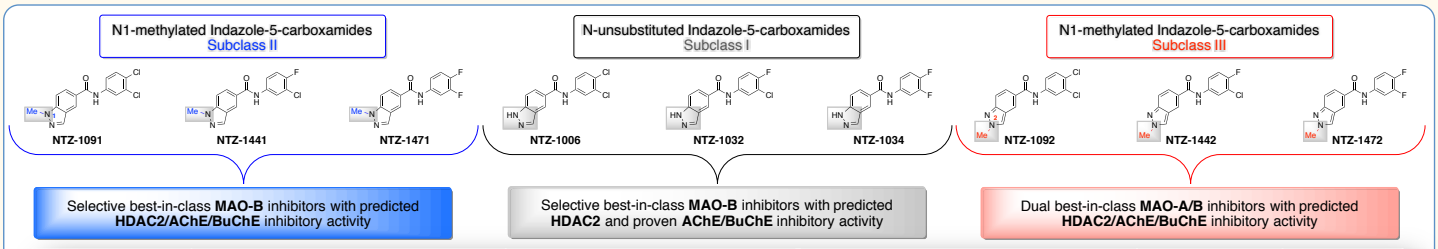
Introduction

A new collection of structurally optimized compounds addressing the central nervous system (CNS) diseases, which have been recently entered into a development and marketing agreement between Key Organics Ltd. and NTZ Lab Ltd., is now available in the BIONET collection [1]. This screening collection with experimentally-determined bioactivity, ADMET, bioavailability, and toxicity will address the need of new, robust, and multi-potent small molecules for the treatment and diagnosis of CNS diseases, such as Parkinson´s disease, Alzheimer´s disease, dementia and/or other neurodegenerative diseases [1–4].

The initial set of nine target indazole-5-carboxamides (subclasses I–III) is part of a large number CNS active compounds [1]. Importantly, the newly discovered molecules are virtually and experimentally evaluated as multi-target acting monoamine oxidase (MAO)/histone technology platform [2,5]. In addition, the neuroprotective effects on TH-positive dopaminergic neurons and the induction of the neurite network outgrowth of the most promising compound **NTZ-1441** have been investigated in a Parkinson´s disease model [4]. These effects are associated with a good BBB penetration of all presented compounds that was confirmed in several *in vitro* assays. This poster will summarize the design, virtual screening with SeeSAR, experimental confirmed "Proof-of-Principle" biological activity of this next generation multi-target compounds that are available for further elaboration for Parkinson´s disease treatment.

Multipotency Screening on Other CNS targets

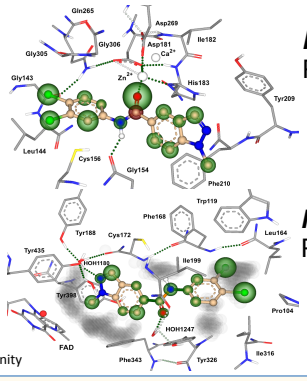


Compd.	IC_{50} ± SEM (nM)		IC_{50} ± SEM (µM)		$K_{i\ HYDE}$ (nM)		K_i ± SEM (nM)	LLE[a] (CNS+ >5)
	*h*MAO-A	*h*MAO-B	*h*AChE	*h*BuChE	*h*HDAC2	*h*MAO-B	*h*MAO-B	
NTZ-1006	>10,000	0.586 ± 0.087	>100	>100	0.19–19.0	5.00 –497.2	0.26 ± 0.04	5.11[b]
NTZ-1032	>10,000	0.679 ± 0.044	>100	>100	0.76–75.1	10.9–1079	0.30 ± 0.02	5.56[b]
NTZ-1034	>10,000	1.59 ± 0.16	>100	>100	1.92–191.5	14.6–1448	0.70 ± 0.07	6.14
NTZ-1091	>10,000	0.386 ± 0.052	78.3 ± 1.1	>100	0.07–7.09	0.98–97.6	0.17 ± 0.02	6.17
NTZ-1441	>10,000	0.662 ± 0.056	>100	>100	0.27–27.1	1.44–142.7	0.29 ± 0.02	6.99
NTZ-1471	>10,000	1.52 ± 0.18	>100	>100	0.85–84.5	2.39–238.0	0.67 ± 0.08	5.72[b]
NTZ-1092	420 ± 24	1.44 ± 0.41	n.t.	n.t.	0.14–14.4	2.29–227.5	0.64 ± 0.18	5.87
NTZ-1442	562 ± 62	8.08 ± 1.05	n.t.	n.t.	0.66–65.8	2.78–276.1	3.57 ± 0.46	5.45
NTZ-1472	764 ± 40	38.4 ± 1.80	n.t.	n.t.	1.92–190.8	3.76–373.3	16.9 ± 0.80	4.32[b]

[a] Ligand-lipophilicity efficiency (LLE) = pIC_{50} (*h*MAO-B) – $logP/D_{7.4}$, [b] Calculated using SeeSAR 8.2, 2019, n.t. = not tested.

Combined X-Ray/SeeSAR CADD & ADME Evaluation

SeeSAR visualization binding of **NTZ-1091** to *h*HDAC2 and *h*MAO-B



***h*HDAC2**
PDB: 4LY1

***h*MAO-B**
PDB: 2V5Z

HYDE colouring:
green = good
red = bad for affinity

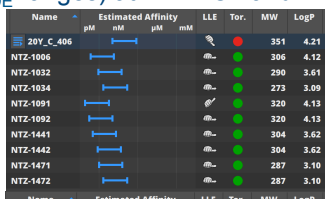
Screening Approach

Selective best-in-class **MAO-B** or dual **MAO-A/B** inhibitors
↓
1. Step: Single X-ray
↓
2. Step: SeeSAR docking
↓
3. Step: HYDE analysis
↓
Multi-target **MAO-A/B /HDAC2** inhibitors

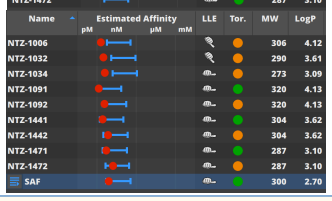
- Multi-target **MAO-A/B / HDAC2** inhibitors
- Reversible mode of action (MoA)
- Competitively mechanism of inhibition
- Stimulation of neurites elongation

HYDE estimated binding affinity ($K_{i\ HYDE}$ ranges) at *h*HDAC2 and hMAO-B

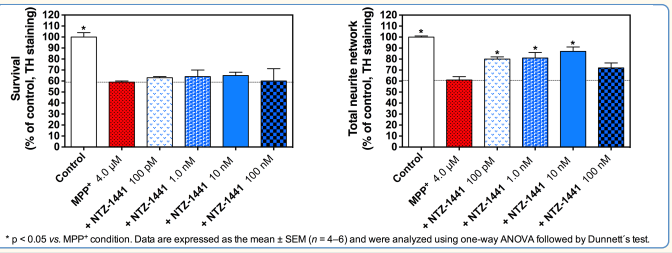
***h*HDAC2**



***h*MAO-B**
● = experiment



Effect of **NTZ-1441** on dopaminergic neurons and neurite network after 48 hrs injury with MPP+



* p < 0.05 *vs.* MPP+ condition. Data are expressed as the mean ± SEM (n = 4–6) and were analyzed using one-way ANOVA followed by Dunnett´s test.

In Summary

- The compounds presented herein belong to the best-in-class selective MAO-B (subclass I and II) or dual MAO-A/B (subclass III) inhibitors with excellent solubility-lipophilicity balance.
- Multi-target *in silico* evaluated inhibitory activity towards human recombinant AChE/BuChE/HDAC1/2, suggesting for selective inhibition of HDAC2 in the sub-nM range (**MAO-A/B and HDAC2 inhibitors**).
- "Proof-of-Principle" experimentally confirmation of selective *h*AChE inhibition for **NTZ-1091** (78.3 µM).
- The most potent compounds are identified as highly GI and BBB permeable CNS active drugs.
- The effect of **NTZ-1441** on the neurite network of TH-positive dopaminergic neurons strongly suggest that this compound stimulated the elongation of neurites after 48 h injury with MPP+ (synaptogenesis).

A new BIONET CNS Compound collection is available for further development and screening on relevant CNS targets. All together these results suggest that the present set of nine compounds are promising drug and radioligand candidates for Parkinson´s disease or even Alzheimer´s disease treatment and diagnosis.

REFERENCES

1. Tzvetkov, N. T. PCT Patent WO 2014/107771 A1, NTZ Lab Ltd., **2014**.
2. Tzvetkov,et al. *Eur. J. Med. Chem.*, **2017**, *127*, 470–492.
3. Tzvetkov,et al. *J. Med. Chem.*, **2014**, *57*, 6679–6703.
4. Tzvetkov,et al. *J. Enz. Inh. Med. Chem.*, **2017**, *32*, 960–967.
5. www.biosolveit.de/SeeSAR (SeeSAR v.8.0, 2018).

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