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COMMUNICATION

Multitarget-Directed Ligands Combining Cholinesterase and Monoamine Oxidase Inhibition with Histamine H_3R Antagonism for Neurodegenerative Diseases

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Abstract: The therapy of complex neurodegenerative diseases raises the need for the development of multitarget-directed drugs (MTD). Novel MTD indole derivatives with inhibitory potencies at acetyl/butyrylcholinesterases and monoamine oxidases A/B as well as at the histamine H_3 receptor (H3R) were obtained by optimization of the neuroprotectant ASS234 by incorporating generally accepted H3R pharmacophore motifs. These small molecule hits demonstrated balanced activities at the targets, mostly in a nanomolar concentration range. Additional in vitro studies showed anti-oxidative, neuroprotective and brain penetration effects. With this promising in vitro profile, compound contilisant (**4** at 1 mg/kg i.p.) also improved significantly the lipopolysaccharide-induced cognitive deficits.

Alzheimer's (AD) and Parkinson´s diseases (PD) are the most prevalent neurodegenerative diseases with complex and variable underlying mechanisms. In studies of the causes and in searches for more efficient therapies, factors such as mitochondrial dysfunction, neuro-inflammation, and especially oxidative stress have been identified as major determinants in progress and development of these diseases. Consequently, an antioxidant drug development strategy for neurodegenerative diseases, especially AD, has been of paramount importance.[1,2] A recent multitarget-directed ligand (MDL) ASS234 (Figure 1)[3–5] is able to irreversibly inhibit monoamine oxidases A and B (MAO A/B), and also reduce production of the second product hydrogen peroxide, a reactive oxygen species (ROS).[6] Thus, ASS234 prevents the catalytic oxidation of biogenic amines, such as serotonin (5-HT), norepinephrine and dopamine, all implicated in cognitive processes, as well as production of ROS associated with neuronal cell death. Additional, ASS234 gives reversible acetylcholinesterase (AChE) inhibition, improving memory and cognition, comparable to that of marketed AChE inhibitors (e.g. donepezil).[7]

The histamine H_3 receptor (H3R) is involved in the central regulation of histamine and other neurotransmitters,[8,9] so was targeted as useful novel pharmacological tool. Blockade of H3R by inverse agonists/antagonists elevates neurotransmitters, like acetylcholine (ACh), 5-HT, dopamine or norepinephrine in the central nervous system. The first H3R inverse agonist pitolisant (WAKIX®) was approved recently for the treatment of narcolepsy, but is also under investigation for diverse cognition and sleep

impairments.[10] Thus, the pro-cognitive use of H3R antagonists/inverse agonists for neurodegenerative disease is being investigated.[11] Although compounds showing multipotent profiles combining H3R affinity with cholinesterase (ChE) inhibition,[12,13] and antioxidant capacity[14] or most recently with MAO inhibition[15] have been reported (for review see [16]), MDLs able to modulate simultaneously H3R, MAO and ChE have not yet been addressed. This multipotent profile might constitute an innovative therapeutic approach for new molecules targeting neurodegenerative diseases with multiple causes.

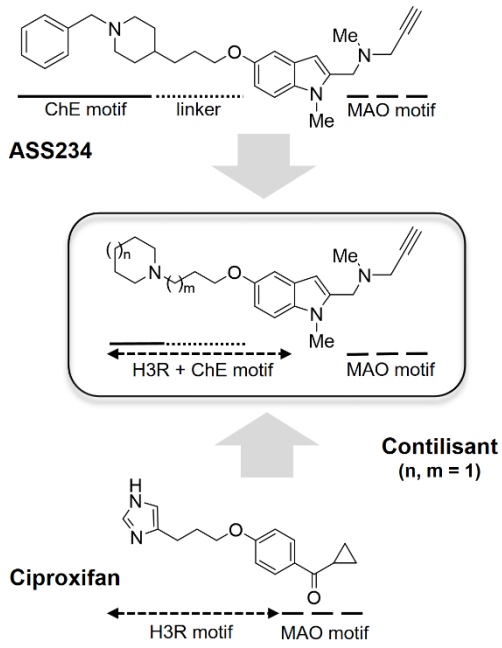
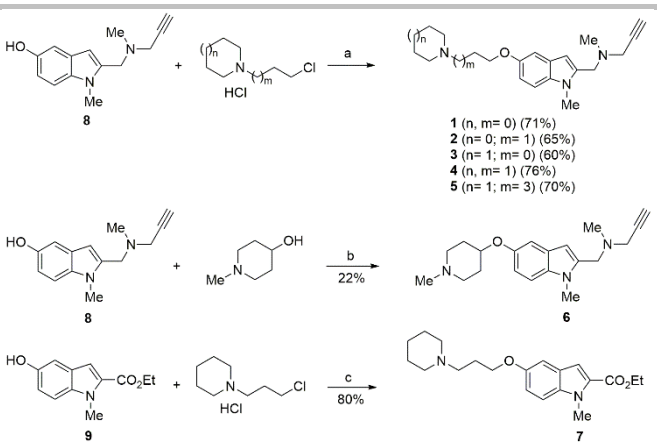


Figure 1. General structure of H3R/MAO/ChE MDLs derived from structural elements of antioxidant ASS234 and H3R antagonist ciproxifan.

ASS234 was structurally modified to fit a generally accepted pharmacophore of H3R antagonists (Figure 1). To avoid adverse effects associated with imidazole-containing H3R antagonists (e.g. ciproxifan), cyclic aliphatic amines like piperidine as the basic center were connected via a (propyloxy)phenyl chain to an eastern arbitrary region. These compounds provide suitable pharmacophores verified by multiple structure-activity relationship (SAR) studies.[17–19] Here, we report the synthesis and biological evaluation of MDLs **1**-**7** (Scheme 1), and the identification of compound **4** (contilisant) providing high antioxidant activity, high affinity at H3R, and excellent inhibition of the target neurotransmitter-catabolizing enzymes. These compounds were evaluated for their affinity at human H3R, H4R and against four neurotransmitter-catabolizing enzymes (AChE and butyrylcholinesterase (BuChE), MAO A/B) (for further off-target screening see Supporting Information).



Scheme 1. Synthesis of MDLs **1**-**7**. Reagents and conditions: a. NaH, DMF, rt; b. PPh_3, DIAD, THF, rt; c. K_2CO_3, DMF, 90 °C.

All MDLs in this small series inhibited ChEs in the micromolar range (Table 1). Contilisant revealed the best inhibition properties with high nanomolar inhibition of AChE. The initial reversible inhibition of MAO A/B (reflecting binding) and inhibition after 30 min preincubation of the inhibitor with the enzymes (due to irreversible inhibition) were determined (Table 1). Without preincubation, all compounds gave low micromolar IC_{50} values. The spacer lengths influence the binding to the active sites of MAO A and B, with a two carbon spacer being optimum when bearing a piperidine ring. Switching to a pyrrolidine ring has little effect in compounds with a three carbon spacer (**2** *vs*. **4**), but decreased inhibition in compounds with a two carbon spacer (**1** *vs*. **3**). After preincubation, IC_{50} values shifted to nanomolar concentrations for most of the propargylamines. Irreversible MAO inhibition was confirmed for contilisant by 50-fold dilution into excess substrate. The IC_{50} value for compound **6** changed very little with preincubation, suggesting that the propargyl group did not form a covalent adduct with MAO B. Compound **7**, lacking the propargyl group, showed no change with preincubation. MAO activity after 50-fold dilution of **7** was >95%, indicating reversible inhibition. Contilisant, showed improved irreversible inhibition of MAO B compared to the MAO A-preferring ASS234. The affinity for binding at human H3R as target and H4R, the structurally most homologous G-protein-coupled receptor, as off-target were measured (Table 1). None of the compounds bound to H4R, indicating good specificity for H3R. Surprisingly, ASS234 showed a remarkable affinity at H3R, but highest affinity was found for **2** and contilisant, both containing the propyloxy linker connected to pyrrolidino and piperidino moieties, respectively. Good affinities were also found for **6**, containing a related H3R pharmacophore motif, and **7**, lacking the propargyl amine group but preserving the propyloxy linker. Compounds with ethyloxy or pentyloxy spacer showed moderate H3R affinities. These findings confirm previously obtained SAR for H3R antagonists.[17,20,21] Since compounds **6** and **7** exhibit comparable H3R affinity, we could demonstrate, that the H3R affinity is positively influenced by the introduction of the second basic moiety, the propargylamine motif, that gives the MAO inactivation properties. Compound **6**, although less effective against AChE, provides structural variation possibilities as the MAO motif could be combined with varied spacers or amine warheads for H3R pharmacophore.

Table 1. IC_{50} and K_i values for the inhibition of hMAO A/B, hAChE/hBuChE and hH3R/hH4R, respectively, and ORAC analysis of compounds **1**-**7**, ASS234, ciproxifan, clorgyline, deprenyl and donepezil.

MTDL	Preinc (min)	hMAO A IC_{50}[a] (μM)	hMAO B IC_{50}[a] (μM)	SR MAO[b]	hAChE IC_{50}[a] (μM)	hBuChE IC_{50}[a] (μM)	SR ChE[c]	ORAC[d] (TE)	hH3R K_i[e] (nM)	hH4R K_i (nM)
1	0	3.00±0.34	5.21±0.82	1.7	37.9±1.5	25.1±5.5	1.5	3.11±0.07	178	>10,000
	30	0.095±0.009	0.140±0.008	1.5					[44, 716]	
2	0	4.01±0.60	1.80±0.24	0.5	18.8±2.7	7.40±1.41	2.5	4.54±0.08	4.5	>10,000
	30	0.073±0.006	0.100±0.020	1.4					[1.8, 11]	
3	0	0.41±0.03	1.32±0.21	3.2	20.6±3.6	8.55±1.48	2.4	1.86±0.06	38.5	>10,000
	30	0.052±0.007	0.017±0.003	0.3					[13, 117]	
4	0	1.85±0.21	1.94±0.15	1.0	0.53±0.05	1.69±0.12	0.3	3.59±0.09	10.8	>100,000
(contilisant)	30	0.145±0.010	0.078±0.006	0.5					[4.2, 27]	
5	0	6.52±0.52	41.3±5.5	6.3	8.3±2.4	3.30±0.71	2.5	2.94±0.04	77.7	>10,000
	30	0.166±0.015	4.65±0.06	28					[19, 311]	
6	0	1.19±0.15	3.80±0.40	3.2	58.3±11.8	31.1±1.8	1.9		14.7	>10,000
	30	0.042±0.004	2.75±0.51	65					[3.8, 57]	
7	0	103±20	12.6±1.0	0.1	20.4±2.0	11.6±1.3	1.8	1.40±0.14	24.4	>10,000
	30	91±1	11.2±0.9	0.1					[12, 50]	
ASS234	0	0.033±0.003	3.20±0.41	97					84.2	>10,000
	30	0.00027±0.00003	0.12±0.02	444	0.81 ± 0.06	1.82±0.14	0.4		[48, 149]	
Ciproxifan	0	11.4±1.2[15]	2.1±0.3[15]	0.2	86.1±20.9	77.3±3.4	1.1		46-180[22–24]	>10,000[23]
Clorgyline	0	0.042 ± 0.003	3.65±0.39	86	not active[25]	not active[25]				
	30	0.00042±0.00008	3.57±0.36	8500						
Deprenyl	0	225±31	0.053±0.005	0.0002	not active[25]	not active[25]				
	30	0.630±0.086	0.0040±0.0009	0.006						
Donepezil[4]	0				0.011 ± 0.001	6.22 ± 0.77	0.002			

[a] The error (SE) is indicated for each value; [b] SR= IC_{50} hMAO B/hMAO A; [c] SR = IC_{50} hAChE/hBuChE; [d] Oxygen Radical Absorbance Capacity [Trolox equivalents (TE)]; [e] The confidence interval (95%) is given in square brackets

Molecular docking studies on the four targets clearly support the in vitro data findings since ASS234 and contilisant fit to the diverse binding cavities for AChE, MAO A/B and H3R (see Supporting Information). Notably in the molecular properties of contilisant obtained with molsoft[26] is its higher hydrophilicity (MolLogP = 3.7) compared to that of ASS234 (MolLogP = 5.5), confirming increased drug-likeness. Further indication for central distribution was obtained from the parallel artificial membrane permeability assay (PAMPA), a tool for prediction of the blood brain barrier (BBB) penetration properties (see Supporting Information). Results clearly indicated the ability of contilisant and ASS234 to pass the BBB via passive diffusion. A complete in silico ADME analysis of the novel hybrids **1**-**7** has been carried out suggesting drug suitability, with special focus to contilisant (see Supporting Information). The antioxidant capacity of hybrids **1**-**5**, and **7** was measured as oxygen radical absorbance capacity (ORAC-FL) (Table 1),[27] with all MDLs presenting good radical scavenging properties, with those for contilisant close to that of the positive control ferulic acid (3.74±0.22 TE).[28] Neuroprotection capacity was studied using three different toxic insults involved in neurodegeneration mechanisms in AD:[29] (a) cocktail of mitochondrial respiratory chain blockers, rotenone and oligomycin A (R/O), a model of ROS generation; (b) protein phosphatase inhibitor okadaic acid (OA), as model of hyperphosphorylation of tau protein; (c) β-amyloid peptides ($A\beta_{25-35}$), involved in ROS and apoptosis pathways. Overall, the data obtained for compounds **1**-**7** revealed an interesting neuroprotection profile (see Supporting Information). At the lowest concentration tested (0.3 μM), contilisant offered significant neuroprotection against the toxic insults assayed (70% vs. R/O, 47% vs. OA and 65% vs. $A\beta_{25-35}$) comparable to those of melatonin (Figure 2). Memory behaviour improvements after ASS234 and contilisant were tested in vivo using the novel object recognition test (NOR) in mice (Figure 3),[30] before and after administration of lipopolysaccharide (LPS) that significantly impaired NOR performance. Mice treated with contilisant after LPS impairment showed a significantly improved discrimination index, but ASS234 (at the same dose) was not able to restore the cognitive deficit.

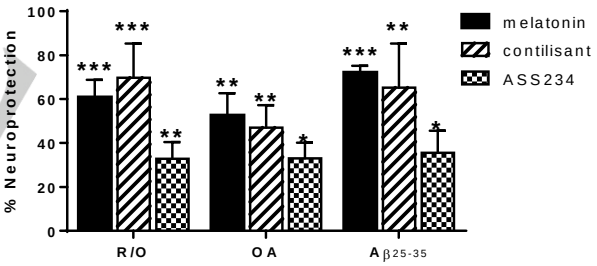


Figure 2. Neuroprotective capabilities of contilisant (0.3 μM), ASS234 (5 μM) and melatonin (0.01 μM) in SH-SY5Y cells following rotenone 30 μM/oligomycin A 10 μM (R/O), okadaic acid 20 nM (OA) or β amyloid peptide 30 μM ($A\beta_{25-35}$) intoxications, respectively. Data expressed as % neuroprotection ± SEM of at least four different cultures performed in triplicates (untreated control set to 100%). ***p≤0.001, **p≤0.01, *p≤0.05 compared to control.

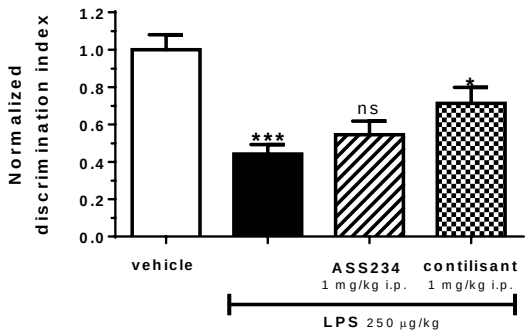


Figure 3. Effects of contilisant and ASS234 on lipopolysaccharide (LPS)-induced memory impairment in the novel object recognition test in mice. ***p≤0.001, *p≤0.05, ns p>0.05.

In conclusion, new MDLs showing inhibitory properties for neurotransmitter-catabolizing enzymes (ChEs and MAOs) alongside H3R affinity, are described for the first time. From this small series, contilisant showed best overall multitargeting properties in nanomolar concentration ranges, with newly designed-in and well-balanced properties of permeation as well as antioxidant and neuroprotective capacity. Contilisant provides a pharmacological profile with improved complexity, possibly beneficial for treatment of neurodegenerative conditions. Compared to the dual targeting H3R/MAO ligand, ciproxifan[15] contilisant is a more potent inhibitor of MAO with irreversible binding properties. Moreover, contilisant at 1 mg/kg restored the cognitive deficit of LPS-treated mice.

As intended, all properties of the small molecule MDL contilisant (**4**) were optimized compared to those of lead compound ASS234, including reduced inhibition of MAO A, and successfully extended by high H3R affinity by taking advantage of the structural blue print for H3R pharmacophores.[31] The resulting unique pharmacological profile, addressing various targets involved in neurodegenerative processes, may be suitable for treatment of Alzheimer´s or Parkinson´s diseases.

Experimental Section

Experimental procedures including, synthesis, analytics, pharmacological assays and modelling studies are available under http://dx.doi.org/.

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Conflict of Interest

Authors SH, HS, JMC, RRR, and FLM declare conflict of interest based on a related patent application, the other authors declare no conflict of interest.

Keywords: Antioxidants • Drug design • Inhibitors • Multi-targeting drugs • Neurological agents

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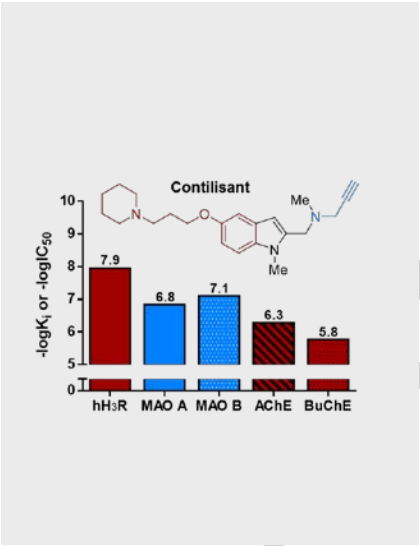
Layout 1:

COMMUNICATION

Hitting multiple targets at once: Therapy of multifactorial disease conditions needs to address multiple targets, an especial-challenge for neurodegenerative diseases. Designing multitarget-directed ligands able to cross the blood brain barrier is a complex task in medicinal chemistry. We described small molecule ligands affecting four neurotransmitter-catabolizing enzymes as well as the histamine H_3 receptor as pro-cognitive G-protein coupled receptor.



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