EXPERT OPINION

Clinical utility of neuroprotective agents in neurodegenerative diseases: current status of drug development for Alzheimer's, Parkinson's and Huntington's diseases, and amyotrophic lateral sclerosis

Petra Dunkel, Christina LL Chai, Beáta Sperlágh, Paul B Huleatt & Péter Mátyus,[†]

[†]*Semmelweis University, Department of Organic Chemistry, and Drug Discovery and Safety Centre, Budapest, Hungary*

Introduction: According to the definition of the Committee to Identify Neuroprotective Agents in Parkinson's Disease (CINAPS), "neuroprotection would be any intervention that favourably influences the disease process or underlying pathogenesis to produce enduring benefits for patients" [Meissner W, *et al.* Trends Pharmacol Sci 2004;25:249-253]. Preferably, neuroprotective agents should be used before or eventually during the prodromal phase of the diseases that could start decades before the appearance of symptoms. Although several symptomatic drugs are available, a disease-modifying agent is still elusive.

Areas covered: The aim of the present review is to give an overview of neuroprotective agents being currently investigated for the treatment of AD, PD, HD and ALS in clinical phases.

Expert opinion: Development of effective neuroprotective therapies resulting in clinically meaningful results is hampered by several factors in all research stages, both conceptual and methodological. Novel solutions might be offered by evaluation of new targets throughout clinical studies, therapies emerging from drug repositioning approaches, multi-target approaches and network pharmacology.

Keywords: neurodegenerative, neuroprotective, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, multi-target-directed ligands

Expert Opin. Investig. Drugs [Early Online]

1. Introduction

It is estimated that neurodegenerative diseases affect nearly 25 million people worldwide. Due to current trends, with changing age structure (aging societies, increasing life expectancies) the incidence of neurodegenerative diseases is on the rise (more people living long enough to be affected), causing significant societal, emotional and economic burdens. Despite the therapeutic agents available, AD, PD, HD, and ALS are at best inadequately treated, with several unmet needs – particularly in terms of slowing/reversing disease progression. Thus there is a pressing need for improved therapies and this offers substantial market potential for the pharma industry.

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Article highlights.

- Neurodegenerative diseases might affect nearly 25 milion people world-wide. Due to current trends, with changing age structure the incidence of neurodegenerative diseases is on the rise, conferring significant societal, emotional and economic burdens.
- Particularly in terms of slowing/reversing disease progression, there are several unmet needs in the therapy of neurodegenerative diseases.
- Low translation rates in the field stem from several factors, as complexity of diseases, lack of knowledge regarding etiopathology or primary factors, inappropriate preclinical disease models, lack of biomarkers for diagnosis and monitoring disease progression, shortcomings in clinical trial methodology.
- With a growing awareness of the rationale of targeting more pathways simultaneosly, particular interest has recently been focused on the design of 'multi-target directed ligands'.
- Besides rational design, novel investigational agents come from various sources, emerging from epidemiological studies, (re)evaluation of herbal medicines or drug repositioning/repurposing programs.

This box summarizes key points contained in the article.

The drug development landscape for neurodegenerative diseases is full of hypes and hopes, however, to date, there is no approved therapeutic agent with an established neuroprotective profile with disease-modifying potential. The antiparkinson agent rasagiline (a propargylamine derivative, (*R*)-*N*-(prop-2-ynyl)-2,3-dihydro-1*H*-inden-1-amine) is close to being the first such drug; however, a disease-modifying indication has recently been dismissed. Despite rejection of an expanded indication, rasagiline trials (ADAGIO, TEMPO) provided important methodological lessons, and maintain the hope in the feasibility of small molecule approaches.

To give an impression of the scientific efforts dedicated toward the design and development of novel drugs for neurodegenerative diseases, according to the lists compiled by Pogačić Kramp and Herrling [1,2], the number of drugs in the preclinical and clinical development pipelines (for all neurodegeneration related indications, including symptomatic and disease-modifying agents) was 1039 in the year 2011, as compared to 605 in 2007. Of the active developments with specific indications, for AD, there were 177 in discovery phase, 54 in Phase I, 56 in Phase II and 11 in Phase III (the numbers reflecting similar tendencies as that of 2005 [3,4]), for PD, there were 62 in discovery phase, 9 in Phase I, 21 in Phase II and 11 in Phase III, for HD, there were 22 in discovery phase, 4 in Phase I, 3 in Phase II and 2 in Phase III and for ALS, there were 12 in discovery phase, 5 in Phase I and 3-3 in Phase II and III. Only time will tell how many of these approaches will result in a successful therapy. To date, the success rate has not been encouraging, with none of the agents in development as of June 2006 reaching the market yet [5].

Despite the distinct clinical, neuropathological features of AD, PD, HD and ALS, several common motifs exist in the processes leading to the progressive loss of anatomically or functionally related systems. The implicated pathways (with various relevance to each disease) are potential targets for intervention and they include: glutamate excitotoxicity, mitochondrial dysfunction, protein mishandling/misfolding and aggregation, neuroinflammation, oxidative stress, ubiquitin/proteasomal dysfunction, disrupted intracellular transport, (contagious) apoptosis and apoptotic signals, microglial activation and disruption of intracellular trafficking and neurofilamental network [6].

The aim of the present review is to provide an overview of neuroprotective agents currently being investigated for the treatment of AD, PD, HD and ALS in clinical phases with a putative neuroprotective effect; for completeness, some agents recently suspended/withdrawn from clinical development are briefly commented on as well. The neuroprotective agents studied which are discussed herein were identified using various databases, in particular the clinicaltrials.gov registry, published lists of investigational drugs and relevant reviews and papers cited by SciFinder. Further information was obtained from published abstracts, papers, reference lists of articles and company websites. 'NCT' codes refer to the clinicaltrials.gov registry identification numbers.

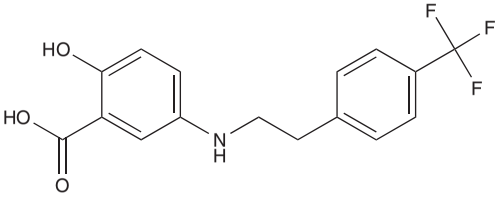
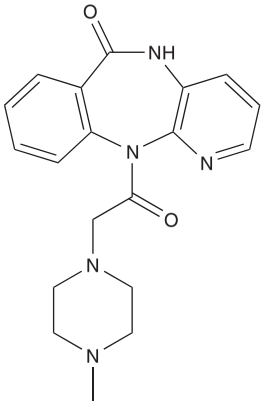
In the following sections we discuss key features of neurodegenerative diseases. Investigational small molecule agents (listed alphabetically in **Table 1**) are presented according to diseases and stages of clinical development, so as to avoid ambiguities in the classification of compounds with complex modes of activity. However, to provide readers with a quick mechanism-guide, neuropathological events together with corresponding targets in AD and PD are shown in Tables 2, where agents are listed according to their main effects. An important emerging field in the pharmaceutical therapy of complex diseases such as neurodegeneration, namely 'the multi-target approach', is also treated. Non-small molecule-based strategies for neuroprotection (immunotherapy or stem cell therapy) are not discussed herein though some relevant articles on the topics are cited. In the Conclusion, specific features and aspects of clinical development of neuroprotective agents are summarized and in the last section, Expert Opinion, some hints and thoughts regarding further trends in the development of neuroprotective agents are provided.

2. Investigational drugs with putative neuroprotective effect

2.1 Alzheimer's disease

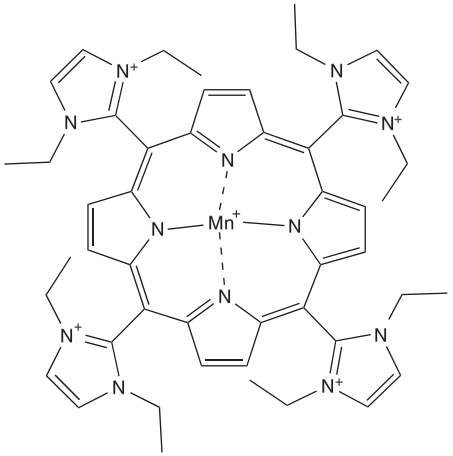
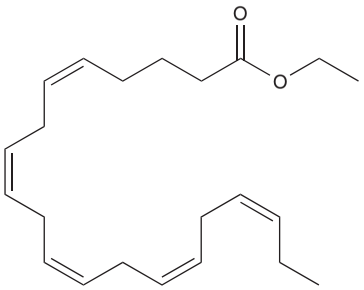
Alzheimer's disease is the most prevalent age-related dementia, with a typical onset age of over 65 years. At present cholinergic and anti-glutamatergic drugs are available for AD therapy, albeit offering only a modest effect. Drug development in the last decade has been dominated by the amyloid hypothesis,

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis.

Name	Structure	Mechanism of action	Investigator	Phase (indication)
AAD-2004		Spin trapping Microsomal prostaglandin E synthase-1 inhibitor	GNT Pharma Co. Ltd	I (ALS, PD, AD)
ABT-126	NA	α_7nAChR agonist	Abbott Laboratories	II (AD)
ACI-91		BACE1 modulator	AC Immune SA	II (AD)

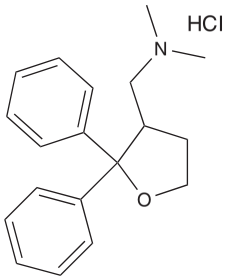
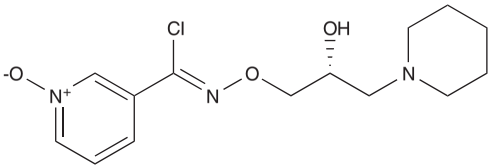
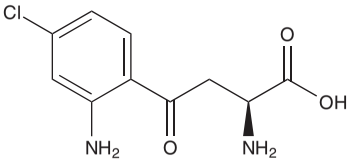
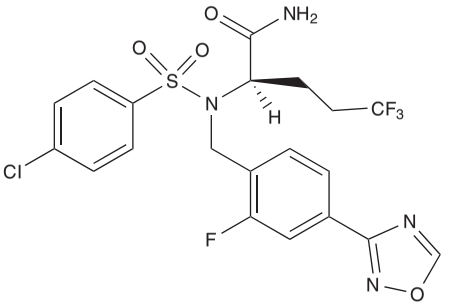
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
AEOL-10150		Metalloporphyrin catalytic antioxidant	Aeolus Pharmaceuticals, Inc.	I (ALS)*
AMR-101		Lipid bi-layer replenisher Anti-apoptotic Mitochondrial integrity stabilizer	Amarin Corp plc	III (HD)

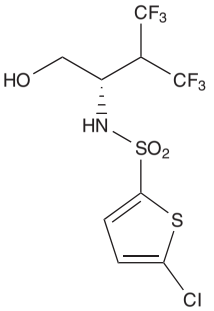
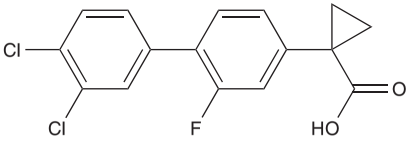
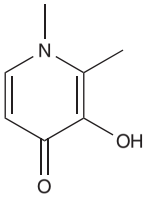
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
ANAVEX-2-73		Mixed muscarinic receptor/σ-1 ligand	Anavex Life Sciences Corp	I (AD)
ARC-031	NA	Soluble amyloid reducing/ clearing agent	Archer Pharmaceuticals, Inc.	I (AD)
Arimoclomol (BRX-220)		Hsp coinducer	Orphazyme ApS	III (ALS)
AV-101		NMDA receptor antagonist	VistaGen Therapeutics, Inc.	I (PD)
Avagacestat (BMS-708163)		γ-secretase inhibitor	Bristol-Myers Squibb Co.	II (AD)

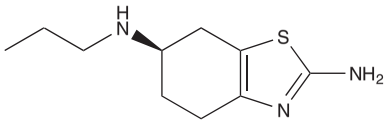
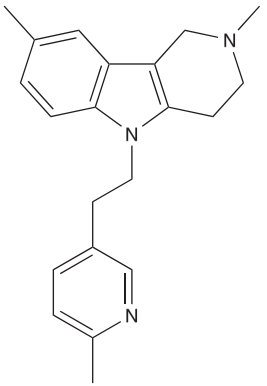
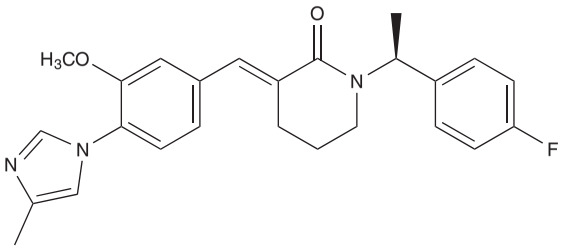
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
AZD-1446 (TC-6683)	NA	$\alpha_4\beta_2$nAChR agonist	AstraZeneca plc/ Targacept, Inc.	II (AD)
Begacestat (GSI953, PF5212362)		γ-secretase inhibitor	Pfizer, Inc.	I (AD)*
CHF5074		γ-secretase modulator	Chiesi Farmaceutici SpA	II (AD)
CTS-21166	NA	BACE1 inhibitor	CoMentis, Inc./ Astellas Pharma, Inc.	I (AD)
Davunetide	NAPVSIPQ	Tau deposition inhibitor Microtubule stabilizer Amyloid protein deposition inhibitor PARP stimulator GSK3 inhibitor	Allon Therapeutics, Inc.	I/II (AD, PD)
Deferiprone		Iron chelator	University Hospital, Lille	II/III (PD)

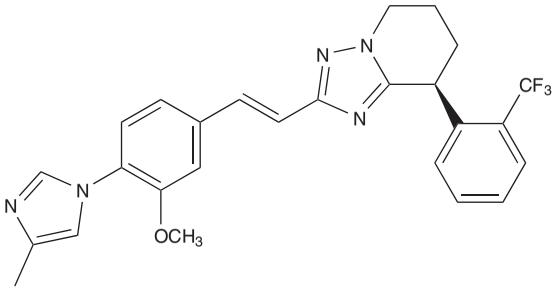
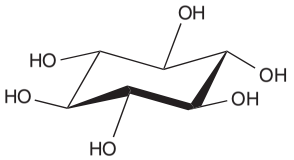
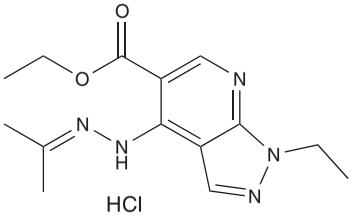
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
Dexpramipexole (KNS-760704)		Mitochondria stabilizer	Biogen Idec, Inc./ Knopp Neurosciences, Inc.	III (ALS)
Dimebon (latrepirdine)		Pleiotropic neurotransmitter signaling actions	Pfizer, Inc./ Medivation, Inc.	III (AD)*
DSP-8658	NA	PPARα/γ modulator β-amyloid deposition inhibitor	Dainippon Sumitomo Pharma Co. Ltd	I (AD)
E-2012		γ-secretase modulator	Eisai Co. Ltd	I (AD)*

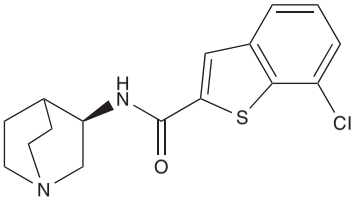
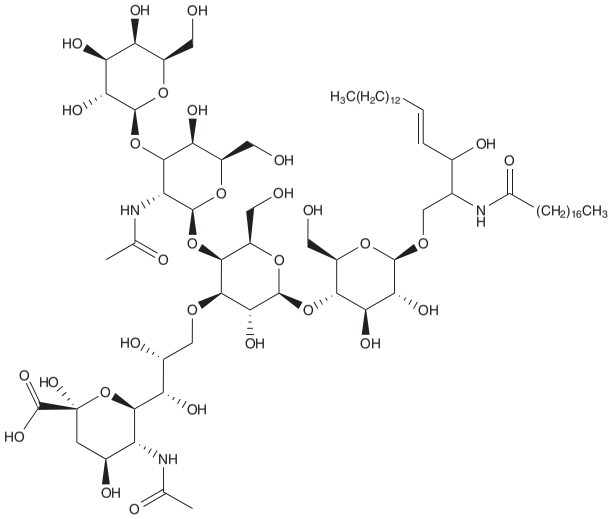
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
E-2212* (presumed structure)		γ-secretase modulator	Eisai Co. Ltd	I (AD)
ELND-005 (AZD-103, scyllo-inositol)		Aβ oligomer accumulation inhibitor Aβ oligomer formation inhibitor	Elan Corp plc/ Transition Therapeutics, Inc.	II (AD)
ELND-006	NA	γ-secretase inhibitor	Elan Corp plc	I (AD)*
Etazolate (EHT-0202)		$GABA_A$ receptor modulator PDE_4 inhibitor α-secretase pathway activator	Exonhit SA	II (AD)
EVP-0334	NA	Histone deacetylase inhibitor	EnVivo Pharmaceuticals, Inc.	I (AD, PD)
EVP-0962	NA	γ-secretase modulator	EnVivo Pharmaceuticals, Inc.	I (AD)

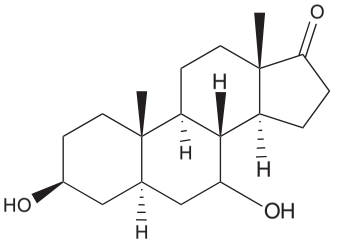
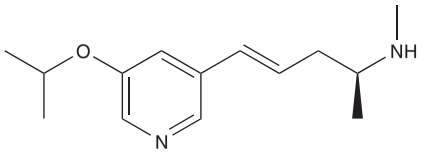
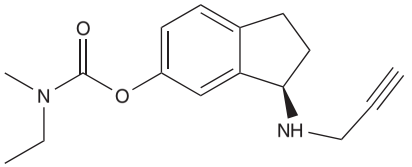
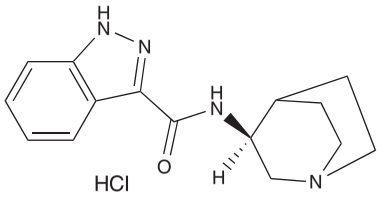
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
EVP-6124		α_7nAChR partial agonist 5-HT_3 receptor antagonist	EnVivo Pharmaceuticals, Inc.	II (AD)
Exebryl-1	NA	β-amyloid formation/ accumulation inhibitor Amyloid plaque deposition inhibitor	ProteoTech, Inc./ Tasly Pharmaceutical Co. Ltd	I (AD)
GM1-ganglioside		Neuronal plasma membrane constituent	Thomas Jefferson University	II (PD)

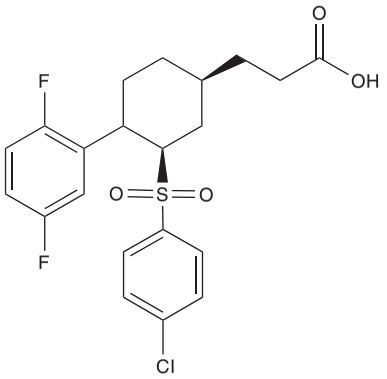
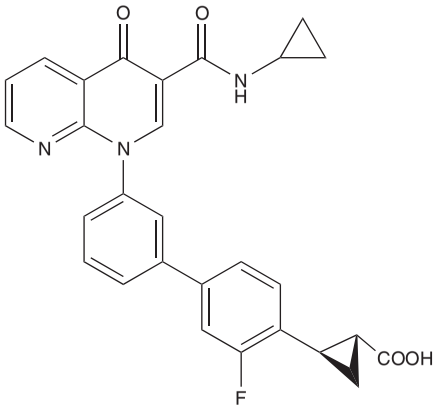
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
HF-0220		Neuroprotective/ cytoprotective (activation of 7-hydroxysteroid-driven neuroprotection pathways)	Newron Pharmaceuticals SpA	II (AD)
HPP-854	NA	BACE1 inhibitor	High Point Pharmaceuticals LLC	I (AD)
Ispronicline (TC-1734, AZD-3480)		$\alpha_4\beta_2$nAChR partial agonist	AstraZeneca plc	II (AD)
Ladostigil (TV-3326)		Acetylcholinesterase inhibitor MAO-B inhibitor	Avraham Pharmaceuticals Ltd	II (AD)
MEM1414	NA	PDE_4 inhibitor	Memory Pharmaceuticals Corp	II (AD)*
MEM3454 (RG3487, RO5313534)		α_7nAChR partial agonist 5-HT_3 receptor antagonist	Memory Pharmaceuticals Corp/ F. Hoffmann – La Roche Ltd	II (AD)*

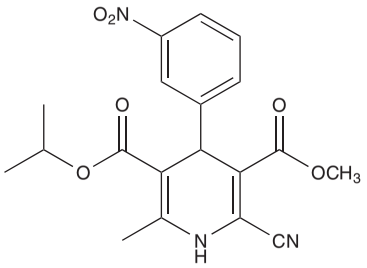
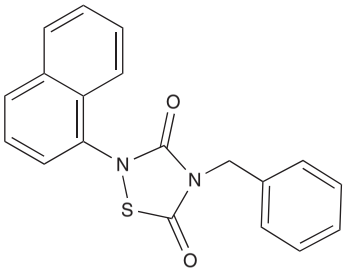
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
MEM-63908	NA	α_7nAChR partial agonist	Memory Pharmaceuticals Corp/ F. Hoffmann – La Roche Ltd	I (AD)*
MK0249	NA	H_3 receptor inverse agonist	Merck & Co., Inc.	II (AD)
MK0752		γ-secretase inhibitor	Merck & Co., Inc.	II (AD)*
MK0952		PDE_4 inhibitor	Merck & Co., Inc.	II (AD)

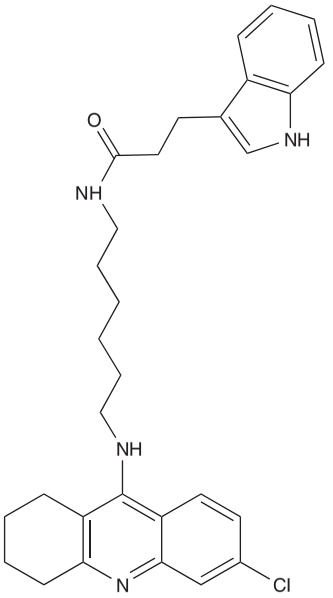
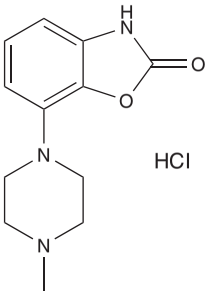
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
Nilvadipine (ARC-029)		Amyloid protein deposition inhibitor Calcium channel blocker	Archer Pharmaceuticals, Inc.	III (AD)
NP-12 (tideglusib)		GSK3 inhibitor	Noscira SA	II (AD)

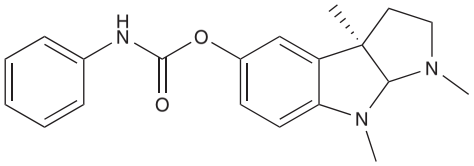
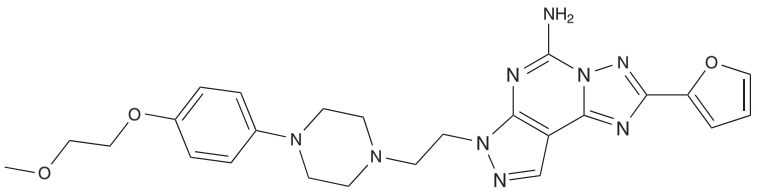
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
NP-61		β-amyloid modulator Acetylcholinesterase inhibitor	Noscira SA	I (AD)
NRM-8499	NA	Amyloid protein deposition inhibitor	Bellus Health, Inc.	I (AD)
Pardoprunox (SLV-308)		D_2 and D_3 receptor partial agonist 5-HT_{1A} receptor full agonist	Abbott Laboratories	III (PD)

*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
PBT2	NA	Metal-protein attenuator Tau protein modulator Amyloid protein deposition inhibitor	Prana Biotechnology Ltd	II (AD, HD)
Posiphen		APP/ tau/ α-synuclein synthesis inhibitor	QR Pharma, Inc.	I (AD)
PQ-912	NA	Glutaminyl cyclase inhibitor	Probiodrug AG	I (AD)
Preladenant		Adenosine A_{2a} receptor antagonist	Merck & Co., Inc.	III (PD)
PU-H71		Hsp90 inhibitor		I (AD)

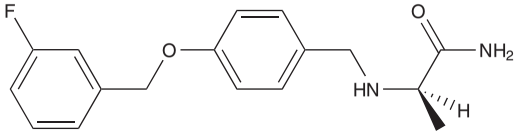
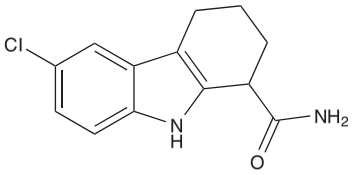
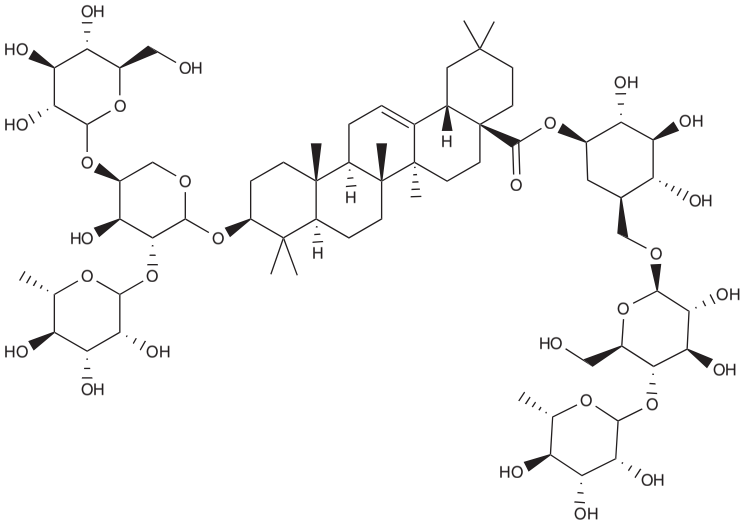
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
			Memorial Sloan-Kettering Cancer Center	
PYM50018	NA	Unspecified target	Phytopharm plc	I (ALS)
PYM50028		GDNF/BDNF activator	Phytopharm plc	II (AD, PD)
RG-1577	NA	MAO-B inhibitor	F. Hoffmann – La Roche Ltd	I (AD)
RG-7129	NA	β-amyloid synthesis inhibitor BACE1 inhibitor	F. Hoffmann – La Roche Ltd	I (AD)
RQ-00000009	NA	5-HT_4 partial agonist	RaQualia Pharma, Inc.	I (AD)
S47445 (CX-1632)	NA	Ampakine BDNF modulator	Servier	I (AD)

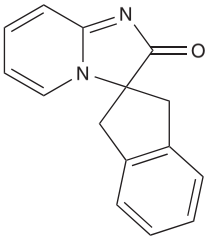
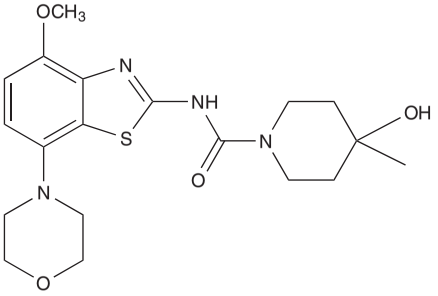
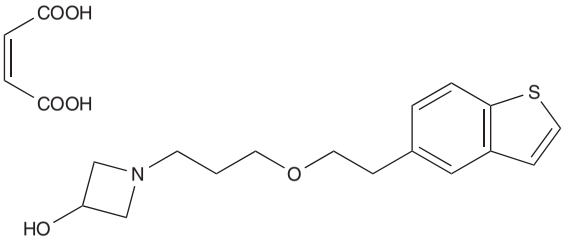
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
Safinamide		MAO-B inhibitor Glutamate release inhibitor Dopamine reuptake inhibitor Sodium and calcium channel blocker	Merck & Co., Inc.	III (PD)
Selisistat (SEN00141196/EX-527)		SIRT1 inhibitor	Siena Biotech SpA	II (HD)
SK-PC-B70M		β-amyloid modulator	SK Chemicals Life Science	III (AD)

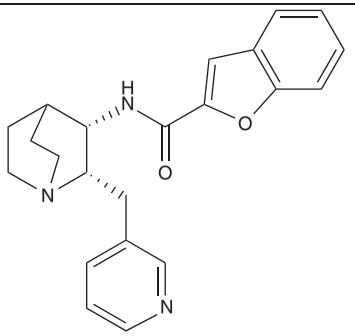
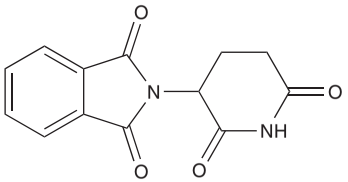
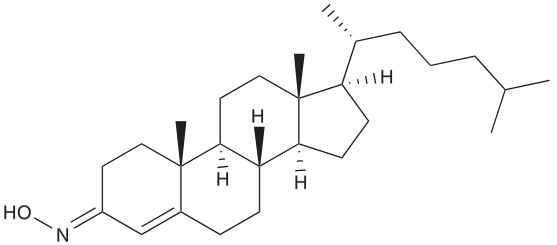
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
SKL-PD (YKP10461)	NA	MAO-B inhibitor (selective and reversible)	SK Biopharmaceuticals Co. Ltd	I (PD)
ST-101 (ZSET-1446)		Cholinergic modulator APP processing modulator	Sonexa Therapeutics, Inc./ Zenyaku Kogyo Co. Ltd	II (AD)
SYN115 (tozadenant)		A_{2A} receptor inhibitor	Biotie Therapies Corp/ UCB Pharma	II (PD)
T-817MA		Neurotrophic modulator	Toyama Chemical Co. Ltd	II (AD)

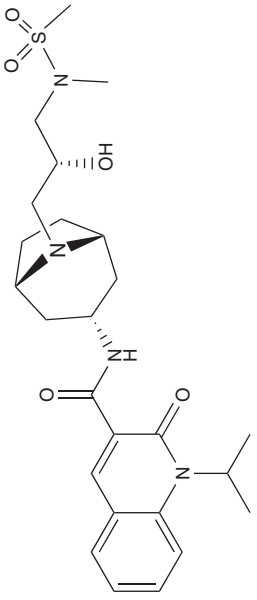
*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
TC5619		α_7nAChR agonist	Targecept, Inc.	II (AD)
Thalidomide		Immunomodulator Angiogenesis inhibitor TNF-α/VEGF/ FGF-2 inhibitor	Celgene Corp	II (AD)
TRO19622 (olesoxime)		Mitochondrial pore modulator	Trophos SA	III (ALS)
TT-301	NA	Anti-inflammatory (inhibits overproduction/ release of (pro) inflammatory cytokines (e.g., IL-1ß, TNF-α) Macrophage and glial cell activation suppressor	Transition Therapeutics, Inc.	I (AD)

*Suspended/withdrawn investigational agents.

Table 1. Selected investigational drugs with putative neuroprotective effect for Alzheimer's disease/Parkinson's disease/ Huntington's disease/ amyotrophic lateral sclerosis (continued).

Name	Structure	Mechanism of action	Investigator	Phase (indication)
Velusetrag		5-HT_4 receptor agonist	Theravance, Inc.	II (AD)

*Suspended/withdrawn investigational agents.

although recently other mechanisms are being intensively studied offering novel targets (**Table 2A**) [7-9].

2.1.1 Phase III trials

In several clinical trials with patients taking dihydropyridine antihypertensives, the results seem to delineate a tendency toward a protective effect of the drugs against AD [10]. Previous studies have also suggested that the protective effect does not follow from the antihypertensive action *per se*, and the protective effect is not a uniform characteristic of the drug class. **Nilvadipine** – a dihydropyridine calcium channel blocker already in use in Europe as an antihypertensive – will enter Phase III multicenter trials recruiting mild to moderate AD patients in early 2012. Nilvadipine inhibited amyloid-β (Aβ)-induced vasoconstriction in an *in vitro* setting and restored the cerebral blood flow in an AD transgenic mouse model overexpressing Aβ. Beneficial effects on cerebral blood flow were also observed in an AD patient, with concomitant amelioration in cognitive function. Preclinical experiments demonstrated that nilvadipine has a direct effect on Aβ production *in vitro* (presumably via an indirect inhibition of the β-cleavage of amyloid precursor protein [APP]), moreover it was able to reduce brain Aβ levels and increase Aβ clearance in an AD transgenic mouse model [11]. The effects might be due to the NFκB inhibition by nilvadipine as NFκB regulates both β-secretase (BACE1) and receptor for advanced glycation end products (RAGE, responsible for Aβ brain influx) expression. Similar results observed with nitrendipine suggest that these agents might serve as useful starting points for the design and development of more potent Aβ modulating derivatives devoid of the antihypertensive activity (in fact, several dihydropyridine hybrid drugs are being studied, as discussed later). Safety studies with nilvadipine in AD patients raised no concerns. Moreover positive results on efficacy were obtained in small-scale AD trials [12], enabling nilvadipine's progress to Phase III. Further supportive results were obtained in small-scale trials with mild cognitive impairment (MCI) patients, with nilvadipine stabilizing cognitive functions. The follow-up compound of nilvadipine, **ARC031** is in Phase I, exerting similar mechanism of action (lowering soluble amyloid levels), but not acting as a calcium channel blocker.

SK-PC-B70M (oleanolic-glycoside saponins enriched fraction from the root of *Pulsatilla koreana*) is currently undergoing Phase III trials in mild to moderate AD [NCT01249196]. *P. koreana* used as a herbal medicine for amoebic dysentery and malaria emerged as a potential neuroprotective agent following a screen on human neuroblastoma SK-N-SH cells incubated with $A\beta_{1-42}$ [13]. Hederacolchiside-E, an oleanolic glycoside was isolated as the active component. Orally administered SK-PC-B70M exhibited beneficial effects against impairments of memory consolidation and spatial working memory induced by scopolamine in a rat model, comparable to the effects of donepezil. In AD mice model, SK-PC-B70M treatment reduced $A\beta_{1-42}$ levels and plaque

Table 2A. Neuroprotective agents in AD.

Neuropathology	Target	Investigational agents (exemplary)
APP processing/ Aβ production, accumulation of toxic Aβ aggregates	α-secretase stimulation	etazolate, M_1 receptor agonists (AF267B, talsaclidine)
	β-secretase inhibitors (modulators)	ACI-91, LY2886721, HPP854, CTS-2116
	γ-secretase inhibitors	semagacestat, BMS708163, ELND006/007
	γ-secretase modulators	EVP0962, tarenflurbil, CHF-5074, E2212, E2012, begacestat
	Aβ aggregation inhibitors (e.g., GAG mimetics, dual-binding-site AChEIs)	ELND005, NRM8499, tramiprosate, colostrinin, NP-61
	Metal ion chelators, metal protein attenuators	Clioquinol, PBT2
	Agents facilitating amyloid removal - immunotherapy (passive, active)	Bapineuzumab, solanezumab, ACC001, CAD106, LY2062430, AAB002, AN1792, IVIg, gantenerumab, MABT5102A
	Miscellaneous Aβ-related targets (APP translation inhibitors, 3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme inhibitors, RAGE modulators)	Posiphen, statins, nilvadipine
Neurotransmitter/ receptor signaling dysfunction	Cholinesterase inhibitors	Dimebon, huperzine A
	Nicotinic receptor agonists ($\alpha_4\beta_2/\alpha_2\beta_2$nAChR, α_7nAChR)	Ispronicline, RG3487, EVP-6124
	Serotonin receptor modulators (5-HT_4 agonists, 5-HT_{1A} antagonists, 5-HT_6 antagonists)	PRX03140, RQ-00000009, TD-5108, velusetrag
	H_3 receptor inverse agonists	MK0249
	MAO-B inhibitors	EVT-302
Abnormal tau hyperphosphorylation, aggregation	Tau kinase inhibitors (e.g., GSK3β)	NP-12
	Tau aggregation inhibitors	Methyltioninium, davunetide
(Neuro)inflammation	Anti-inflammatory agents, immunomodulators	NSAIDs, TT301/302
	PPARγ agonists	Rosiglitazone, DSP8658
Oxidative stress	ROS scavengers, (dietary) antioxidants	Mitoquinone, melatonin, vitamin E, coenzyme Q10
Trophic factor deficiency	Nerve growth factor (NGF) delivery, NGF agonists	CERE 110, PYM50028
	BDNF expression activation	Ampakines
Transcriptional dysfunctions	Histone deacetylase inhibitors	EVP0334
Excitotoxicity, calcium homeostasis dysregulation	NMDA receptor modulators, Ca^{2+} channel blockers	Nimodipine

deposition in the brain, besides exerting antioxidant effects and restoring/enhancing phospho-CREB (cAMP response element binding protein), calbindin and transthyretin levels. In a transgenic ALS mouse model overexpressing the mutant human superoxide dismutase (SOD) 1, SK-PC-B70M treatment increased survival, ameliorated motor function deficits, exerted antioxidant effects and protection against neuronal cell loss [13].

Of the recent casualties, **dimebon** (latrepirdine) is another failed attempt on the field, with the negative results of the multicenter Phase III CONCEPT study [NCT00829374] having been released in January 2012. Patients with mild to moderate AD treated with dimebon and donepezil (vs. donepezil and placebo) did not achieve significantly better results for the two co-primary endpoints, Alzheimer's Disease Assessment Scale – Cognitive Subscale (ADAS-cog) or the Alzheimer's Disease Cooperative Study – Activities of Daily Living (ADCS-ADL), which is not a completely unexpected result considering previous negative outcomes in the Phase III CONNECTION study [NCT00675623] [14]. Dimebon has not performed better in Phase III HD trials either. Dimebon was originally approved as an antihistamine in the 1980's in Russia and the interest in its development as a neuroprotective drug was fuelled by the promising effects observed in preclinical studies and early smaller scale clinical trials (see e.g., [15]). Dimebon exhibits pleiotropic effects, including cholinesterase inhibition, *N*-methyl-*D*-aspartate (NMDA) receptor inhibition, and activities on a number of other targets as well (e.g., 5-HT_7, 5-HT_6, α_{1A}, dopaminergic receptor subtypes). Its effect on Aβ neurotoxicity has been studied, and an action on mitochondrial permeability transition pores has been suggested and debated [16].

2.1.2 *Phase II trials*

Agents affecting the neuronal nicotinic acetylcholine (ACh) receptors (nAChR) are well-represented among the investigational drugs in Phase II. The role of nAChRs in impaired cognitive functions in AD, as well as the interaction of (α_7, $\alpha_4\beta_2$) nAChRs and Aβ peptides and its consequences under normal and pathological conditions has been extensively studied and reviewed (e.g., [17,18]), effects including shift toward the non-amyliodogenic pathway, lowered Aβ production, increased neuroprotective sAPPα formation. α_7nAChRs may be involved in AD pathogenesis and selective activation could have beneficial effects on cognitive functions in addition to the putative neuroprotective and disease-modifying effect [19]. **ABT-126** is Abbott's selective α_7nAChR agonist. A Phase II trial has already been carried out [NCT00948909] and one is to be initiated in mild to moderate AD [NCT01527916]. **EVP-6124** is a quinuclidine structure having a 5-HT_{3A} antagonist effect as well as showing partial agonism at α_7nAChRs that can be beneficial in potential nicotinic agonist side effects [20]. Following oral administration, EVP-6124 showed good brain penetration. In *in vivo* experiments, EVP-6124 showed memory enhancing effects in a dose-dependent manner (object recognition task: reversal of scopolamine-induced deficit, prevention of natural forgetting), the procognitive effect could be blocked by a selective α_7nAChR antagonist. Potential (beneficial) interaction with acetylcholinesterase inhibitors (AChEIs) was studied due to potential co-administration of the agents in a clinical setting. For low EVP-6124 agonist concentrations, a co-agonism with ACh as a novel mechanism of action was suggested as that could be important from a drug safety viewpoint as well as for the design of combinations with AChEIs. EVP-6124 exhibited procognitive effects in normal volunteers, and increased cognitive functions in AD patients on donepezil or rivastigmine therapy in a small-scale study [21]. Phase II data for AD [NCT01073228] for EVP-6124 are expected in the first half of 2012. An already discontinued representative of the class, **MEM3454** (R3487, RO5313534) – developed for cognitive impairment associated with schizophrenia and AD – shares the α_7nAChR partial agonist/5-HT_{3A} antagonist profile with EVP-6124, with no activity at $\alpha_4\beta_2$nACh or other nicotinic receptors and other off-targets [22]. In operant visual signal detection task (a sustained attention model) in rats, MEM3454 showed improved performance. MEM3454 demonstrated various procognitive *in vivo* effects – presumably mediated via α_7nACh activation (episodic, spatial, working memory, executive functions – young and aged subjects, acute and repeated dosing) – and improved sensorimotor gating deficits [22]. Procognitive effect was maintained following repeated administration, suggesting an equilibrium between receptor activation and desensitization. Beneficial effects on attention and working memory function were verified in non-human primate model with MEM3454 exhibiting an inverted U-shaped dose-response curve (characteristic of nicotinic agonists) [23]. In Phase I study in healthy volunteers, MEM3454 improved Cognitive Drug Research (CDR) battery test performance. In a Phase IIa trial in mild to moderate AD patients [NCT00454870], MEM3454 provided a cognitive benefit, consistent with the results of the Phase I trials. A second trial of MEM3454 was initiated in H12009 as adjunct therapy to donepezil [NCT00884507], however as reported in Q12011, further development was discontinued. A second Roche (formerly Memory Pharmaceuticals) molecule, **MEM63908** (RG4996) is a selective α_7nAChR partial agonist with no 5-HT_3R action. In animal studies, MEM63908 improved learning and memory (young and age-impaired subjects). Phase I/II studies have been completed (safety, tolerability, pharmacokinetics, food interactions), however, no further development has been reported. **TC-5619** shows full α_7nAChR agonism and no activity on 5-HT_3 receptors or other nicotinic receptors [24]. A Phase I study for AD has commenced [NCT01254448] (development program for negative symptoms and cognitive dysfunction in schizophrenia and attention deficit hyperactivity disorder (ADHD) being in a more advanced state). Future development of the agent for this indication is under evaluation, with 'enabling studies' being reported. **Ispronicline** (AZD-3480, TC-1734) is an orally active, brain-selective $\alpha_4\beta_2/\alpha_2\beta_2$nAChR agonist (devoid of α_7 activity), with memory enhancing and neuroprotective effects. The cognition enhancing effects of nicotinic agonists is well established by preclinical data, in addition to a neuroprotective action against various damages (e.g., excitotoxicity, 1-methyl-4-phenylpyridinium (MPP^+) toxicity, Aβ-induced toxicity). Ispronicline showed efficacy in different cognition models (scopolamine-induced cognitive deficits, object recognition, radial arm maze), stimulated ACh release, exerted antidepressant activity and neuroprotection (glutamate excitotoxicity, decreased perfusion model), with low addiction liability and no toxicity concerns (reviewed in [25]). Safety and pharmacokinetic profile was verified in two Phase I studies (single and multiple dose) in healthy volunteers. Short-term ispronicline administration resulted in cognitive improvement (attention, episodic memory) in healthy volunteers, both in young and elderly subjects with age associated memory impairment. In Phase IIb mild to moderate AD study, no significant improvement was achieved on the primary outcome (ADAS-Cog) [26] for either ispronicline or the active control donepezil, rendering the study inconclusive. Improvements were detected however on secondary outcome measures (Mini Mental State Examination (MMSE), AD Cooperation Study – Clinical Global Impression of Change (ADCS-CGIC), Disability Assessment for Dementia (DAD)). Ispronicline did not improve cognition in patients with schizophrenia either. The second compound of the class (with a distinct pharmacological profile), is the selective $\alpha_4\beta_2$nAChR agonist **AZD-1446** (TC-6683). Further studies in mild to moderate AD as an adjunct to donepezil are awaited, following announcement by AstraZeneca of an intention to develop AZD-1446 further.

The second well-represented class is that of agents interacting with γ-secretase, the enzyme generating Aβ (γ-secretase inhibitor/modulator-related literature has recently been reviewed in [27]). γ-Secretase inhibitors (GSIs) were demonstrated to lower brain Aβ levels and therefore deemed to have a potential for neuroprotection in AD. Among the most studied agents, semagacestat (LY-450139) of Eli-Lilly was subjected to Phase III trials in 2008 (IDENTITY trials), however development was stopped following preliminary results of cognitive worsening. Negative outcomes may be due to a lack of selectivity of semagacestat toward other γ-secretase substrates (particularly Notch), preferential inhibition of $A\beta_{1\text{-}40}$ generation and accumulation of the Aβ precursor. Challenges faced with γ-secretase inhibitor development turned attention toward γ-secretase modulators (GSMs, first described among non-steroidal anti-inflammatory drugs (NSAIDs)) [28], promoting generation of shorter Aβ species, without interfering with the processing of other γ-secretase substrates. A representative of the class, tarenflurbil (the *R*-enantiomer of flurbiprofen) proved to be ineffective in Phase III, presumably due to low intrinsic activity and pharmacokinetic problems (insufficient brain penetration). Negative outcomes with γ-secretase interacting agents (and data accumulating with Aβ-targeted vaccines) cast doubt on clinical utility/potential of γ-secretase-related approaches (and the amyloid hypothesis) in general [29]. However, important lessons were learned and facilitated the design of novel agents with improved properties. **Avagacestat** (BMS-708163) is a GSI with improved selectivity versus Notch protein (involved in tissue regeneration in the skin and the gastrointestinal tract), which is a crucial issue for clinical application [30]. It has been thoroughly characterized in the course of Phase I and II trials, appearing to be safe and well-tolerated. BMS-708163 dose-dependently decreased plasma and cerebrospinal fluid (CSF) Aβ levels [31] in healthy volunteers and mild to moderate AD patients. Further development depends on the results of a recent Phase II trial in early-stage AD [NCT00890890], however, previously in higher doses, a trend for cognitive worsening was detected. An issue raised recently is the potential synaptotoxic effect of the β-C-terminal fragment of APP, accumulated as a consequence of γ-secretase inhibition; of note, cognitive impairment was observed in a mouse model following subchronic avagacestat treatment [32]. Merck's GSI, **MK0752** was verified to lower CSF $A\beta_{40}$ concentrations in humans (in healthy volunteers) and in preceding animal models, was brain permeable and well tolerated, although the drug potently inhibits Notch cleavage. In a mechanism study in rhesus monkeys, MK0752 reduced central nervous system (CNS) Aβ formation, with no rebound phenomenon following cessation of treatment. A shift of APP metabolism toward alternative nonamyloidogenic pathways during inhibitor treatment was suggested [33]. MK0752 has already been discontinued for AD indication, however it was characterized for anticancer indications (breast cancer, T-cell acute lymphoblastic leukemia, acute myeloid leukemia). Indeed, the Notch pathway might be important in several human malignancies and the exploitation of GSIs in this direction warrants further studies [34]. **NIC5-15** – a monosaccharide of natural origin – might have direct (Notch-sparing) GSI effect besides an indirect insulin-sensitizing, glucose transport enhancer property. In small-scale Phase IIa AD trial, NIC5-15, while stabilizing cognitive performance over the trial course, was safe and well-tolerated [35].

In further approaches targeting Aβ, **posiphen** is the cholinergically inactive (+)-enantiomer of phenserine that has been formerly developed to AD trials [36]. Like phenserine, posiphen lowers APP and Aβ levels *in vitro* and in mice (independent of acetylcholinesterase (AChE) activity), acting on the translation level (suppressing APP translation by interacting with an iron responsive element (IRE) in the 5'UTR of APP mRNA) [36]. Moreover, lower β-secretase activities have been observed and a putative neurotrophic effect has been suggested. The enantiomers exert similar effects on APP expression, however lack of AChEI effect by posiphen allows higher dose levels to be used. Slow formation of cholinergically active metabolites has been confirmed, i.e., posiphen acts as an AChEI prodrug (therefore providing symptomatic relief). Posiphen blocks α-synuclein expression *in vitro*, making it a potential lead to PD as well [37]. In MCI patients, posiphen administration (10-day treatment) lowered CSF secreted APP-α and -β, tau and phosphorylated tau and inflammatory marker levels back to that found in healthy volunteers [36].

The mechanism of action of the cognitive enhancer **ZSET1446** (ST 101) is not completely clear. It was however found to ameliorate cognitive dysfunctions caused by $A\beta_{1\text{-}40}$ or scopolamine besides having beneficial effects on ACh and choline acetyl transferase (ChAT) function – thus an indirect enhancement of the central cholinergic system was suggested [38]. Another component of ZSET1446's effect might be its protective action against Aβ-induced neurotoxicity (through elevation of glutathione *S*-transferase expression). Recently a novel APP processing pathway induced by ZSET1446 was reported (verified in mice and cynomolgus monkey models). ZSET1446 reduced Aβ levels both *in vitro* and *in vivo* and a novel type of C-terminal APP cleavage was detected, resulting in a product bypassing either α- or β-secretase pathways and consequently Aβ formation [38]. Phase II studies of ZSET1446 as a monotherapy or as an add-on therapy to AChEIs in AD have been completed [NCT00842816, NCT00842673].

The clioquinol analog **PBT2** is a novel 'metal-protein attenuating', 'metal chaperone' compound (a group distinct from 'chelators'), targeting an initial event of the amyloid pathway, i.e., the interaction between Aβ and metals in the synapse, leading to oxidative stress and the formation of toxic oligomers [39]. PBT2's effect is that of restoring impaired metal ion homeostasis. Besides inhibiting Aβ toxicity via its metal (Cu, Zn) chelating effects, the neutral hydrophobic

complexes formed with PBT2 can cross the cell membranes and induce neuroprotective signaling cascades via an effect on PI3K, JNK, glycogen synthase kinase (GSK) 3β phosphorylation and calcineurin [40]. As *in vivo* verification of the metal hypothesis approach, in transgenic mice AD models PBT2 treatment resulted in decreased amyloid burden in the brain and a rapid improvement of cognition, without altering total tissue metal levels. PBT2 might enhance clearance and degradation of amyloid via dissolution of oligomerized Aβ, metal translocation to cells leading to increased matrix metalloproteinase expression and restoration of the activity of interstitial metalloproteinases. In human Phase II trials in early AD [NCT00471211], PBT2 treatment improved cognitive outcomes (neuropsychological test battery (NTB) Executive Factor z-score) in addition to lowering CSF $A\beta_{42}$ levels but no correlation was found between biomarkers ($A\beta_{40}$, $A\beta_{42}$, pTau, tTau) and cognitive function [41]. The parent compound of PBT2, clioquinol showed efficacy in HD and PD disease models – characterized by Cu or Fe overload – as well [42]. This approach is supported by a growing array of information on the role of metal imbalances in neurodegenerative diseases. The beneficial effects of PBT2 against HD were verified, as suggested by a recent announcement that Phase II trials for HD are ready to start.

ELND005 (AZD-103, *scyllo*-inositol) – an endogenous inositol stereoisomer – is an Aβ aggregation inhibitor which is able to modulate Aβ folding, oligomeric assembly and fibril formation thus enhancing its normal clearance and consequently reducing Aβ-induced neurotoxicity both *in vitro* and *in vivo* [43]. In a series of proof-of-concept *in vivo* experiments in AD models, ELND005 prophylactically prevented AD-like phenotype (improved behavioral and cognitive function, reduced brain Aβ levels, plaque burden, synaptic loss, gliosis and decreased mortality). Beneficial effects were also observed following a therapeutic dosing and in advanced stages of AD [43]. ELND005 is able to accumulate in the brain following oral administration with no incorporation into phosphatidylinositol lipids. As an important element of future therapeutic applications, brain inositol transporter levels (sodium/*myo*-inositol transporter (SMIT) 1, SMIT2 expression profile) were found to be unaffected by age and amyloid pathology [44]. Regarding clinical trials, in a Phase II mild to moderate dose-ranging AD study of ELND005 [NCT00568776], the two higher doses (1000, 2000 mg) were discontinued (due to the number of deaths and serious infections), whereas the lowest dose (250 mg) led to decreased CSF Aβ levels, but with no significant effect on primary clinical efficacy outcomes (NTB, ADCS-ADL) [45]. According to a subgroup analysis, a trend toward positive cognitive effects was detected among mild AD patients.

TTP488 (PF-04494700) is an orally available small-molecule RAGE inhibitor (disrupting Aβ-RAGE interactions) recently discontinued [46], due to lack of benefit observed in Phase IIb trials. RAGE has been implicated in Aβ toxicity [47]. In an 18-month Phase IIb trial, interim analysis did not support benefit in primary efficacy outcome measure ADAS-Cog, however results from follow-up visits showed an improvement in ADAS-Cog scores [48].

Reviewing further agents in Phase II, with various other different mechanisms of actions, **RG-1577** (EVT-302) of Roche (licensed from Evotec, developed initially for smoking cessation) is an orally active, reversible, non-covalent MAO-B inhibitor. The neuroprotective effects may be due to actions on mitochondria and suppression of oxidative stress. The compound is currently in Phase II for AD.

ACI-91 and thalidomide represent examples of drug repositioning. The parent compound of **ACI-91**, pirenzepine – a muscarinic receptor antagonist – is approved for the treatment of gastric ulcer. The already established safety profile might be an add-on benefit for the development for novel indications. ACI-91, demonstrating BACE1 modulating effects, reduction of plaque formation and a procognitive activity entered Phase II AD trials in 2008 [49]. Several epidemiological and preclinical data support the role of neuroinflammation and angiogenesis in neurodegenerative diseases [50]. The anti-inflammatory and anti-angiogenic agent, **thalidomide** has been studied in several relevant models. Thalidomide has a long history as a therapeutic agent. First approved in the 1950s as a sedative hypnotic, it was withdrawn due to severe teratogenic effects. Later, interest in thalidomide was raised by its efficacy for the treatment of erythema nodosum leprosum and various cancers. Thalidomide exerts a complex mechanism of actions, one particular activity being the inhibition of the pro-inflammatory/pro-apoptotic cytokine tumor necrosis factor (TNF)-α. *In vitro* it was found to shift the balance of APP processing, stimulating the nonamyloidogenic sAPPα secretion, presumably mediated via α-secretase activity and the PKC- and mitogen-activated protein kinase (MAPK)-dependent pathways [51]. In a model of inflamed AD brain, thalidomide inhibited vascular changes/remodeling, suppressed blood–brain barrier (BBB) leakiness, blocked microgliosis and astrogliosis, and reduced neuronal loss [52]. In a TNF-α targeting *in vivo* proof-of-concept study, thalidomide treatment improved Aβ-induced memory impairment [53]. Thalidomide (and its derivative, lenalidomide) exerted neuroprotective effects in an ALS mouse model such as improved motor performance, decreased motor neuron cell death and increased life span [54]. Thalidomide underwent ALS Phase II trials; no efficacy was demonstrated there. A Phase II/III study is currently ongoing with mild to moderate AD patients [NCT01094340].

Activation of neurotrophic pathways has long been studied as a potential disease-modifying approach for neurodegenerative diseases. **T-817MA**, a novel neurotrophic agent showed protective effects against Aβ- and oxidative stress-induced neurotoxicity *in vitro* and promoted neurite outgrowth. T-817MA administration ameliorated Aβ-induced learning deficits in rats and alleviated neuronal damages, and was demonstrated to increase hippocampal neurogenesis in a follow-up study [55]. To assess its role in tau-related

neurodegenerative disorders, T-817MA has been studied in transgenic mice overexpressing human mutant tau. T-817MA treatment attenuated motor deficit, prevented motor neuron loss, and improved cognitive dysfunction [55]. In a further experiment, T-817MA attenuated tau-induced synaptic abnormalities. Addressing another oxidative stress-related disease, T-817MA improved motor dysfunction and had protective effects against 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP)-induced neurotoxicity in mice, presumably via blocking formation of lipid peroxidation products [56]. Phase II trials in mild to moderate AD started in 2008 [NCT00663936].

H_3 receptor is a G-protein-coupled receptor (GPCR) expressed throughout the brain, particularly in regions associated with cognition, regulating the release of other neurotransmitters [57]. H_3 receptors are suggested to modulate sleep–wake cycles and cognitive processes, demonstrated by several antagonist preclinical studies. Inverse agonists increase the release of histamine, ACh, dopamine, serotonin and norepinephrine. In AD-related actions, activation of postsynaptic receptor pathways (CREB, GSK3β) might be of relevance as well. Several H_3R antagonists have advanced to clinical phases, for treatment of ADHD, AD, cognitive deficits in schizophrenia or sleep disorders [58]. **MK0249** is a histamine H_3R inverse agonist developed by Merck. Besides clinical PET studies to assess receptor occupancy, it has completed Phase II studies in AD, ADHD and schizophrenia with several negative outcomes (lack of efficacy) [59]. Results of a mild to moderate AD study have been recently published and a once-daily MK0249 administration apparently did not improve cognitive function.

MK0952 is a selective phosphodiesterase $(PDE)_4$ inhibitor (with no isozyme specificity) developed for long-term memory loss and MCI [60], with an improved side effect profile and therapeutic window compared to first-generation agents, which exhibited several dose-limiting adverse reactions (e.g., emesis, nausea). PDEs hydrolyze cAMP, thereby regulating its intracellular levels and affecting multiple cellular processes – including memory-related processes (via PKA and CREB pathways) [61]. PDE_4 inhibitors demonstrated neuroprotective, neuroregenerative and anti-inflammatory effects in a series of *in vivo* experiments. Indeed, the prototype PDE_4 inhibitor rolipram demonstrated several beneficial cognitive effects in preclinical models, particularly reversing $A\beta_{40}$ induced memory impairment and it was also neuroprotective in an MPTP-induced toxicity PD model [62,63]. Function, targeting of PDE_4 subpopulations is still the subject of ongoing research (see e.g., [64]). Of note, tumor therapy may be a novel direction for PDE_4 inhibitors (as cAMP metabolism dysregulation in several cases is thought to play a role in tumorigenesis) [65]. For MK0952, design principles involving exploitation of intrinsic activity with low whole blood potency, improved oral bioavailability with rapid absorption and sufficient brain penetration were applied for better CNS targeting. MK0952 improved long-term recognition memory and cognition in preclinical models and had a superior therapeutic window compared to agents formerly studied [60]. Phase I and II studies [NCT00362024] were completed, however no further clinical trials have as yet been reported. In the PDE_4 inhibitor family, **MEM1414** was developed by Roche and the former Memory Pharmaceuticals. It was effective in preclinical cognitive tests, was well-tolerated in Phase I trials, and Phase II studies were planned; however there has been no information on further development following the merger of the parent company. Proof-of-concept preclinical studies of etazolate provided a link between γ-aminobutyric acid $(GABA)_A$ (associated with neuroprotection in several experimental settings) and APP pharmacology [66]. The anxiolitic **etazolate** (SQ-20009, EHT-0202) is a selective $GABA_A$ receptor modulator, demonstrated to exert neuroprotection against Aβ-induced toxicity, via induction of the nonamyloidogenic α-secretase pathway (offering amyloid plaque reduction) and formation of the neurotrophic, procognitive sAPPα [67], in addition to presumably suppressing Aβ-induced neuronal overexcitation. Procognitive effects were verified in an age-related cognitive deficit rat model (spatial learning and memory) [66]. Etazolate has a PDE_4 inhibitory action as well, that might contribute to its effects (CREB pathway). Etazolate was well tolerated in Phase I, and underwent Phase IIa studies in mild to moderate AD as adjunctive therapy to an AChEI [68]. A trend toward cognitive improvement was shown and the response was dependent on the apolipoprotein (Apo)E4 status.

HF0220 (7β-hydroxyepiandrosterone) is an endogenous neurosteroid, with potent anti-inflammatory effects and putative efficacy in relevant diseases (e.g., colitis, psoriasis, rheumatoid arthritis, neurodegenerative diseases). HF0220 was neuroprotective in several *in vitro* and *in vivo* models (e.g., Aβ-induced toxicity) [69]. In AD, HF0220 might exert beneficial effects via increasing the anti-inflammatory prostaglandin J2 (15d-PGJ_2) levels and peroxisome proliferator-activated receptor (PPAR)-γ activation. Phase II studies have been successfully completed in mild to moderate AD in 2008 [NCT00357357].

Ladostigil (TV3326) is a multifunctional hybrid drug combining rasagiline (exploiting the intrinsic neuroprotective activity of the propargylamine moiety [70]) and rivastigmine scaffolds designed for the treatment of AD. It is a cholinesterase and brain selective MAO inhibitor while the MAO-A profile confers antidepressant activity. Ladostigil was observed to display a multitude of parent compound/propargylamine-related AD-relevant actions, i.e., exerted neuroprotective effects and improved/prevented cognitive/memory deficits in various animal models, altered APP synthesis and processing (via PKC/MAPK pathways), showed antioxidant activity (direct scavenging and indirect effect on antioxidant enzymes), led to elevation of neurotrophic factors (brain-derived neurotrophic factor (BDNF), glial-cell line derived neurotrophic factor (GDNF)), had anti-apoptotic effect (via the Bcl-2 pathway) (complex neuroprotective actions

reviewed in, e.g., [71,72]). As in the case of aminoindane from rasagiline, the ladostigil metabolite hydroxyaminoindane itself has several neuroprotective effects, contributing to the overall action. Phase II studies are ongoing in MCI [NCT01429623] and mild to moderate AD [NCT01354691].

Davunetide (NAP, AL-108) is a neuronal peptidic tubulin interacting agent (thereby affecting tau phosphorylation), the active fragment of the activity-dependent neuroprotective protein (ADNP), showing efficacy in various neurotoxicity models (e.g., oxidative stress, Aβ) *in vitro* [73]. In ADNP-deficiency model davunetide reduced tau hyperphosphorylation and improved cognitive performance. In AD mouse models, davunetide administration in pre-pathological states reduced brain Aβ levels and tau hyperphosphorylation, changing tau distribution (increased soluble tau) and improving cognitive functions in later stages. Effects on cognitive impairments (spatial learning, memory) and tau pathology were confirmed in a tau-transgenic model [74]. In synucleinopathy model, davunetide improved motor performance and reduced α-synuclein inclusions (a long-lasting effect) [75]. In Phase I trials, intranasally administered davunetide was safe and well-tolerated. In Phase II studies in amnesic MCI – an AD precursor – [NCT00422981], davunetide treatment improved cognitive outcomes (visual matching/delayed visual recognition memory, working memory) [76].

Methylthioninium chloride (methylene blue, Trx0014) entered AD clinical trials as a tau aggregation inhibitor [77]. In Phase II mild to moderate AD study, substantial slowing of the rate of disease progression was reported.

2.1.3 *Phase I trials*

Begacestat (GSI-953, PF-5212362) is a Notch-sparing (17-fold selectivity) GSI (resulting from high-throughput screening (HTS) and further lead optimization) [78]. In AD mouse models, orally administered begacestat reduced brain, plasma, CSF $A\beta_{40}/A\beta_{42}$ levels and was able to reverse cognitive deficits (contextual fear-conditioning). Dose-dependent transient reductions of plasma $A\beta_{40}$ levels (less intensive effect observed on plasma $A\beta_{42}$) were verified in healthy human volunteers and AD patients following single doses, with later rebounds [79]. Further development was discontinued in 2010. **ELND006** is an APP-selective GSI with 15-/70-fold selectivity as determined in enzymatic and cellular assays. In rodent models, ELND006 was highly brain permeable following oral dosing and reduced brain and CSF Aβ levels, higher doses being needed for plasma Aβ lowering. In an aged AD mouse model, ELND006 treatment reduced amyloid burden and brain Aβ levels, but not dystrophic neuritis [80]. In non-human primate single and repeated dose studies, brain Aβ was reduced by approximately 25% (in a dose-dependent manner), whereas a rebound effect was observed for plasma Aβ [81]. ELND006 was subjected to Phase I trials, however further development has been halted due to observed liver toxicity, suggested to being unrelated to the mechanism of action [82]. GSMs emerging as an alternative to GSIs [83] are represented by several agents in Phase II trials. GSMs modulate the ratio of Aβ isoforms (either via direct enzyme- or substrate-targeting or conformational changes induced, the mechanism is still being explored), while the rate of APP processing remains unchanged. In addition, no Notch inhibition occurs, resulting in a more favorable safety profile. **E2012**, an orally active cinnamide (with a non-NSAID structure)-derived GSM entered the clinic in 2006. In rat cortical neuron culture, E2012 reduced $A\beta_{40/42}$ and increased $A\beta_{38}$ (less prone to aggregation) without changing total Aβ levels and causing APP-carboxy terminal fragments (CTF) accumulation. $A\beta_{40/42}$ levels were decreased following oral treatment *in vivo* (rat brain, plasma, CSF) preclinically and after single doses in healthy volunteers (plasma levels) in a dose-related way, without a rebound effect characteristic of GSIs [84]. The effect on Aβ isoform pattern was further characterized in dogs (CSF levels, matrix-assisted laser desorption/ ionization - time of flight (MALDI-TOF) analysis), indicating a shift in γ-secretase cleavage site preference [85]. Phase I clinical studies were suspended due to lenticular opacity observed in a rodent safety test, but subsequently resumed in 2008 following re-evaluation of safety. Further development was put on hold in favor of E2212. The follow-up GSM **E2212** has recently entered Phase I trials [NCT01221259]. It has improved *in vitro*/*in vivo* activity and a better safety profile compared to E2012. **EVP0962** (EVP-0015962) is a selective (Notch-sparing) GSM, which entered Phase I trials in H12011, following successful preclinical studies in AD models, i.e., reducing $A\beta_{1\text{-}42}$ levels, amyloid plaques, brain inflammation and reversing behavioral deficits in AD transgenic mice [86]. **CHF5074** is a flurbiprofen analog GSM, devoid of cyclooxygenase (COX) activity and interactions with Notch signaling. *In vitro* CHF5074 showed dose-dependent inhibition of $A\beta_{42}$ secretion. In mice AD model, CHF5074 dose-dependently reduced plasma Aβ levels and the $A\beta_{42}/A\beta_{40}$ ratio. Chronic CHF5074 treatment in AD mice reduced brain amyloid burden and plaque-associated activated microglia fraction, an effect that might result from the anti-amyloidogenic action [87]. In behavioral testing, CHF5074 improved performance in spatial memory impairment. Chronic administration of CHF5074 was also effective in reducing Aβ associated tau pathology (reduced level of hyperphosphorylated and total tau, via secondary action on GSK3β (decreased active and total level)) [88]. Activity on brain plasticity was studied, assessing effects on contextual memory, measures of synaptic density and neurogenesis [87]. Beneficial effects were described in young transgenic mice, without plaque deposition (attenuated memory and long-term potentiation impairment, reduced brain Aβ and hyperphosphorylated tau levels) [89]. CHF5074 entered Phase I trials in 2009 and a Phase II study is ongoing in MCI [NCT01303744, NCT01421056].

AAD-2004, a drug candidate for AD, displays Aβ lowering action and antioxidative properties (reducing free radicals

and PGE_2) as well as anti-inflammatory effects. The two latter properties might be common motifs in the neuropathology of neurodegenerative diseases; therefore, ALS and PD could also be among the putative future indications. Anti-inflammatory, antioxidative and neuroprotective effects were verified in preclinical AD, ALS and PD models, in addition to beneficial disease-specific actions [90,91]. The design paradigm for AAD-2004 started from sulfasalazine and involved the development of derivatives that prevent free radical formation and that possess anti-inflammatory effects without causing gastric side effects typical of NSAIDs. From such a combination of the two approaches, a synergistic neuroprotective effect was anticipated and sought in a clinical setting as no previous monotherapies with antioxidative or anti-inflammatory agents alone showed clinical benefit.

Although β-secretase is a promising target, the challenges of related medicinal chemistry hampered the design and synthesis of BACE1 inhibitors [92]. The most potent inhibitors are hydrophilic peptides which have unfavorable pharmacokinetic properties. The selective, brain penetrant **CTS21166** (ASP1702) – a transition state analog – was the first agent of the class to enter the clinic [93]. Preclinically, in transgenic mice CTS21166 reduced brain $A\beta_{40/42}$ levels and plaque load presumably via equilibrium shift. Central and peripheral $A\beta_{40}$ was reduced dose-dependently following single doses of CTS21166 in non-human primates [94]. In Phase I study [NCT00621010], single doses of CTS21166 significantly reduced plasma Aβ, with no rebound effect observed. Of the β-secretase targeting agents, **RG7129** is a BACE1 inhibitor from Roche, which recently entered Phase I. **HPP-854** is a BACE1 inhibitor, demonstrated to dose-dependently lower brain Aβ levels in animal studies. Phase I studies in subjects with MCI or mild AD are due to be finished in Q12012 [NCT01482013].

Of the further Aβ-related approaches, **NRM8499** is a prodrug of the controversial Aβ antagonist agent tramiprosate (Alzhemed). The latter has been marketed as a nutraceutical (under the name Vivimind) after Phase III studies failed to demonstrate efficacy in terms of cognitive improvement [95]. Tramiprosate is a sulfonated glycosaminoglycan (GAG) mimetic agent, disrupting Aβ aggregation and plaque formation by binding to soluble Aβ and maintaining it in non-fibrillar form thus competing with GAGs [96]. In human trials tramiprosate lowered $A\beta_{42}$ in the CSF of mild to moderate AD patients. According to recent reports, it may also act on tau aggregation. NRM8499 is expected to increase brain exposure to tramiprosate, thereby improving cognitive and other clinical outcomes in AD. Phase I studies were undertaken in Q12010, and verified an improved safety and tolerability profile.

NP-61 is a rationally designed dual binding site AChEI, i.e., binding to catalytic and peripheral sites of the enzyme. In transgenic AD models, orally administered NP-61 reduced brain Aβ levels, plaque burden and attenuated behavioral dysfunctions. Two Phase I trials have been conducted, demonstrating its safety [97]. AChE is suggested to have a role in Aβ deposition, and acts as a pathological chaperone enabling the formation of the amyloidogenic form. Dual binding site AChEIs could exert cholinergic function and simultaneously inhibit the AChE-induced Aβ aggregation, thereby offering both symptomatic and disease-modifying potential (design, structural features have been reviewed in, e.g., [98]).

Exebryl-1 is a small molecule α- and β-secretase modulator, also inhibiting tau aggregation according to *in vitro* results. Exebryl-1 emerged from a research program on the identification and pharmacological evaluation (Aβ inhibition) of *Uncaria tomentosa* components followed by the design, synthesis and testing of analogs of the active agent [99]. In a series of preclinical tests, orally administered exebryl-1 reduced Aβ formation and accumulation of fibrillar and soluble forms, amyloid load in transgenic AD mouse models at all stages of disease with an increase in CSF Aβ (presumably as a consequence of improved clearance), improved memory function and reduced astrocytosis and microgliosis [100,101]. Exebryl-1 entered Phase I trials in 2008.

GSK3 is a ubiquitous serine/threonine protein kinase also involved in several AD-related processes (tau hyperphosporylation, memory impairment, APP cleavage – Aβ production, inflammatory responses, cholinergic deficit, apoptosis), inasmuch, that a 'GSK hypothesis' of AD has been recently put forward [102]. GSK3 inhibitors therefore could affect AD pathogenesis at multiple points. A clinically applied drug with GSK3 inhibitory action, lithium has been thoroughly studied in this respect. Novel specific inhibitors emerged from inhibitor screening and structure-based rational design programs. **NP-12** (NP031112, tideglusib, Nypta) is a thiazolidinedione ATP noncompetitive irreversible GSK3 inhibitor [103]. Preclinically, the effects of NP-12 on amyloid and tau pathology were addressed in a double transgenic mouse model. NP-12 treatment prevented spatial memory impairment, decreased tau phosphorylation, amyloid deposition, glial activation and increased neuronal survival respectively. Interestingly, NP-12 treatment increased internalization and brain content of the neurotrophic insulin-like growth factor I (IGF-I) in an AD model [104]. NP-12 entered clinical trials in 2006 while Phase II studies in mild to moderate AD patients were started in 2009. In a Phase IIa study [NCT00948259] promising efficacy outcomes were obtained and a Phase IIb trial [NCT01350362] is currently ongoing.

Velusetrag (TD-5108) – a selective 5-HT_4 receptor agonist – is in Phase II for GI motility disorders. Regarding AD applications, 5-HT_4 receptor agonists have been reported to increase ACh release, attenuate cognitive impairment and modulate amyloid protein levels, favoring sAAPα formation and the non-amyloidogenic pathway [105,106]. Moreover, a 5-HT_4 agonist, PRX-03140 was reported to improve cognitive functions in a Phase IIa AD clinical trial. Velusetrag enhanced cognitive functions in a scopolamine-induced

spatial learning deficit model [107]. A Phase I study has recently been completed in healthy elderly subjects [NCT01467726]. **RQ-00000009** is a selective partial 5-HT_4 receptor agonist [108], which was demonstrated to improve cognitive and memory functions in rodent models (rat object recognition test, scopolamine impaired spontaneous alteration rat model), increase ACh release and dose-dependently decrease brain cortex Aβ protein levels. Safety, tolerability and pharmacokinetics have been studied in a recently completed Phase I study.

PQ-912 is a representative of the glutaminyl cyclase inhibitor class [109], the first to enter clinical development. Experimental data suggest an important role for glutaminyl cyclase in pyroglutamate (pE)-modified Aβ formation, which might act as a seed in amyloidogenic aggregation, thereby contributing to neurotoxicity and disease progression [110,111]. Proof-of-concept preclinical studies with glutaminyl cyclase inhibitors verified the approach, with inhibitor treatment leading to reduced $Aβ_{3(pE)–42}$ burden, Aβ plaque formation and gliosis and improvement in cognitive outcomes (context memory, spatial learning). Phase I results for PQ-912 have recently been announced and the findings show that the oral treatment was safe and well tolerated.

The targets of further approaches include, e.g., neuroinflammation, transcriptional dysfunction or neurotrophic factors-related pathways. **DSP-8658** is a (non-thiazolidinedione) PPARα/γ modulator with potent antihyperglycemic and lipid lowering activities, developed for the treatment of diabetes. The relationship between PPARγ and Alzheimer disease is a well-established one [112]. PPARγ is a ligand-activated nuclear receptor, involved in multiple functions related to the lipid, glucose metabolism and the modulation of inflammatory responses. In preclinical studies, PPARγ agonists were found to attenuate AD pathophysiology and exert functional benefits, therefore, human clinical trials were initiated with the available PPARγ agonists and contradictory results were obtained. DSP-8658 itself enhanced microglial Aβ uptake *in vitro* and *in vivo* via upregulation of CD36, and had beneficial cognitive effects [113].

TT301 and **302** inhibit glial cell activation and the release/overproduction of pro-inflammatory cytokines (e.g., IL-1β, TNF-α), thereby reducing glial cell derived inflammatory cycles and their long-term neurotoxic effects. TT301 was found to be effective in several preclinical disease models, such as rheumatoid arthritis or traumatic brain injury [114]. A Phase I study of iv administered TT301 has been conducted, evaluating the effects of TT301 on LPS-induced changes in blood cytokine levels [NCT01357421].

The complex role of σ1 receptors – a ligand-regulated molecular chaperone in the endoplasmic reticulum (ER) – remains to be unraveled. Known functions include the regulation and modulation of voltage-regulated and ligand-gated ion channels which affects, e.g., calcium mobilization or signal transduction pathways [115]. Moreover, recently a function as an inter-organelle signaling modulator has been suggested [116]. The *in vitro* studies of Marazzo *et al.* provided the first evidence, that σ1-ligands might exert neuroprotection against amyloid toxicity [117], which was confirmed later in *in vivo* studies. **ANAVEX-2-73** is a mixed muscarinic receptor ligand/σ1 receptor agonist, with actions on ER, mitochondrial and oxidative stress. The active metabolite of ANAVEX-2-73, **ANAVEX-19-44** is in preclinical development, as is a novel back-up compound, **ANAVEX-1-41**. ANAVEX-2-73 in a rat model attenuated Aβ peptide-induced learning deficits, or prevented them when administered before the Aβ peptides, suggesting neuroprotection against amyloid toxicity. Contemporaneously, anti-apoptotic and antioxidant effects were observed [118]. In a non-transgenic AD mice model, ANAVEX-2-73 attenuated Aβ-induced tau hyperphosphorylation via Akt activation and GSK3β inactivation [119].

Besides being a well-established cancer target, the implication of the molecular chaperone Hsp90 in neurodegenerative diseases is supported by several experimental findings [120]. Disease-relevant activities include maintaining aberrant neuronal protein activity and expression (inhibition of Hsp90 leading to degradation of the client proteins) leading to the formation of toxic aggregates, and regulation of the so-called heat shock factor-1 (HSF-1) transcription factor – Hsp90 inhibition therefore leading to the induction of heat shock proteins (e.g., Hsp70) via its activation. **PU-H71** is a synthetic purine-scaffold class Hsp90 inhibitor [121], thoroughly studied in cancer models. In a PD proof-of-concept study, PU-H71 *in vitro* suppressed LRRK2 expression – the data indicating the importance of Hsp90 in maintaining LRRK2 stability, and ameliorated mutant LRRK2-elicited loss of axonal outgrowth [122]. Preclinical data with Hsp90 inhibitors support, that this class might have a wider relevance in the treatment of neurodegenerative diseases.

Epigenetic targeting is a relatively novel approach for the treatment of age-related diseases, however a growing amount of *(in vitro* and *in vivo)* preclinical data confirms the neuroprotective, neurotrophic and anti-inflammatory activity of histone deacetylase (HDAC) inhibitors [123,124]. HDAC inhibitors could restore acetylation homeostasis and transcriptional dysfunctions and, in addition to histones, could act on several non-histone protein targets (resulting in transcriptional activation of disease-modifying genes) and counteract gene silencing by DNA methylation. **EVP0334** is an orally available, brain penetrant HDAC inhibitor (selective inhibition of a subset of class I and II HDACs), which improves cognitive performance and short- and long-term memory in preclinical studies [125,126]. EVP-0334 has finished Phase I studies and further development is planned according to preclinical evaluation in various neurodegenerative indications.

Finally, **S47445** (CX1632) is a first-in-class ampakine compound [127], which positively modulates AMPA receptor activity, increases magnitude and duration of glutamate

answer, synaptic transmission and plasticity activating expression of neurotrophic factors such as BDNF. Application of growth factors in neurodegenerative diseases has long been in the focus of research. A major drawback however is an appropriate central delivery. Activation of AMPA receptors and consequent BDNF production might offer an alternative solution. The approach has been verified in a series of preclinical and early clinical studies [127], with different agents of the class (e.g., PD, HD, neurotoxicity or cognition-impairment models). Importantly for chronic application, with different dosing regimes, the effect on BDNF expression and signaling and AMPA receptor concentration could be separately addressed [128].

2.2 Parkinson's disease

The major hallmark of Parkinson's disease is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, leading to the characteristic motor symptoms: resting tremors, bradykinesia and rigidity. However, other neurons are also affected and late-stage disabilities emerge from the involvement of nondopaminergic systems [129,130]. Parkinson's disease is not fatal itself, with secondary causes leading to death, resulting from severe motor dysfunction. From a therapeutic point of view, dopaminergic substitution treatments (levodopa, dopamine receptor agonists, MAO-B inhibitors, catechol *O*-methyltransferase inhibitors) were real breakthroughs in the management of the disease, particularly for motor symptoms. Efficacy is hampered in later disease progression due to the emergence of dyskinesia and motor fluctuations. Potential targets for neuroprotection are summarized in **Table 2B**.

2.2.1 *Phase III trials*

A_{2A} adenosine receptor antagonists might offer an alternative to dopaminergic therapies for improving PD symptoms (without the dopaminergic side effect profile) in addition to offering putative neuroprotective effect [131]. A_{2A} receptor antagonists were reported to improve motor functions in PD models via modulating basal ganglia neurotransmission (reducing the inhibitory output of the basal ganglia indirect pathway), which could potentially offer an alternative symptomatic treatment for PD, without provoking dyskinesia [132]. Moreover, for antagonists attenuating dopaminergic neurodegeneration, epidemiological and preclinical data also support a neuroprotective role [133]. A_{2A} receptors are colocalized in the striatum with dopamine D_2 receptors on GABAergic striatopallidal neurons and are involved in fine motor movement modulation. Dopaminergic hypofunction (as in PD) disrupts the balance of direct and indirect movement pathways, which could be restored by the A_{2A} antagonists. Investigational agents indeed provided motor benefits in preclinical models, and several representatives of the class have already entered the clinic. A selective A_{2A} antagonist, istradefylline (KW-6002) reached advanced clinical trial status for the treatment of PD motor complications [134]. **Preladenant** (SCH 420814) is a selective (> 1000 fold selectivity over A_1, A_{2B} and A_3 subtypes), competitive non-xanthine A_{2A} receptor antagonist [135]. The pharmacological profile of preladenant has been assessed preclinically in a series of PD and depression models (reversal of hypolocomotion induced by A_{2A} agonist treatment, L-Dopa induced contralateral rotation potentiation in unilaterally 6-OHDA-lesioned rats, attenuation of D_2 antagonist induced catalepsy, inhibition of L-Dopa-induced behavioral sensitization) – which point toward a compensating effect for loss of D_2 receptor-mediated actions in the indirect (striatopallidal) pathway and reduced risk of dyskinesias (as monotherapy or combined with L-Dopa) [136]. In a non-human primate MPTP model of PD, preladenant demonstrated an antiparkinsonian effect comparable to L-Dopa and was additive in combination, with no dyskinesia liability. Preladenant was safe and well-tolerated in Phase I studies. In a Phase II [NCT00406029] PD study [137], the primary efficacy measure of mean daily off time was reduced in the two higher preladenant dose groups without significant increases in dyskinesia. The effect on nonmotor symptoms warrant further studies in later large-scale trials. Phase III studies of preladenant in early and moderate to severe PD – as adjunct to levodopa or as a monotherapy – are ongoing [NCT01155466, NCT01227265, NCT01215227, NCT01155479].

Pardoprunox (SLV-308) – is more for symptomatic treatment [138] - is a combined dopamine $D_{2/3}$ receptor partial agonist (expected to result in DA receptor activation without motor complications, the action depending on the dopaminergic tone) and 5-HT_{1A} receptor agonist (expected to ameliorate dyskinesia, depression and cognitive impairment) in development for PD [139]. It exerted antiparkinsonian effects and improved motor symptoms in several preclinical PD models. In early PD patients pardoprunox was well tolerated and improved motor function (UPDRS-motor score, UPDRS-ADL) as a monotherapy; efficacy was confirmed in two follow-up studies, in addition to assessing safety and tolerability of different dosing regimes [140]. A trend toward efficacy was observed in advanced PD as an adjunct to levodopa.

Altered brain iron levels reflecting iron homeostasis dysregulations have been described in several neurodegenerative diseases (it is still unresolved whether as a cause or as a consequence of cell death). Reactive free iron load may interact with H_2O_2 (Fenton reaction) formed, for example in dopamine metabolism yielding toxic free radical species or may contribute to α-synuclein aggregation. Consequent oxidative stress could result in various cell damage (protein misfolding, DNA damage, lipid peroxidation of cell membrane, proteasomal dysfunction), with an outcome of apoptotic cell death (reviewed in, e.g., [141]). Iron chelation has been considered as a potential therapeutic approach for PD – supported by clinical findings in PD patients. **Deferiprone** (Ferriprox) is an oral, brain permeable bidentate iron chelating agent, used in the treatment of peripheral iron load

Table 2B. Neuroprotective agents in PD.

Neuropathology	Target	Investigational agents (exemplary)
Neurotransmitter/receptor signaling dysfunction	adenosine A_{2A} antagonists	istradefylline, preladenant, SYN-115, vipadenant
Oxidative stress	MAO-B inhibitors	Safinamide
	Metal ion chelators, metal protein attenuators kynurenine pathways modulators	Deferiprone
Mitochondrial dysfunction	(dietary) antioxidants, mitochondrial function modulators	coenzyme Q10, creatine, green tea polyphenols
Excitotoxicity, calcium homeostasis dysregulation	Ca^{2+} channel blockers	Isradipine
Trophic factor deficiency	Nerve growth factor (NGF) delivery, NGF agonists	CERE 110, PYM50028

disorders, as thalassemia major. In *in vitro* studies, deferiprone was neuro/cytoprotective against AD and PD relevant insults (ferric iron, H_2O_2, $A\beta_{1\text{-}40}$, MPP^+ induced neuronal cell death) [142]. In a 6-OHDA *in vivo* PD model, deferiprone attenuated dopaminergic neuron loss, with normalization of dopamine content [143]. Small-scale pilot studies with deferiprone in neurodegeneration with brain iron accumulation, Friedreich's ataxia confirmed the potential benefits of the iron chelation/relocation approach [144], supporting initiation of small-scale phase II/III studies in PD [NCT00943748, NCT01539837].

Safinamide (developed initially as an anticonvulsant) has multiple (symptomatic and neuroprotective) actions, it is a reversible, non-covalent MAO-B inhibitor (5000-fold selectivity vs. MAO-A), inhibits dopamine uptake, (in state-dependent manner) voltage-dependent Na^+ and Ca^{2+} channels (without affecting peripheral L-type Ca^{2+} channels) and glutamate release [145]. Preclinically efficacy was confirmed in different neurotoxicity and PD models (e.g., restoration of levodopa response in 6-OHDA lesion, prevention of MPTP neurotoxicity/neurodegeneration). Antidyskinetic activity was confirmed in a non-human primate L-Dopa induced dyskinesia model, where safinamide prolonged the antiparkinsonian effect [146]. In a small-scale open pilot study, safinamide, as adjunct to levodopa, significantly improved motor performance (UPDRS scale) and decreased motor fluctuations. Phase III trials were initiated in 2007 in early PD as an add-on to a dopamine agonist ('MOTION' trials) or mid-late PD (with motor fluctuations) as add-on to levodopa ('SETTLE' trials). In two-year mid-late PD [NCT01286935, NCT01187966] trials, safinamide improved UPDRS Part II/III/IV, PDQ-39 (emotional well-being) and GRID HAM-D scores (depressive symptoms), increased ON time and decreased OFF time without worsening dyskinesia [147,148]. In patients with more severe dyskinesia at baseline, safinamide showed efficacy assessed by DRS scores [149]. Merck has recently announced the return to Newron Pharmaceuticals SpA of all rights to safinamide due to a more limited market potential for the drug than originally anticipated.

2.2.2 *Phase II/I trials*

SYN115 (tozadenant) is a (non-xanthine) adenosine A_{2A} antagonist. Based on promising preclinical results, SYN115 has been subjected to Phase I and IIa trials (mild to moderate PD patients taking levodopa) [150] and is currently in Phase IIb trials, with results due in H1 2013. The Phase IIb trial will evaluate four SYN115 doses as adjunctive therapy in levodopa-treated PD patients with 'end-of-dose' wearing off. With the aim of obtaining more information on the mechanism of action of SYN115, as well as accelerating dose finding, perfusion magnetic resonance imaging was carried out, assessing cerebral blood flow responses. SYN115 treatment improved several measures in clinical rating scales of motor function and cognition [133]. Of the class, the non-xanthine **vipadenant** (BIIB014, V2006) entered Phase II trials in 2007 [133]. It increased ON time without dyskinesia and decreased OFF time. Receptor occupancy was confirmed by PET studies [151]. Further development was discontinued in 2010, due to concerns raised by preclinical toxicology assays. The next generation compound V81444 entered Phase I trials in H22011. **ST1535** was well tolerated in Phase I studies, moreover two of its metabolites (ST3932, ST4206) were described to have antiparkinsonian action in preclinical models *in vivo* [133].

One therapeutic approach is to exploit endogenous neuroprotective mechanisms such as the brain-derived and glial cell-derived neurotrophic factors. A major challenge in developing neurotrophic factor based therapies is the delivery of active agents. As an alternative, **PYM50028** (Cogane, smilagenin) is an orally available steroidal sapogenin originally isolated from traditional Chinese medicines *(Rhizoma anemarrhenae, Radix asparagi*), with a potential to induce endogenous growth factor actions in PD. In cultured dopaminergic neurons, PYM50028 showed a protective effect against MPP^+ toxicity, via stimulation of GDNF expression [152]. In MPTP-lesioned mice, PYM50028 treatment increased striatal GDNF and BDNF levels and attenuated MPP^+ induced neuronal loss. The preclinical data suggested both neuroprotective and neurorestorative effects [153]. Oral PYM50028 reversed parkinsonian disability in MPTP-lesioned macaques [154]. In aged rats, PYM50028 improved memory by increasing the stability of

M_1-receptor mRNA [155] and demonstrated efficacy in preclinical AD and ALS models. Oral PYM50028 was safe and well tolerated in healthy volunteers and mild to moderate PD sufferers [154]. A Phase IIa study in mild to moderate AD has been conducted, and a Phase II study in early PD is ongoing [NCT01060878]. Due to positive preclinical data, PYM50028 has recently been granted an orphan drug status for ALS by the FDA and the European Commission.

GM1 ganglioside (a sialic acid-containing glycosphingolipid) is a component of neuronal plasma membranes, especially in synaptic regions. GM1 has a role in neuronal growth, development and regeneration, modulation of cell surface and receptor activities and may enhance endogenous neurotrophic mechanisms. In an abundance of *in vivo* experiments, GM1 treatment improved outcomes in neuro-injury (CNS lesions of different origins, neurotoxin exposure) or aging models [156]. GM1 was effective in testing dopaminergic deficits, eliciting improved cognitive and motor functioning. GM1 effect may involve restoration of damaged but viable DAergic neurons, stimulation of sprouting of neurites, enhanced dopamine synthesis and interaction with neurotrophic pathways. A role in the structure and function of lipid membrane rafts might be a crucial component in the activity. In a recent study, the causal role of brain ganglioside abnormality in PD pathogenesis was suggested, rendering GM1 administration a kind of replacement therapy [157]. Positive results in murine and non-human primate PD models supported proceeding forward to human trials. In a pilot open-label PD study, GM1 treatment (administered sc) was safe and well-tolerated, resulting in functional improvements. In a small-scale double-blind placebo-controlled study in mild to moderate PD, significant improvements (UPDRS, timed motor test) were observed in the GM1 group. Long term safety and efficacy (UPDRS, UPDRS-ADL, timed motor tests) were verified in an open 5-year study [158].

SKL-PD (YKP10461) is a selective and reversible inhibitor of MAO-B, with MAO-B independent neuroprotective and neurorestorative effects in neuronal cell and animal models [159].

AV-101 (L-4-chlorokynurenine) is an orally available prodrug (to enhance brain delivery) of 7-chlorokynurenic acid, a kynurenic acid derivative NMDA receptor antagonist. The kynurenine pathway contributes to tryptophan metabolism with experimental/clinical findings in PD, AD, HD suggesting its alteration causes a shift toward routes yielding neurotoxic intermediates (reviewed in, e.g., [160,161]). As a therapeutic approach, use of the alternative intrinsic neuroprotective kynurenic acid was suggested. Phase I studies of AV-101 have been started in neuropathic pain [NCT01483846], a positive result might enable its development for neurodegenerative indications.

2.3 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (Lou Gehrig's disease, motor neuron disease) affecting brain and spinal cord neurons controlling voluntary muscles was first described in 1873. Progressive degeneration of motor neurons leads to muscle weakening and atrophy, leading to final respiratory failure. There is an average survival of 3 – 5 years from symptom onset. Mostly people between 60 and 70 years of age are affected, with the incidence being 1 – 2/100000 and the prevalence 4 – 6/100000 of the total population. Approximately 10% disease cases are familiar, with a known genetic cause and the remainders are sporadic. Of the potential genetic causes, mutation of the antioxidant SOD1 is the most studied and has yielded animal disease models. The only approved drug at present is the antiglutamatergic riluzole, which has demonstrated modest life span extension in clinical trials [162,163].

2.3.1 *Phase III trials*

TRO19622 (olesoxime) – a cholesterol-like small molecule - was identified in a phenotypic cell-based screening program (motor neuron survival endpoint) as a potential candidate for ALS [164]. *In vitro*, TRO19622 dose-dependently improved motor neuron survival in the absence of trophic factors and promoted neurite outgrowth and branching. *In vivo* efficacy was tested in lesion models (motor neuron death, axonal degeneration/regeneration) and mutant SOD1 mice (familial ALS). In the latter, TRO19622 treatment resulted in improved motor performance, delayed disease onset and increased life span. Studies directed toward elucidating the mechanism of action implied that TRO19622 may act on mitochondria, inhibiting mPTP opening, interacting with its protein components (VDAC, TSPO) [165]. Further studies demonstrated that TRO19622 suppresses microglial and astrocyte activation, and transiently protects neuromuscular junctions [166]. Phase I studies in healthy volunteers and Phase Ib in ALS patients have been successfully completed. Phase II/III trials were funded by the EU FP7 'Mitotarget' program, results of which were announced at the end of 2011. TRO19622 treatment did not result in significant increase in survival (vs. placebo).

Arimoclomol (BRX-220), an analog of bimoclomol [167] – a co-inducer of Hsp expression – was first studied as an agent against insulin resistance and diabetic complications. Arimoclomol amplifies the cytoprotective heat shock response, presumably via prolonging the activation of heat shock transcription factor-1, resulting in an increase in Hsp70 and Hsp90 expression [168]. Of note, arimoclomol is a co-inducer and not an activator of heat shock response, exerting its effect in cells already under stress, which is a crucial feature from a drug safety/side effect point of view. Arimoclomol proved to be effective in a nerve injury model of motor neuron degeneration. In mice overexpressing mutant human SOD1, arimoclomol treatment delayed disease progression (even when administered after symptom onset), improved muscle function and motor neuron survival and led to a prolonged lifespan [168]. The post-symptomatic efficacy of arimoclomol treatments started in early or late symptomatic stages was

verified in a later SOD1 mouse model trial [169]. Arimoclomol was safe and well-tolerated in ALS patients [170] and a Phase II/III trial is ongoing in SOD1 familial ALS patients [NCT00706147].

Dexpramipexole (KNS760704) is the chirally pure *R*-enantiomer of pramipexole, a dopamine agonist used in PD. Dexpramipexole is devoid of dopamine agonist effect – exhibiting 1000-fold lower agonist affinity than pramipexole – and dopaminergic side effects in concentrations needed for neuroprotection. Its development was facilitated by reports on the neuroprotective effects of pramipexole, unrelated to its dopamine agonist activity (reviewed in, e.g., [171]). Dexpramipexole demonstrated antioxidant, anti-apoptotic and neuroprotective effects preclinically and increased survival in SOD1 mutant mice. The precise mechanism of action remains to be determined, however, dexpramipexole was suggested to target mitochondria via stabilization of mitochondrial function, inhibition of mPTP opening, inhibition of stress-induced membrane currents and pathological conductance and consequent effect on oxidative phosphorylation – bioenergetic efficiency [172]. In a pilot open label ALS study, non-significant reductions were observed in disease progression (slope of decline on the ALSFRS-R). In a two-part Phase II study [NCT00647296, NCT00931944], dexpramipexole was safe and well-tolerated. A dose-dependent slowing of functional decline (slope of decline on the ALSFRS-R) and decreased mortality was observed in the highest dose group, encouraging further trials [173]. A large-scale Phase III study ('EMPOWER') was initiated in 2011.

Edaravone (MCI-186) is a free radical scavenger approved for acute cerebral infarction. In ALS disease models, edaravone slowed disease progression, motor neuron degeneration and reduced abnormal SOD1 deposition [174]. Safety and efficacy in ALS (ALSFRS-R score, 3-nitrotyrosine level in CSF as a marker of oxidative stress) were studied in a small-scale open-label trial and Phase III studies are ongoing in Japan.

2.3.2 *Phase III/I trials*

AEOL-10150 is a manganoporphyrin designed to mimic the effect of SOD by scavenging the reactive oxygen and nitrogen species (cycling between Mn(III) and Mn(IV) states), affecting ROS signaling. In addition, it has shown anti-inflammatory and anti-apoptotic effects. AEOL-10150 acts as a catalytic antioxidant, i.e., it is not consumed in the reaction [175]. In a G93A mouse model, AEOL-10150 treatment prolonged survival and slowed disease progression, global motor function being maintained until an end-stage rapid decline. Gliosis, as well as the levels of oxidative injury markers was reduced [176]. Safety, tolerability, pharmacokinetics were characterized in small-scale Phase I studies but further development toward ALS indication were suspended due to financial reasons. Manganoporphyrins could have a pro-oxidative action, thereby suppressing escalation of inflammation and immune responses. Other multiple effects contributing to neuroprotection have been also suggested, like an action on Ca^{2+} metabolism [177].

PYM50018 (Myogane), a sapogenin-type small molecule related to PYM50028 has demonstrated neuroprotective effects in several preclinical models, via induction of neuroprotective factors. Upon completion of a Phase I trial, a shift in the focus of PYM50018 development from ALS toward an ophthalmological neurodegenerative disease, glaucoma may be anticipated [154].

2.4 Huntington's disease

Huntington's disease has a well-defined genetic cause, i.e., autosomal dominant inheritance, such that the huntingtin protein gene has an expansion of CAG (glutamine) repeat (> 38, number of repeats correlating with disease onset). The *N*-terminal polyQ fragments produced via cleavage are able to aggregate (self/with other protein) and form inclusions, leading to neuronal toxicity. HD is characterized by cognitive and memory impairments, motor symptoms (choreic movements) and behavioral alterations and has an average survival of 15 – 20 years from diagnosis. Symptomatic treatments are used at present, with no drug able to slow disease progress [178,179].

2.4.1 *Phase III trials*

AMR101 (ultra pure ethyl-eicosapentaenoic acid) – a long chain highly unsaturated fatty acid – is the pro-drug of eicosapentaenoic acid (EPA) and its exact mechanism of action for treating HD remains unknown. EPA is involved in a number of biological functions; reducing the NFκB pathway activity and expression of the prostaglandin synthesizing enzyme cascade, as well as suppression of JKN activation might contribute to its activity against HD [180]. In a transgenic HD mouse model, EPA ameliorated Huntington's phenotype, whereas ethyl-EPA improved motor dysfunction. Beneficial effects of EPA on motor function were described in a human study, followed by a small-scale HD trial [181]. Two Phase III HD trials have been conducted (one in Europe and one in North-America), where beneficial effects emerged during the longer run (12 *vs*. 6 months treatment) [182].

2.4.2 *Phase II/I trials*

The role of sirtuins in neuroprotection and in neurodegenerative diseases and consequently the relevance of their pharmacological modulation are at best controversial. In Drosophila HD models the Sirt1 ortholog Sir2 demonstrated distinct effects on neuronal survival and lifespan, where reducing Sir2 improved the former [183]. Sirt1 inhibition exerted neuroprotection against oxidative stress in cultured neurons and Sir2 inhibition suppressing neuronal degeneration has been observed in other Drosophila models as well. HD is characterized by a (mutant Htt-induced) transcriptional dysregulation that might be restored by HDAC and particularly Sir2/SIRT1 inhibition (acting via histones and other targets

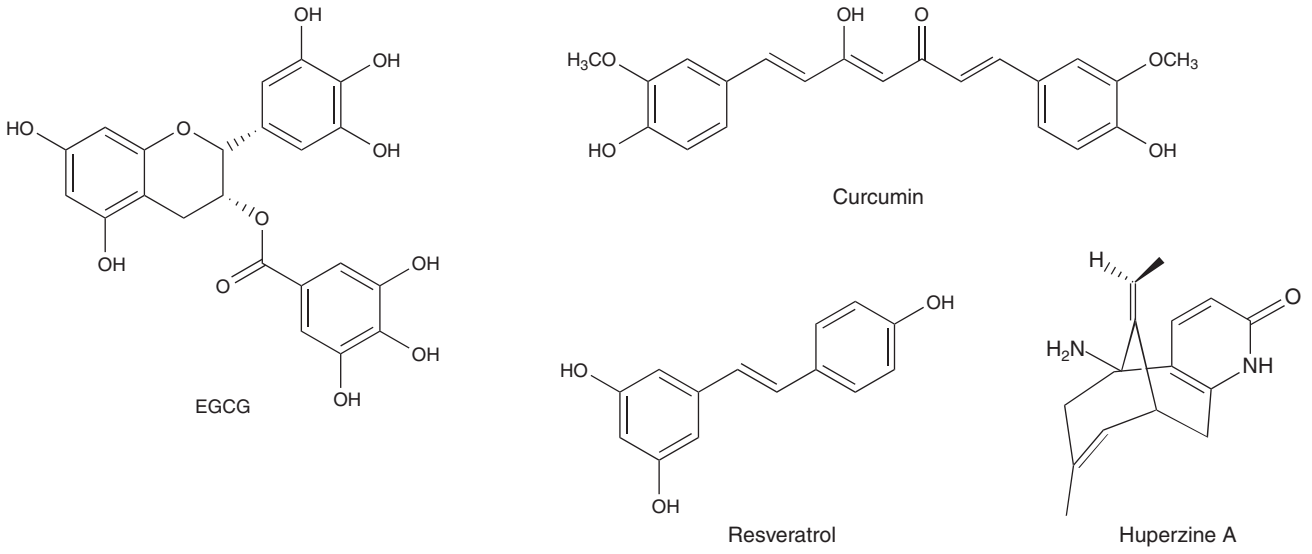


Figure 1. Examples of natural products studied as therapeutic agents for neurodegenerative diseases.

as CBP, UCP2 or PGC1α) [184]. Adding to the complexity of the issue, of the most recent studies, Jiang *et al.* and Jeong *et al.* reported the beneficial effects (on motor function, brain atrophy, metabolic changes, survival) of Sirt1 overexpression against mutant Htt-toxicity in mice HD models [185,186]. In the mechanism of action, the role of a physical inhibitory interaction between mutant Htt and Sirt1 (and consequently its downstream targets) and restoration of BDNF actions were suggested. **Selisistat** (SEN0014196/EX-527) is a first-in-class selective inhibitor of SIRT1 [187], an NAD-dependent deacetylase, involved among a broad array of actions [188] also in the acetylation of mutant huntingtin. Selisistat expressed protective effects against HD in cellular and animal disease models, showing a trend toward disease-modification and it is suggested to increase mutant huntingtin clearance. The drug has been granted orphan drug status for HD by the FDA, EMEA and the Australian Department of Health and Ageing and has recently entered Phase II trials (co-funded by an EU FP7 programme (PADDINGTON)), after completion of Phase I studies in 2010.

Cysteamine (RP103) [189] – an approved drug for nephropathic cystinosis – is undergoing Phase II HD trials. Safety and maximum tolerable dose were determined in a small-scale open-label study in 2006.

3. Designed multi-target ligands for the treatment of neurodegenerative diseases

Successes with some plant isolates in the prevention and treatment of diseases have received increasing attention. Of natural products, especially green tea constituents – particularly (-)-epigallocatechin-3-gallate (EGCG), the red wine antioxidant resveratrol, curry spice component curcumin, ginkgo biloba preparations (e.g., EGb-761, containing quercetine and bilobalid), CoQ10, omega-3 fatty acids and the *Huperzia serrata* alkaloid huperzine A have been currently the focus of interest, and the subject of clinical trials in various neurodegenerative diseases (reviewed in e.g., [190,191], representative structures summarized in **Figure 1**) (regarding natural products, see also [192]).

There is a growing awareness of the rationale of applying multifaceted approaches for the treatment of neurodegenerative diseases, targeting more pathways simultaneously (i.e., using 'magic buckshots/shotguns' instead of 'magic bullets'). Several names have been suggested for such single agents designed to have a multi-mechanistic action (reviewed in, e.g., [193]), in the present section the term 'designed multiple ligand' (DML) is used. Such agents can emerge from various sources [194,195].

The various DML strategies for AD/PD/HD/ALS indications have been extensively reviewed by Cavalli *et al.* in 2008 [196], **Figure 2** exemplifying some design principles. For AD, the most prevalent approach is to combine the activities of an AChEI with some other disease-relevant effects such as: i) blocking of Aβ aggregation (aimed at the class of dual binding AChEIs, which when used as a starting point can be endowed with additional BuChE, BACE1, metal chelating (e.g., bis-tacrine derivatives, PBT2-tacrine heterodimers), anti-platelet activating factor (a pro-inflammatory mediator) or antioxidant (e.g., lipocrine, a lipoic acid-tacrine derivative or tacrine-melatonin hybrids) activity as well [197]), ii) action on other neurotransmitter systems (MAO inhibition, serotonin transporter inhibition, 5-HT_3 ligand activity, H_3 inhibition, cannabinoid CB1 antagonism) – conferring beneficial effects on other disease related symptoms such as anxiety or depression, iii) antioxidant properties, iv) calcium channel blocking (e.g., tacrine-dihydropyridine hybrids – reviewed

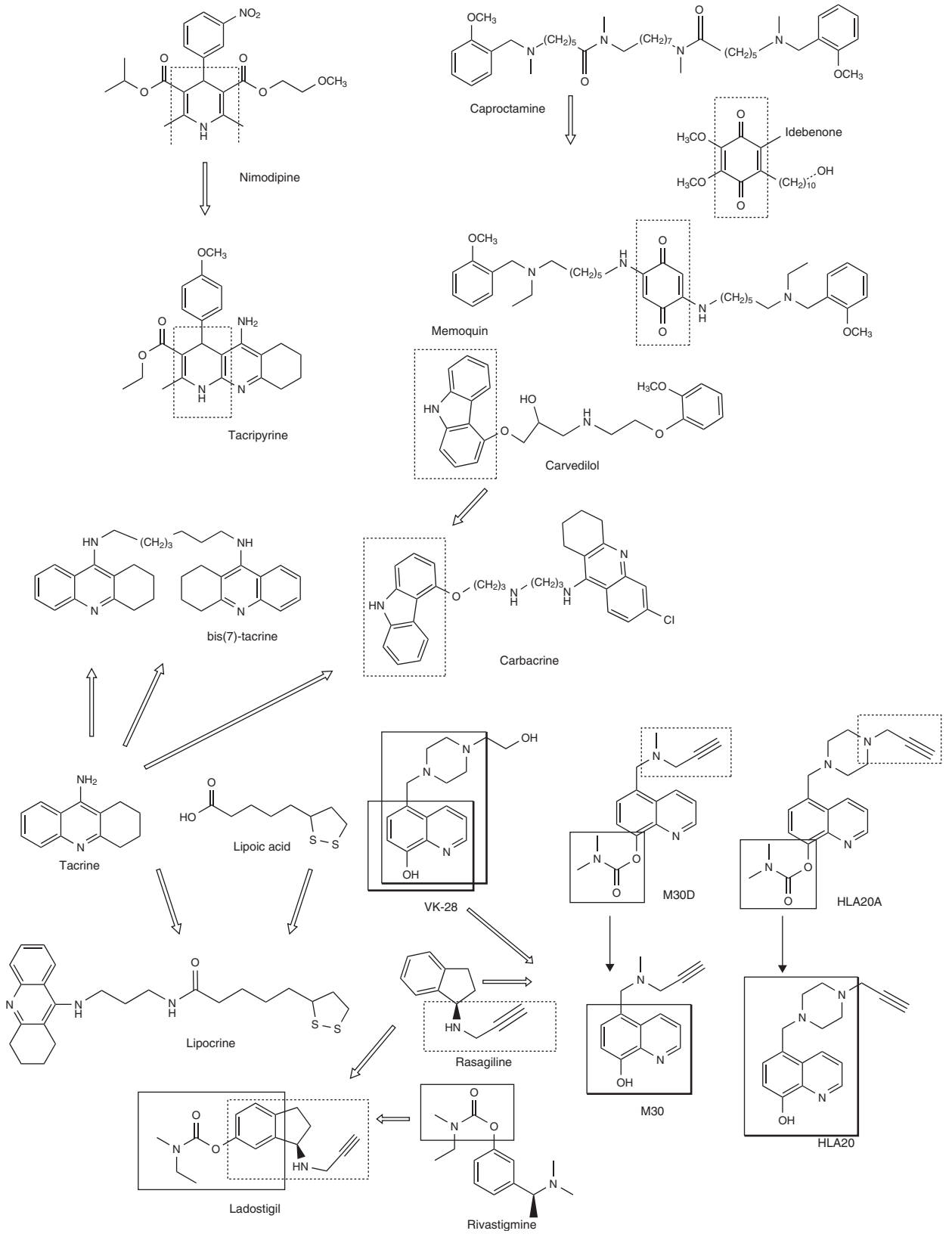


Figure 2. Selected examples for 'multi-target directed ligands' for neurodegenerative diseases: structures, design strategies (continued).

in [198,199]). Of the former strategies, the combined MAO/AChE inhibitor ladostigil is already in Phase II. Design, characteristics of recent tacrine-based hybrid approaches (dual binding site AChEIs, dual binding site AChEIs with additional antioxidant (e.g., ferulic acid, melatonin hybrids) or metal-chelating action, tacrine/NO-donor vasorelaxant hybrids) have been reviewed recently by Rampa *et al.* [200]. Another interesting DML drug candidate for AD is memoquin – an agent derived from the AChEI – M_2 antagonist caproctamine and the synthetic CoQ10 derivative idebenone, that is an AChEI (interacting with both the catalytic and peripheral anionic site) with activity against ROS formation and Aβ aggregation (self and AChE-induced) in addition to inhibiting BACE1. A promising *in vivo* efficacy profile in AD models (reduced Aβ accumulation, prevention of tau hyperphosphorylation, rescuing behavioral impairment, restored cholinergic deficit [201]) makes memoquin a potential candidate for further development. Based on the metal homeostasis dysfunction hypothesis, metal chelating agents with add-on activities such as amyloidogenesis targeting (e.g., BACE1 inhibition) or ROS scavenging have been designed and studied. Of calcium overload/excitotoxicity-driven approaches, design of dual NMDA receptor/neuronal calcium channel antagonists – exemplified by NGP1-01, NMDA receptor antagonist/glutamate release inhibitors and AChEI/NMDA receptor antagonists (as carbacrine, reducing oxidative stress, self- and AChE-induced Aβ aggregation) has been suggested [193,199]. A novel class of DMLs is that of combined PPARγ antagonists/γ-secretase modulators.

For DML design for PD, MAO-B inhibition is sought after [202] and rasagiline in particular has been used as a parent scaffold. HLA20 and M30 (a neuroprotective/neurorestorative agent according to *in vitro*/*vivo* AD/PD/ALS models [203,204]) emerged from the combination of an iron-chelating moiety (8-hydroxyquinoline of VK28) and the propargyl group (responsible for neuroprotective profile) of the MAO-B inhibitor rasagiline. Recently, further pro-chelating, site-activated derivatives were described, with enhanced target selectivity, releasing parent M30/HLA20 upon binding to and inhibition of AChE [205]. A potential designed-in second activity besides MAO inhibition could be that of A_{2A} antagonism [206] or antioxidant effect (ROS/RNS scavenging, neuronal NOS inhibition). Istradefylline (KW-6002) can be considered as the prototype MAO-B/A_{2A} dual agent, its recent clinical trials offering important proof-of-concept data.

Several approved drugs were reported to have additional pharmacological activities and in fact were found to behave as multifunctional agents. Identifying neurodegenerative disease-relevant actions of agents approved for other indications might facilitate drug repositioning. Considerable attention has been focused on PPAR-γ agonists with the thiazolidinedione scaffold, particularly the antidiabetics rosiglitazone and pioglitazone have been reported to exert MAO-B inhibition, attenuation of neuroinflammation and neuroprotection in PD or AD relevant models. The mitochondrial target and action of pioglitazone has also recently been delineated. The anti-epileptic, Na^+/Ca^{2+} channel blocker zonisamide was also reported to exert MAO-B inhibition and neuroprotection in PD or AD relevant models [202]. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase enzyme inhibitors – statins – were described to have various AD- and PD-relevant, e.g., neuroinflammation- or amyloid production-related actions. Clinical trials in AD were carried out with simvastatin, atorvastatin and pravastatin (reviewed in, e.g., [8]). Another class arising from epidemiological studies is that of NSAIDs, which have been implicated in reduced risk of AD. A number of long-term prospective (primary prevention) studies were carried out with several representatives of the class (e.g., the 'ADAPT' trial). Of antibiotics, the brain permeable tetracycline antibiotic, minocycline has been extensively studied in experimental and clinical setting as a neuroprotective agent. Of β-lactam antibiotics, particularly ceftriaxone was studied following reports on its action via glutamate transporter-1 and NMDA receptor. The macrolid, immunosuppressant rapamycin (sirolimus) exerting pleiotropic effects, has been effective in various neurodegenerative disease models.

Figure 2. Selected examples for 'multi-target directed ligands' for neurodegenerative diseases: structures, design strategies (continued). Memoquin: derived from caproctamine with a well-balanced dual-binding site AChEI and competitive muscarinic M2 receptor antagonist (facilitating ACh release via presynaptic receptors) profile, adding antioxidant activity via incorporating the 1,4-benzoquinone scaffold from coenzyme Q10/idebenone. **Tacripyrine:** derived from combining the tetrahydroaminoquinoline scaffold of tacrine with 1,4-dihydropyridine calcium channel blockers, yielding an AChEI-VDCC antagonist, with modest Aβ aggregation inhibitor potency. **Carbacrine**: derived by linking the carbazole part of the antioxidant, NMDAR antagonist, β-blocker carvedilol with the tetrahydroacridine scaffold of tacrine, yielding a dual-binding site AChEI, with antioxidant (neuroprotective against ROS formation) and NMDAR modulator (noncompetitive open-channel blocker) profile. **Bis(7)-tacrine**: tacrine homodimer, with dual-binding site AChEI, NMDAR antagonist, L-type calcium channel and Aβ processing (BACE1 inhibitor, modest α-secretase activator) modulator profile. **Lipocrine**: derived by linking the antioxidant, neuroprotective lipoic acid with tacrine, yielding dual-binding site AChEIs with antioxidant (neuroprotective against ROS formation) effect. **Ladostigil**: MAO/AChE inhibitor, derived by inserting an AChEI carbamate moiety in the structure of rasagiline. **M30**, **HLA20**: derived by combining the iron-chelator VK-28/clioquinol parent structures and the propargylamine moiety of rasagiline. Introducing a novel function by the AChEI carbamate moiety of rivastigmine, site-activated pro-chelators **M30D** and **HLA20** were obtained.

4. Conclusion

Besides suboptimal target design, low translation rates can partly be ascribed to shortcomings in preclinical models. Better animal models are needed, reflecting more closely human pathology and predicting outcomes. With regards to clinical trials, an issue often raised in connection with recent failures is whether a neuroprotective effect could have been clearly demonstrated with the adopted clinical trial designs and methodology if the agent is devoid of a quick, potent and robust action (for a detailed discussion refer to reviews (e.g., [207]). Some important clinical trial-related issues of major importance for neuroprotective agents could be summarized as follows:

1) Choosing an optimal target population and patient stratification - including more specific subgroups of patients in trials, due to the substantial heterogeneity of the different subtypes and therefore their therapeutic responses which can confound statistical analysis. Targeting specific subgroups might lead to a market constraint, however, this could be compensated for by future early intervention protocols. Accurate clinical diagnosis (especially for early stages) is still a challenging issue as diagnostic biomarkers are lacking and a wide variety of clinical signs and symptoms and disease etiologies are prevalent. (Moreover, the trials usually address already symptomatic patients, potentially with irreversible functional impairments.);
2) Distinguishing between symptomatic and real disease-modifying effects (*vs.* natural disease progression). Delayed-start trial design is one solution that has been used (e.g., for rasagiline neuroprotection trials) for overcoming this problem;
3) A lack of objective, sensitive, reliable biomarkers for assessing disease progression and neuroprotective effects (importantly not confounded by symptomatic effects) for monitoring throughout the course of clinical trials. Rating scales and clinical endpoints currently used can be confounded by several factors (e.g., placebo influence, intersite variances in larger trials, insensivity of rating scales, rater bias (inexperience, reliability));
4) The time-scale of disease progression (especially in mild to moderate patients) *vs.* the length of neuroprotective trials means, that only modest effects could be expected, hardly yielding statistically significant efficacy measures. On the other hand, trial durations might be suboptimal for a meaningful effect. Modest effects to be detected and the need for statistical significance often bring about the launch of large-scale trials, conferring further potential errors (e.g., intersite variances);
5) Establishing optimal dosing regimes enabling sufficient brain penetration via thorough preclinical and early clinical testing, evolving subtherapeutic dosing – although assessing dose–response relationships could be a further challenge for neuroprotective agents.

Neuroprotective drugs for neurodegenerative diseases are likely to be used for long-term and safety aspects have to be designed accordingly. As the diseases affect mainly aged populations, age-related changes in pharmacokinetics and pharmacodynamics should be considered [208], such as the likely presence of other diseases and possible interactions with other drugs administered.

5. Expert opinion

If the future success of an investigational agent is to come from a sound knowledge on the etiopathology of the targeted disease, there is still a long way to go. Despite substantial progresses in the last decades, there are still several open questions regarding the etiology and pathophysiology of AD, PD, HD and ALS, hampering drug development programs. Therapeutic challenges are inherent in the nature of the diseases: cases are mostly sporadic, stemming from complex interplays of genetic, endogenous and environmental factors, leading to diverse outcomes and confounding diagnosis and consequent trial designs. Besides these factors, the chronic, multifactorial nature of the diseases renders preclinical testing also challenging. In fact, there is a growing awareness, that instead of single diseases, clinical syndromes must be managed. The relative importance and interdependence of the various key players is still to be unravelled, such as the nature of the primary factor, if there is any. Not unexpectedly therefore, diseases are mostly unresponsive to single target approaches, warranting novel development and study methods, based on systems-oriented and network approaches to address the robustness and fragility of biological systems. There is also an increasing effort dedicated to a better comprehension, design and application of preventive interventions.

In parallel with basic research programs providing a better understanding of the diseases (offering novel targets or highly needed biomarkers), future directions might shift toward targeting early disease states and an increasing effort on the design and exploitation of multi-target approaches (via single agents or appropriate combinations). Such (early) multi-directed interventions could have the potential for slowing or modifying neurodegenerative processes, albeit a definite cure for already symptomatic cases seems to be at present elusive.

Novel investigational agents and/or ideas may frequently come from unexpected sources, in particular, from drug repositioning programs.

Last but not least, organizational issues make also a strong influence on the outcome of research projects oriented toward the development of clinically useful neuroprotective agents. Substantial contribution to the field might be expected from academic programs and small/medium biotech companies, due to their flexible environment suitable also to non-conventional drug discovery strategies. Undoubtedly, neuroprotective drug discovery and development represent a field where academic and industrial cooperation is inevitable.

Acknowledgement

The authors apologize for omitted references, due to space constraints reviews or the most recent reports were preferably cited wherein references to primary literature could be achieved.

Dedication

This paper is kindly dedicated to Professor Ferenc Fülöp on the occasion of his 60th birthday.

Declaration of interest

The authors have no relevant disclosures. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. The support of the Hungarian Scientific Research Fund (OTKA, K73389) and the National Development Agency – A*STAR Fund (TET_10-1-2011-0050) is gratefully acknowledged.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1 Pogacic V, Herrling P. List of drugs in development for neurodegenerative diseases: update June 2007. Neurodegenerative Dis 2007;4:443-86

2 Pogacic Kramp V, Herrling P. List of drugs in development for neurodegenerative diseases: update October 2011. Neurodegenerative Dis 2012;published online 30 December 2011; doi:10.1159/000335520

3 Scatena R, Martorana GE, Bottoni P, et al. An update on pharmacological approaches to neurodegenerative diseases. Expert Opin Investig Drugs 2007;16:59-72

4 Kwon MO, Herrling P. List of drugs in development for neurodegenerative diseases: update September 2005. Neurodegenerative Dis 2005;2:61-108

5 Stanzione P, Tropepi D. Drugs and clinical trials in neurodegenerative diseases. Ann Ist Super Sanita 2011;47:49-54

6 Forman MS, Trojanowski JQ, Lee VM. Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. Nat Med 2004;10:1055-63

7 Hampel H, Prvulovic D, Teipel S, et al. The future of Alzheimer's disease: the next 10 years. Prog Neurobiol 2011;95:718-28

• **A review about future research priorities for AD management.**

8 Tayeb HO, Yang HD, Price BH, Tarazi FI. Pharmacotherapies for Alzheimer's disease: beyond cholinesterase inhibitors. Pharmacol Ther 2012;134:8-25

• **A review on AD pathomechanism, targets and investigational agents.**

9 Citron M. Alzheimer's disease: strategies for disease modification. Nat Rev Drug Discov 2007;9:387-98

• **A review on AD targets for disease-modifying interventions.**

10 Fournier A, Oprisiu-Fournier R, Serot JM, et al. Prevention of dementia by antihypertensive drugs: how AT1-receptor-blockers and dihydropyridines better prevent dementia in hypertensive patients than thiazides and ACE-inhibitors. Expert Rev Neurother 2009;9: 1413-31

11 Paris D, Bachmeier C, Patel N, et al. Selective antihypertensive dihydropyridines lower Abeta accumulation by targeting both the production and the clearance of Abeta across the blood-brain barrier. Mol Med 2011;17:149-62

12 Kennelly S, Abdullah L, Kenny RA, et al. Apolipoprotein E genotype-specific short-term cognitive benefits of treatment with the antihypertensive nilvadipine in Alzheimer's patients-an open-label trial. Int J Geriatr Psychiatry 2012;27:415-22

13 Seo JS, Baek IS, Leem YH, et al. SK-PC-B70M alleviates neurologic symptoms in G93A-SOD1 amyotrophic lateral sclerosis mice. Brain Res 2011;1368:299-307

14 Bezprozvanny I. The rise and fall of dimebon. Drug News Perspect 2010;23(8):518-23

15 Sabbagh MN, Shill HA. Latrepirdine, a potential novel treatment for Alzheimer's disease and Huntington's chorea. Curr Opin Investig Drugs 2010;11:80-91

16 Naga KK, Geddes JW. Dimebon inhibits calcium-induced swelling of rat brain mitochondria but does not alter calcium retention or cytochrome C release. Neuromolecular Med 2011;13:31-6

17 Schliebs R, Arendt T. The cholinergic system in aging and neuronal degeneration. Behav Brain Res 2011;221:555-63

18 Kawamata J, Shimohama S. Stimulating nicotinic receptors trigger multiple pathways attenuating cytotoxicity in models of Alzheimer's and Parkinson's diseases. J Alzheimer Dis 2011;24:95-109

19 Wallace TL, Porter RHP. Targeting the nicotinic alpha7 acetylcholine receptor to enhance cognition in disease. Biochem Pharmacol 2011;82:891-903

20 Prickaerts J, van Goethem NP, Chesworth R, et al. EVP-6124, a novel and selective alpha7 nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of alpha7 nicotinic acetylcholine receptors. Neuropharmacology 2012;62:1099-110

21 Hilt D, Safirstein B, Hassman D, et al. EVP-6124: safety, tolerability and cognitive effects of a novel alpha7 nicotinic receptor agonist in Alzheimer's disease patients on stable donepezil or rivastigmine therapy. Alzheimer Dement 2009;5:e32

22 Wallace TL, Callahan PM, Tehim A, et al. RG3487, a novel nicotinic alpha7 receptor partial agonist, improves cognition and sensorimotor gating in rodents. J Pharmacol Exp Ther 2011;336:242-53

23 Wallace TL, Chiu G, Dao H, et al. R3487/MEM 3454, a novel nicotinic alpha 7 receptor partial agonist, improves attention and working memory performance in cynomolgus macaques. Biochem Pharmacol 2009;78:912

24 Hauser TA, Kucinski A, Jordan KG, et al. TC-5619: an alpha7 neuronal nicotinic receptor-selective agonist that demonstrates efficacy in animal models

of the positive and negative symptoms and cognitive dysfunction of schizophrenia. Biochem Pharmacol 2009;78:803-12

25 Gatto GJ, Bohme GA, Caldwell WS, et al. TC-1734: an orally active neuronal nicotinic acetylcholine receptor modulator with antidepressant, neuroprotective and long-lasting cognitive effects. CNS Drug Rev 2004;10:147-66

26 Frohlich L, Ashwood T, Nilsson J, Eckerwall G. Effects of AZD3480 on cognition in patients with mild-to-moderate Alzheimer's disease: a phase IIb dose-finding study. J Alzheimer Dis 2011;24:363-74

27 Panza F, Frisardi V, Solfrizzi V, et al. Interacting with gamma-secretase for treating Alzheimer's disease: from inhibition to modulation. Curr Med Chem 2011;18:5430-47

- **A comprehensive review of γ-secretase inhibitor/modulator approaches.**

28 Petterson M, Kauffman GW, am Ende CW, et al. Novel gamma-secretase modulators: a review of patents from 2008 to 2010. Expert Opin Ther Patents 2011;21:205-26

29 Hardy J. The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. J Neurochem 2009;110:1129-34

30 Gillman KW, Starrett JE, Parker MF, et al. Discovery and evaluation of BMS-708163, a potent, selective and orally bioavailable gamma-secretase inhibitor. ACS Med Chem Lett 2010;1:120-4

31 Albright C, Andreasen N, Minthon L, et al. The gamma-secretase inhibitor BMS-708163 causes dose-dependent reductions in CSF Abeta40 levels in patients with mild-to-moderate Alzheimer's disease (AD). Alzheimer Dement 2011;7:S311

32 Mitani Y, Yarimizu J, Saita K, et al. Differential effects between gamma-secretase inhibitors and modulators on cognitive function in amyloid precursor protein-transgenic and nontransgenic mice. J Neurosci 2012;32:2037-50

33 Cook JJ, Wildsmith KR, Gilberto DB, et al. Acute gamma-secretase inhibition of nonhuman primate CNS shifts amyloid precursor protein (APP) metabolism from amyloid-beta production to alternative APP fragments without amyloid-beta rebound. J Neurosci 2010;30:6743-50

34 Shih I, Wang T. Notch signalling, gamma-secretase inhibitors, and cancer therapy. Cancer Res 2007;67:1879-82

35 Pasinetti G, Rosen Z, Grossman H. Nic5-15: a novel natural gamma-secretase inhibitor that attenuates brain beta-amyloid content and improves cognition. Alzheimer Dement 2009;5:e28

36 Available from: www.qrpharma.com/presentation.html [Last accessed 15 March 2012]

37 Rogers JT, Mikkilineni S, Cantuti-Castelvetri I, et al. The alpha-synuclein 5'untranslated region targeted translation blockers: anti-alpha synuclein efficacy of cardiac glycosides and Posiphen. J Neural Transm 2011;118:493-507

38 Green KN, Khashwji H, Estrada T, Laferla FM. ST101 induces a novel 17kDa APP cleavage that precludes Abeta generation in vivo. Ann Neurol 2011;69:831-44

39 Kenche VB, Barnham KJ. Alzheimer's disease & metals: therapeutic opportunities. Br J Pharmacol 2011;163:211-19

40 Crouch PJ, Savva MS, Hung LW, et al. The Alzheimer's therapeutic PBT2 promotes amyloid beta-degradation and GSK3 phosphorylation via a metal chaperone activity. J Neurochem 2011;119:220-30

41 Faux NG, Ritchie CW, Gunn A, et al. PBT2 rapidly improves cognition in Alzheimer's disease: additional phase II analyses. J Alzheimer Dis 2010;20:509-16

42 Nguyen T, Hamby A, Massa SM. Clioquinol down-regulates mutant huntingtin expression in vitro and mitigates pathology in a Huntington's disease mouse model. Proc Natl Acad Sci USA 2005;102:11840-5

43 Fenili D, Brown M, Rappaport R, McLaurin J. Properties of scyllo–inositol as a therapeutic treatment of AD-like pathology. J Mol Med 2007;85:603-11

44 Fenili D, Weng Y, Aubert I, et al. Sodium/myo-inositol transporters: substrate transport requirements and regional brain expression in the TgCRND8 mouse model of amyloid pathology. PLoS ONE 2011;6:e24032

45 Salloway S, Sperling R, Keren R, et al. A phase 2 randomized trial of ELND005, scyllo-inositol, in mild to moderate Alzheimer disease. Neurology 2011;77:1253-62

46 Sabbagh MN, Agro A, Bell J, et al. PF-04494700, an oral inhibitor of receptor for advanced glycation end products (RAGE), in Alzheimer disease. Alzheimer Dis Assoc Disord 2011;25:206-12

47 Srikanth V, Maczurek A, Phan T, et al. Advanced glycation endproducts and their receptor RAGE in Alzheimer's disease. Neurobiol Aging 2011;32:763-77

48 Galasko DR, Van Dyck C, Sabbagh M, et al. A randomized clinical trial of an inhibitor of RAGE-Abeta interactions in patients with mild to moderate AD. J Nutr Health Aging 2011;15:S9

49 Available from: http://www.acimmune.com/content/?p=41 [Last accessed 17 February 2012]

50 De Filippis D, Cipriano M, Esposito G, et al. Are anti-angiogenic drugs useful in neurodegenerative disorders? CNS Neurol Disord Drug Targets 2010;9:807-12

51 Avramovich Y, Amit T, Youdim MBH. Non-steroidal anti-inflammatory drugs stimulate secretion of non-amyloidogenic precursor protein. J Biol Chem 2002;277:31466-73

52 Ryu JK, McLarnon JG. Thalidomide inhibition of perturbed vasculature and glial-derived tumor necrosis factor-alpha in an animal model of inflamed Alzheimer's disease brain. Neurobiol Dis 2008;29:254-66

53 Alkam T, Nitta A, Mizoguchi H. Restraining tumor necrosis factor-alpha by thalidomide prevents the amyloid beta-induced impairment of recognition memory in mice. Behav Brain Res 2008;189:100-6

54 Kiaei M, Petri S, Kipiani K, et al. Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis. J Neurosci 2006;26:2467-73

55 Fukushima T, Nakamura A, Iwakami N, et al. T-817MA, a neuroprotective agent, attenuates the motor and cognitive impairments associated with neuronal degeneration in P301L tau transgenic mice. Biochem Biophys Res Commun 2011;407:730-4

56 Kawasaki T, Ago Y, Kitao T, et al. A neuroprotective agent, T-817MA (1-{3-[2-(1-benzothiophen-5-yl)ethoxy] propyl} azetidin-3-ol maleate), prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity in mice. Neuropharmacology 2008;55:654-60

57 Esbenshade TA, Browman KE, Bitner RS, et al. The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. Br J Pharmacol 2008;154:1166-81

58 Brioni JD, Esbenshade TA, Garrison TR, et al. Discovery of histamine H3 antagonists for the treatment of cognitive disorders and Alzheimer's disease. J Pharmacol Exp Ther 2011;336:38-46

59 Kuhne S, Wijtmans M, Lim HD, et al. Several down, a few to go: histamine H3 receptor ligands making the final push towards the market? Expert Opin Investig Drugs 2011;20:1629-48

60 Gallant M, Aspiotis R, Day S, et al. Discovery of MK-0952, a selective PDE4 inhibitor for the treatment of long-term memory loss and mild cognitive impairment. Bioorg Med Chem Lett 2010;20:6387-93

61 Halene TB, Siegel SJ. PDE inhibitors in psychiatry – future options for dementia, depression and schizophrenia? Drug Discov Today 2007;12:870-8

62 Cheng Y, Wang C, Lin H, et al. Inhibition of phosphodiesterase-4 reverses memory deficits produced by Abeta25–35 or Abeta1–40 peptide in rats. Psychopharmacology 2010;212:181-91

63 Yang L, Calingasan NY, Lorenzo BJ, Beal MF. Attenuation of MPTP neurotoxicity by rolipram, a specific inhibitor of phosphodiesterase IV. Exp Neurol 2008;211:311-14

64 Li Y, Cheng Y, Huang Y, et al. Phosphodiesterase-4D knock-out and RNA interference-mediated knock-down enhance memory and increase hippocampal neurogenesis via increased cAMP signaling. J Neurosci 2011;31:172-83

65 Sengupta R, Sun T, Warrington NM, Rubin JB. Treating brain tumors with PDE4 inhibitors. Trends Pharmacol Sci 2011;32:337-44

66 Drott J, Desire L, Drouin D, et al. Etazolate improves performance in a foraging and homing task in aged rats. Eur J Pharmacol 2010;634:95-100

67 Lichtenthaler SF. Alpha-secretase in Alzheimer's disease: molecular identity, regulation and therapeutic potential. J Neurochem 2011;116:10-21

68 Vellas B, Sol O, Snyder PJ, et al. EHT0202 in Alzheimer's disease: a 3-month, randomized, placebo-controlled, double-blind study. Curr Alzheimer Res 2011;8:203-12

69 Dudas B, Hanin I, Rose M, Wulfert E. Protection against inflammatory neurodegeneration and glial cell death by 7beta-hydroxy epiandrosterone, a novel neurosteroid. Neurobiol Dis 2004;15:262-8

70 Youdim MB, Bar Am O, Yogev-Falach M, et al. Rasagiline: neurodegeneration, neuroprotection, and mitochondrial permeability transition. J Neurosci Res 2005;79:172-9

71 Bar-Am O, Amit T, Weinreb O, et al. Propargylamine containing compounds as modulators of proteolytic cleavage of amyloid-beta protein precursor: involvement of MAPK and PKC activation. J Alzheimer Dis 2010;21:361-71

72 Weinreb O, Amit T, Bar-Am O, et al. The neuroprotective mechanism of action of the multimodal drug ladostigil. Front Biosci 2008;13:5131-7

73 Gozes I. NAP (Davunetide) provides functional and structural neuroprotection. Curr Pharm Des 2011;17:1040-4

74 Shiryaev N, Jouroukhin Y, Giladi E, et al. NAP protects memory, increases soluble tau and reduces tau hyperphosphorylation in a tauopathy model. Neurobiol Dis 2009; 34:381-8

75 Fleming SM, Mulligan CK, Richter F, et al. A pilot trial of the microtubule-interacting peptide (NAP) in mice overexpressing alpha-synuclein shows improvement in motor function and reduction of alpha-synuclein inclusions. Mol Cell Neurosci 2011;46:597-606

76 Available from: www.allontherapeutics.com/product-development/davunetide/ [Last accessed 20 March 2012]

77 Schirmer RH, Adler H, Pickhardt M. Mandelkow E. "Lest we forget you-methylene blue...". Neurobiol Aging 2011;32:e7-e16

78 Mayer SC, Kreft AF, Harrison B, et al. Discovery of begacestat, a notch-1-sparing gamma-secretase inhibitor for the treatment of Alzheimer's disease. Curr Med Chem 2011;18:5430-47

79 Martone RL, Zhou H, Atchison K, et al. Begacestat (GSI-953): a novel, selective thiophene sulfonamide inhibitor of amyloid precursor protein gamma-secretase for the treatment of Alzheimer's disease. J Pharmacol Exp Ther 2009;331:598-608

80 Schroeter S, Brighma E, Motter R, et al. APP-selective gamma-secretase inhibitor ELND006 effects on brain parenchymal and vascular amyloid-beta in the PDAPP mouse model of Alzheimer's disease. Alzheimer Dement 2010;6:S546

81 Brigham E, Quinn K, Motter R, et al. Effects of single and multiple dose oral administration of ELND006, a novel APP-selective gamma-secretase inhibitor, on amyloid-beta concentrations in the brain and CSF of cynomolgus monkeys. Alzheimer Dement 2010;6:S546-7

82 Hopkins CR. ACS chemical neuroscience molecule spotlight on ELND006: another gamma-secretase inhibitor fails in the clinic. ACS Chem Neurosci 2011;2:279-80

83 Oehlrich D, Berthelot DJ, Gijsen HJM. gamma-Secretase modulators as potential disease modifying anti-Alzheimer's drugs. J Med Chem 2011;54:669-98

84 Nagy C, Schuck E, Ishibashi A, et al. E2012, a novel gamma-secretase modulator, decreases plasma amyloid-beta (Abeta) levels in humans. Alzheimer Dement 2010;6:S574

85 Portelius E, Van Broeck B, Andreasson U, et al. Acute effect on the Abeta isoform pattern in CSF in response to gamma-secretase modulator and inhibitor treatment in dogs. J Alzheimer Dis 2010;21:1005-12

86 Available from: http://www.envivopharma.com/news-item.php?id=28 [Last accessed 07 March 2012]

87 Imbimbo BP, Giardino L, Sivilia S, et al. CHF5074, a novel gamma-secretase modulator, restores hippocampal

neurogenesis potential and reverses contextual memory deficit in a transgenic mouse model of Alzheimer's disease. J Alzheimer Dis 2010;20:159-73

88 Lanzillotta A, Sarnico I, Benarese M, et al. The gamma-secretase modulator CHF5074 reduces the accumulation of native hyperphosphorylated tau in a transgenic mouse model of Alzheimer's disease. J Mol Neurosci 2011;45:22-31

89 Balducci C, Mehdawy B, Mare L, et al. The gamma-secretase modulator CHF5074 restores memory and hippocampal synaptic plasticity in plaque-free Tg2576 mice. J Alzheimer Dis 2011;24:799-816

90 Available from: http://gntpharma.com/web/bbs/board.php?bo_table=eb_0302&sca=AAD-2004 [Last accessed 17 February 2012]

91 Shin JH, Lee YA, Lee JK, et al. AAD-2004, a potent spin trapping molecule and microsomal prostaglandin E synthase-1 inhibitor, shows safety and efficacy in a mouse model of ALS. Nature Precedings 2010. Available from: http://hdl.handle.net/10101/npre.2010.5237.1 [Last accessed 17 February 2012]

92 Ghosh AK, Brindisi M, Tang J. Developing beta-secretase inhibitors for treatment of Alzheimer's disease. J Neurochem 2012;120:71-83

93 Yu J, Koelsch G, Li A, et al. In vivo efficacy of BACE-1 inhibitor CTS21166 (ASP1702) in rat CNS compartments. Alzheimer Dement 2009;5:P430-1

94 Yu J, Koelsch G, Li A, et al. Real-time microdialysis for Abeta inhibition and PK/PD analysis in a nonhuman primate model: in vivo efficacy of a BACE-1 inhibitor. Alzheimer Dement 2010;6:E73

95 Aisen PS, Gauthier S, Ferris SH, et al. Tramiprosate in mild-to-moderate Alzheimer's disease – a randomized, double-blind, placebo-controlled, multi-centre study (the Alphase Study). Arch Med Sci 2011;7:102-11

96 Gandhi NS, Mancera RL. Heparin/heparan sulphate-based drugs. Drug Discov Today 2010;15:1058-69

97 Available from: http://www.noscira.com/investigacion.cfm?mS=228&mSS=252 [Last accessed 07 March 2012]

98 Galdeano C, Viayna E, Arroyo P, et al. Structural determinants of the multifunctional profile of dual binding site acetylcholinesterase inhibitors as anti-Alzheimer agents. Curr Pharm Des 2010;16:2818-36

99 Snow AD, Cummings JA, Lake TP, et al. P4-319: development of exebryl-1: a disease modifying small molecule therapeutic that causes a marked clearance of brain amyloid load and improved memory in transgenic mouse models of Alzheimer's disease. Alzheimer Dement 2006;2:S610

100 Hu Q, Cam J, Lake T, et al. Identification of Exebryl-1® and other novel small molecules as tau protein aggregation inhibitors. Alzheimer Dement 2011;7:S481

101 Snow A, Cummings J, Lake T, et al. Exebryl-1: a novel small molecule currently in human clinical trials as a disease-modifying drug for the treatment of Alzheimer's disease. Alzheimer Dement 2009;5:P418

102 Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. J Neurochem 2008;104:1433-9

103 Dominguez JM, Fuertes A, Orozco L, et al. Evidence for irreversible inhibition of glycogen synthase kinase-3beta by tideglusib. J Biol Chem 2012;287:893-904

104 Bolos M, Fernandez S, Torres-Aleman I. Oral administration of a GSK3 inhibitor increases brain insulin-like growth factor I levels. J Biol Chem 2010;285:17693-700

105 Madsen K, Neumann WJ, Holst K, et al. Cerebral serotonin 4 receptors and amyloid-beta in early Alzheimer's disease. J Alzheimers Dis 2011;26:457-66

106 Lezoualc'h F. 5-HT4 receptor and Alzheimer's disease: the amyloid connection. Exp Neurol 2007;205:325-9

107 Shen F, Smith JA, Chang R, et al. 5-HT4 receptor agonist mediated enhancement of cognitive function in vivo and amyloid precursor protein processing in vitro: a pharmacodynamic and pharmacokinetic assessment. Neuropharmacology 2011;61:69-79

108 Available from: http://www.raqualia.com/product/5-ht4.html [Last accessed 20 February 2012]

109 Buchholz M, Hamann A, Aust S, et al. Inhibitors for human glutaminyl cyclase by structure based design and bioisosteric replacement. J Med Chem 2009;52:7069-80

110 Schilling S, Zeitschel U, Hoffmann T, et al. Glutaminyl cyclase inhibition attenuates pyroglutamate Abeta and Alzheimer's disease–like pathology. Nat Med 2008;14:1106-11

111 Alexandru A, Jagla W, Graubner S, et al. Selective hippocampal neurodegeneration in transgenic mice expressing small amounts of truncated Abeta is induced by pyroglutamate–Abeta formation. J Neurosci 2011;31:12790-801

112 Jiang Q, Heneka M, Landreth GE. The role of peroxisome proliferator-activated receptor-gamma (PPARgamma) in Alzheimer's disease. Therapeutic implications. CNS Drugs 2008;22:1-14

113 Yamanaka M, Kurzwelly D, Reyes E, Heneka M. PPAR gamma/RXR induced and CD36-mediated Abeta phagocytosis results in cognitive improvement in APP/PS1 mice. Alzheimer Dement 2011;7:S401

114 Available from: http://www.transitiontherapeutics.com/media/news.php [Last accessed 20 February 2012]

115 Maurice T, Su TP. The pharmacology of sigma-1 receptors. Pharmacol Ther 2009;124:195-206

116 Su TP, Hyashi T, Maurice T, et al. The sigma-1 receptor chaperone as an inter-organelle signaling modulator. Trends Pharmacol Sci 2010;31:557-66

117 Marrazzo A, Caraci F, Salinaro ET, et al. Neuroprotective effects of sigma-1 receptor agonists against beta-amyloid-induced toxicity. Neuroreport 2005;16:1223-6

118 Villard V, Espallergues J, Keller E, et al. Anti-amnesic and neuroprotective potentials of the mixed muscarinic receptor/sigma 1 (tau1) ligand ANAVEX2-73, a novel aminotetrahydrofuran derivative. J Psychopharmacol 2011;25:1101-17

119 Available from: www.anavex.com/publications.html [Last accessed 20 March 2012]

120 Luo W, Rodina A, Chiosis G. Heat shock protein 90: translation from cancer to Alzheimer's disease treatment? BMC Neurosci 2008;9:S7

121 Taldone T, Sun W, Chiosis G. Discovery and development of heat shock protein 90 inhibitors. Bioorg Med Chem 2009;17:2225-35

122 Wang L, Xie C, Greggio E, et al. The chaperone activity of heat shock protein 90 is critical for maintaining the stability of leucine-rich repeat kinase 2. J Neurosci 2008;28:3384-91

123 Dietz KC, Casaccia P. HDAC inhibitors and neurodegeneration: at the edge between protection and damage. Pharmacol Res 2010;62:11-17

124 Chuang D, Leng Y, Marinova Z, et al. Multiple roles of HDAC inhibition in neurodegenerative conditions. Trend Neurosci 2009;32:591-601

125 Patzke H, Albayya FP, Besterman JM, et al. Development of the novel histone deacetylase inhibitor EVP-0334 for CNS indications. 2008. p. 831.21/I12

126 Leventhal L, Tran A, Gallager I, et al. The histone deacetylase inhibitor EVP-0334 is pro-cognitive in mice. 2008. p. 831.20/I11

127 Lynch G. Memory enhancement: the search for mechanism-based drugs. Nat Neurosci 2002;5:1035-8

128 Lauterborn JC, Pineda E, Chen LY, et al. Ampakines cause sustained increases in brain-derived neurotrophic factor signaling at excitatory synapses without changes in AMPA receptor subunit expression. Neuroscience 2009;159:283-95

129 Gallagher DA, Schapira AHV. Etiopathogenesis and treatment of Parkinson's disease. Curr Top Med Chem 2009;9:860-8

130 Meissner WG, Frasier M, Gasser T, et al. Priorities in Parkinson's disease research. Nat Rev Drug Discov 2011;10:377-93

• **A review about future research priorities for PD management.**

131 Szabo N, Kincses ZT, Vecsei L. Novel therapy in Parkinson's disease: adenosine A(2A) receptor antagonists. Expert Opin Drug Metab Toxicol 2011;7:441-55

132 Jenner P, Mori A, Hauser R, et al. Adenosine, adenosine A2A antagonists, and Parkinson's disease. Parkinsonism Relat Disord 2009;15:406-13

133 Armentero MT, Pinna A, Ferre S, et al. Past, present and future of A2A adenosine receptor antagonists in the therapy of Parkinson's disease. Pharmacol Ther 2011;132:280-99

134 Park A, Stacy M. Istradefylline for the treatment of Parkinson's disease. Expert Opin Pharmacother 2012;13:111-14

135 Muller CE, Jacobson KA. Recent developments in adenosine receptor ligands and their potential as novel drugs. Biochim Biophys Acta 2011;1808:1290-308

136 Hodgson RA, Bertorelli R, Varty GB, et al. Characterization of the potent and highly selective A2A receptor antagonists Preladenant and SCH 412348 [7-[2-[4-2,4-difluorophenyl]-1-piperazinyl]ethyl]-2-(2-furanyl)-7H-pyrazolo[4,3-e][1,2,4] triazolo[1,5-c]pyrimidin-5-amine] in rodent models of movement disorders and depression. J Pharmacol Exp Ther 2009;330:294-303

137 Hauser RA, Cantillon M, Pourcher E, et al. Preladenant in patients with Parkinson's disease and motor fluctuations: a phase 2, double-blind, randomised trial. Lancet Neurol 2011;10:221-9

138 Schapira AHV. Molecular and clinical pathways to neuroprotection of dopaminergic drugs in Parkinson disease. Neurology 2009;72:S44-50

139 Jones CA, Johnston LC, Jackson MJ, et al. An in vivo pharmacological evaluation of pardoprunox (SLV308) - a novel combined dopamine D2/D3 receptor partial agonist and 5-HT1A receptor agonist with efficacy in experimental models of Parkinson's disease. Eur Neuropsychopharmacol 2010;20:582-93

140 Sampaio C, Bronzova J, Hauser RA, et al. Pardoprunox in early Parkinson's disease: results from 2 large, randomized double-blind trials. Mov Disord 2011;26:1464-76

141 Sian-Hulsmann J, Mandel S, Youdim MBH, Riederer P. The relevance of iron in the pathogenesis of Parkinson's disease. J Neurochem 2011;118:939-57

142 Molina-Holgado F, Gaeta A, Francis PT, et al. Neuroprotective actions of deferiprone in cultured cortical neurones and SHSY-5Y cells. J Neurochem 2008;105:2466-76

143 Dexter DT, Statton SA, Whitmore C, et al. Clinically available iron chelators induce neuroprotection in the 6-OHDA model of Parkinson's disease after peripheral administration. J Neural Transm 2011;118:223-31

144 Velasco-Sanchez D, Aracil A, Montero R, et al. Combined therapy with idebenone and deferiprone in patients with Friedreich's ataxia. Cerebellum 2011;10:1-8

145 Caccia C, Maj R, Calabresi M, et al. Safinamide: from molecular targets to a new anti-Parkinson drug. Neurology 2006;67:S18-23

146 Gregoire L, Roach A, Di Paolo T. Safinamide reduces levodopa-induced dyskinesia in MPTP lesioned primates while prolonging anti-parkinsonian efficacy. Mov Disord 2010;25:S411-12

147 Anand R, Borgohain R, Bhatt M, et al. Long-term efficacy of safinamide as add-on to levodopa in Parkinson's disease (PD) using an 'ON' and 'ON-OFF' treatment analysis. Parkinsonism Relat Disord 2012;18:S132

148 Borgohain R, Mehta NA, Bajenaru OA, et al. Effect of safinamide on depressive symptoms in patients with mid-late stage Parkinson's disease. Mov Disord 2010;25:S291

149 Anand R, Borgohain R, Stocchi F, et al. First 2-year, placebo-controlled study in Parkinson's disease patients with motor fluctuations indicates safinamide may benefit patients with more severe dyskinesia. Parkinsonism Relat Disord 2012;18:S132-3

150 Black KJ, Koller JM, Campbell MC, et al. Quantification of indirect pathway inhibition by the adenosine A2a antagonist SYN115 in Parkinson disease. J Neurosci 2010;30:16284-92

151 Brooks DJ, Papapetropoulos S, Vandenhende F, et al. An open-label, positron emission tomography study to assess adenosine A2A brain receptor occupancy of vipadenant (BIIB014) at steady-state levels in healthy male volunteers. Clin Neuropharmacol 2010;33:55-60

152 Zhang Y, Xia Z, Hua Y, et al. Role of glial cell derived neurotrophic factor in the protective effect of smilagenin on rat mesencephalic dopaminergic neurons damaged by MPP+. FEBS Lett 2008;582:956-60

153 Visanji NP, Orsi A, Johnston TH, et al. PYM50028, a novel, orally active, nonpeptide neurotrophic factor inducer, prevents and reverses neuronal damage induced by MPP+ in mesencephalic neurons and by MPTP in a mouse

model of Parkinson's disease. FASEB J 2008;22:2488-97

154 Available from: http://www.phytopharm.co.uk/index.php?option=com_content&view=article&id=51&Itemid=37 [Last accessed 20 March 2012]

155 Hu Y, Wang Z, Zhang R, et al. Regulation of M1-receptor mRNA stability by smilagenin and its significance in improving memory of aged rats. Neurobiol Aging 2010;31:1010-19

156 Hadjiconstantinou M, Neff NH. GM1 ganglioside: in vivo and in vitro trophic actions on central neurotransmitter systems. J Neurochem 1998;70:1335-45

157 Wu G, Lu Z, Kulkarni N, et al. Mice lacking major brain gangliosides develop parkinsonism. Neurochem Res 2011;36:1706-14

158 Schneider JS, Sendek S, Daskalakis C, Cambi F. GM1 ganglioside in Parkinson's disease: results of a five year open study. J Neurol Sci 2010;292:45-51

159 Available from: http://eng.skbp.com/index.asp [Last accessed 19 February 2012]

160 Kincses ZT, Vecsei L. Pharmacological therapy in Parkinson's disease: focus on neuroprotection. CNS Neurosci Ther 2011;17:345-67

161 Szabo N, Kincses ZT, Toldi J, Vecsei L. Altered tryptophan metabolism in Parkinson's disease: a possible novel therapeutic approach. J Neurol Sci 2011;310:256-60

162 Glicksman MA. The preclinical discovery of amyotrophic lateral sclerosis drugs. Expert Opin Drug Discov 2011;6:1127-38

163 Habib AA, Mitsumoto H. Emerging drugs for amyotrophic lateral sclerosis. Expert Opin Emerg Drugs 2011;16:537-58

164 Bordet T, Berna P, Abitbol J, Pruss RM. Olesoxime (TRO19622): a novel mitochondrial-targeted neuroprotective compound. Pharmaceuticals 2010;3:345-68

165 Duffy LM, Chapman AL, Shaw PJ, Grierson AJ. The role of mitochondria in the pathogenesis of amyotrophic lateral sclerosis. Neuropathol Appl Neurobiol 2011;37:336-52

166 Sunyach C, Michaud M, Arnoux T, et al. Olesoxime delays muscle denervation, astrogliosis, microglial activation and motoneuron death in an ALS mouse model. Neuropharmacology 2012;62:2346-53

167 Phukan J. Arimoclomol, a coinducer of heat shock proteins for the potential treatment of amyotrophic lateral sclerosis. IDrugs 2010;13:482-96

168 Kieran D, Kalmar B, Dick JR, et al. Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. Nat Med 2004;10:402-5

169 Kalmar B, Novoselov S, Gray A, et al. Late stage treatment with arimoclomol delays disease progression and prevents protein aggregation in the SOD1 mouse model of ALS. J Neurochem 2008;107:339-50

170 Cudkowicz ME, Shefner JM, Simpson E, et al. Arimoclomol at dosages up to 300 mg/day is well tolerated and safe in amyotrophic lateral sclerosis. Muscle Nerve 2008;38:837-44

171 Gribkoff VK, Bozik ME. KNS-760704 [(6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine dihydrochloride monohydrate] for the treatment of amyotrophic lateral sclerosis. CNS Neurosci Ther 2008;14:215-26

172 Alavian KN, Dworetzky SI, Bonanni L, et al. Effects of dexpramipexole on brain mitochondrial conductances and cellular bioenergetic efficiency. Brain Res 2012;1446:1-11

173 Cudkowicz M, Bozik ME, Ingersoll EW, et al. The effects of dexpramipexole (KNS-760704) in individuals with amyotrophic lateral sclerosis. Nat Med 2011;17:1652-6

174 Ito H, Wate R, Zhang J, et al. Treatment with edaravone, initiated at symptom onset, slows motor decline and decreases SOD1 deposition in ALS mice. Exp Neurol 2008;213:448-55

175 Orrell RW. AEOL-10150 (Aeolus). Curr Opin Investig Drugs 2006;7:70-80

176 Crow JP, Calingasan NY, Chen J, et al. Manganese porphyrin given at symptom onset markedly extends survival of ALS mice. Ann Neurol 2005;58:258-65

177 Batinic-Haberle I, Spasojevic I, Tse HM, et al. Design of Mn porphyrins for treating oxidative stress injuries and their redox-based regulation of cellular transcriptional activities. Amino Acids 2012;42:95-113

178 Hersch SM, Rosas HD. Neuroprotection for Huntington's disease: ready, set, slow. Neurotherapeutics 2008;5:226-36

179 Stack EC, Ferrante RJ. Huntington's disease: progress and potential in the field. Expert Opin Investig Drugs 2007;16:1933-53

180 Murck H, Manku M. Ethyl-EPA in Huntington disease – potentially relevant mechanism of action. Brain Res Bull 2007;72:159-64

181 Puri BK, Leavitt BR, Hayden MR, et al. Ethyl-EPA in Huntington disease: a double-blind, randomized, placebo-controlled trial. Neurology 2005;65:286-92

182 Huntington Study Group TREND-HD Investigators. Randomized controlled trial of ethyl-eicosapentaenoic acid in Huntington disease: the TREND-HD study. Arch Neurol 2008;65:1582-9

183 Pallos J, Bodai L, Lukacsovich T, et al. Inhibition of specific HDACs and sirtuins suppresses pathogenesis in a drosophila model of Huntington's disease. Hum Mol Gen 2008;17:3767-75

184 Sadri-Vakili G, Cha JH. Histone deacetylase inhibitors: a novel therapeutic approach to Huntington's disease (complex mechanism of neuronal death). Curr Alzheimer Res 2006;3:403-8

185 Jeong H, Cohen DE, Cui L, et al. Sirt1 mediates neuroprotection from mutant huntingtin by activation of the TORC1 and CREB transcriptional pathway. Nat Med 2012;18:159-65

186 Jiang M, Wang J, Fu J, et al. Neuroprotective role of Sirt1 in mammalian models of Huntington's disease through activation of multiple Sirt1 targets. Nat Med 2012;18:153-8

187 Cen Y. Sirtuins inhibitors: the approach to affinity and selectivity. Biochim Biophys Acta 2010;1804:1635-44

188 Lavu S, Boss O, Elliott PJ, Lambert PD. Sirtuins - novel therapeutic targets to treat age-associated diseases. Nat Rev Drug Discov 2008;7:841-53

189 Gibrat C, Cicchetti F. Potential of cystamine and cysteamine in the treatment of neurodegenerative diseases. Prog Neuro Psychopharmacol Biol Psychol 2011;35:380-9

190 Williams P, Sorribas A, Howes MR. Natural products as a source of Alzheimer's drug leads. Nat Prod Rep 2011;28:48-77

191 Joyner PM, Cichewicz RH. Bringing natural products into the fold – exploring the therapeutic lead potential of secondary metabolites for the treatment of protein-misfolding-related neurodegenerative diseases. Nat Prod Rep 2011;28:26-47

192 Georgiou NA, Garssen J, Witkamp RF. Pharma–nutrition interface: the gap is narrowing. Eur J Pharmacol 2011;651:1-8

193 Geldenhuys WJ, Youdim MBH, Carroll RT, Van der Schyf CJ. The emergence of designed multiple ligands for neurodegenerative disorders. Prog Neurobiol 2011;94:347-59

194 Morphy R, Rankovic Z. Designing multiple ligands – medicinal chemistry strategies and challenges. Curr Pharm Des 2009;15:587-600

195 Kitano H. A robustness-based approach to systems-oriented drug design. Nat Rev Drug Discov 2007;6:202-10

196 Cavalli A, Bolognesi ML, Minarini A, et al. Multi-target-directed ligands to combat neurodegenerative diseases. J Med Chem 2008;51:347-72

•• **A comprehensive review of single drug - multi-target approaches for neurodegenerative diseases; design principles, mechanisms of actions.**

197 Bolognesi ML, Minarini A, Rosini M, et al. From dual binding site acetylcholinesterase inhibitors to multi-target-directed ligands (MTDLs): a step forward in the treatment of Alzheimer's disease. Mini Rev Med Chem 2008;8:960-7

198 Leon R, Marco-Contelles J. A step further towards multitarget drugs for Alzheimer and neuronal vascular diseases: targeting the cholinergic system, amyloid-beta aggregation and Ca2+ dyshomeostasis. Curr Med Chem 2011;18:552-76

199 Bajda M, Guzior N, Ignasik M, Malawska B. Multi-target-directed ligands in Alzheimer's disease treatment. Curr Med Chem 2011;18:4949-75

200 Rampa A, Belluti F, Gobbi S, Bisi A. Hybrid-based multi-target ligands for the treatment of Alzheimer's disease. Curr Top Med Chem 2011;11:2716-30

201 Bolognesi ML, Cavalli A, Melchiorre C. Memoquin: a multi-target–directed ligand as an innovative therapeutic opportunity for Alzheimer's disease. Neurotherapeutics 2009;6:152-62

202 Pisani L, Catto M, Leonetti F, et al. Targeting monoamine oxidases with multipotent ligands: an emerging strategy in the search of new drugs against neurodegenerative diseases. Curr Med Chem 2011;18:4568-87

• **A recent review of MAO inhibitor based approaches for the design of multi-target ligands for neurodegenerative diseases.**

203 Youdim MBH. M30, a brain permeable multi target neurorestorative drug in post nigrostriatal dopamine neuron lesion of parkinsonism animal models. Parkinsonism Relat Disord 2012;18:S151-4

204 Mandel S, Amit T, Bar-Am O, Youdim MBH. Iron dysregulation in Alzheimer's disease: multimodal brain permeable iron chelating drugs, possessing neuroprotective-neurorescue and amyloid precursor protein-processing regulatory activities as therapeutic agents. Prog Neurobiol 2007;82:348-60

205 Zheng H, Youdim MB, Fridkin M. Site-activated chelators targeting acetylcholinesterase and monoamine oxidase for Alzheimer's therapy. ACS Chem Biol 2010;5:603-10

206 Petzer JP, Castagnoli N, Schwarzschild MA, et al. Dual-target–directed drugs that block monoamine oxidase B and adenosine A2A receptors for Parkinson's disease. Neurotherapeutics 2009;6:141-51

207 Becker RE, Greig NH, Giacobini E. Why do so many drugs for Alzheimer's disease fail in development? Time for new methods and new practices? J Alzheimer Dis 2008;15:303-25

•• **A review on clinical trial related challenges and shortcomings, particularly for AD studies.**

208 Mangoni AA, Jackson SHD. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. Br J Clin Pharmacol 2004;57:6-14

Affiliation

Petra Dunkel[1], Christina LL Chai[2,3], Beáta Sperlágh[4], Paul B Huleatt[2] & Péter Mátyus,[†1,5]

[†]Author for correspondence

[1]Semmelweis University, Department of Organic Chemistry, Hőgyes Endre utca 7, 1094 Budapest, Hungary

[2]Institute of Chemical and Engineering Sciences, 8 Biomedical Grove, Neuros, #07-01, Singapore 138665, Singapore

[3]National University of Singapore, Department of Pharmacy, 18 Science Drive 4, Singapore 117543, Singapore

[4]Institute of Experimental Medicine of the Hungarian Academy of Sciences, Szigony utca 43, 1083 Budapest, Hungary

[5]Semmelweis University, Drug Discovery and Safety Centre, Högyes Endre utca 7, 1094 Budapest, Hungary

Tel: +36 1 2170851;

Fax: +36 1 2170851;

E-mail: matyus.peter@pharma.semmelweis-univ.hu