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

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†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


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.
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-1H-inden-1-amine) is close to being the first such drug; however, a disease-modifying indication 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.

Article highlights.

- Neurodegenerative diseases might affect nearly 25 million people worldwide. 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 simultaneously, 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.

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 misfolding/misassembly, neuroinflammation, oxidative stress, ubiquitin/proteasomal dysfunction, disrupted intracellular transport, (contiguous) 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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Mechanism of action</th>
<th>Investigator</th>
<th>Phase (indication)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAD-2004</td>
<td><img src="image" alt="AAD-2004 Structure" /></td>
<td>Spin trapping Microsomal prostaglandin E synthase-1 inhibitor</td>
<td>GNT Pharma Co. Ltd</td>
<td>I (ALS, PD, AD)</td>
</tr>
<tr>
<td>ABT-126</td>
<td>NA</td>
<td>α2nAChR agonist</td>
<td>Abbott Laboratories</td>
<td>II (AD)</td>
</tr>
<tr>
<td>ACI-91</td>
<td><img src="image" alt="ACI-91 Structure" /></td>
<td>BACE1 modulator</td>
<td>AC Immune SA</td>
<td>II (AD)</td>
</tr>
</tbody>
</table>

*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).

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</tr>
</thead>
<tbody>
<tr>
<td>AEOL-10150</td>
<td></td>
<td>Metalloporphyrin catalytic antioxidant</td>
<td>Aeolus Pharmaceuticals, Inc.</td>
<td>I (ALS)*</td>
</tr>
<tr>
<td>AMR-101</td>
<td></td>
<td>Lipid bi-layer replenisher</td>
<td>Amarin Corp plc</td>
<td>III (HD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-apoptotic Mitochondrial integrity stabilizer</td>
<td></td>
<td></td>
</tr>
</tbody>
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<tbody>
<tr>
<td>ANAVEX-2-73</td>
<td><img src="image" alt="ANAVEX-2-73 Structure" /></td>
<td>Mixed muscarinic receptor/α-1 ligand</td>
<td>Anavex Life Sciences Corp</td>
<td>I (AD)</td>
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<tr>
<td>ARC-031</td>
<td>NA</td>
<td>Soluble amyloid reducing/clearing agent</td>
<td>Archer Pharmaceuticals, Inc.</td>
<td>I (AD)</td>
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<tr>
<td>Arimoclomol (BRX-220)</td>
<td><img src="image" alt="Arimoclomol Structure" /></td>
<td>Hsp coinducer</td>
<td>Orphazyme ApS</td>
<td>III (ALS)</td>
</tr>
<tr>
<td>AV-101</td>
<td><img src="image" alt="AV-101 Structure" /></td>
<td>NMDA receptor antagonist</td>
<td>VistaGen Therapeutics, Inc.</td>
<td>I (PD)</td>
</tr>
<tr>
<td>Avagacestat (BMS-708163)</td>
<td><img src="image" alt="Avagacestat Structure" /></td>
<td>γ-secretase inhibitor</td>
<td>Bristol-Myers Squibb Co.</td>
<td>II (AD)</td>
</tr>
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<tr>
<td>AZD-1446 (TC-6683)</td>
<td>NA</td>
<td>α&lt;sub&gt;4&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;nAChR agonist</td>
<td>AstraZeneca plc/Targacept, Inc. II (AD)</td>
</tr>
<tr>
<td>Begacestat (GSI953, PF5212362)</td>
<td><img src="image" alt="Structure" /></td>
<td>γ-secretase inhibitor</td>
<td>Pfizer, Inc. I (AD)*</td>
</tr>
<tr>
<td>CHF5074</td>
<td><img src="image" alt="Structure" /></td>
<td>γ-secretase modulator</td>
<td>Chiesi Farmaceutici SpA II (AD)</td>
</tr>
<tr>
<td>CTS-21166</td>
<td>NA</td>
<td>BACE1 inhibitor</td>
<td>CoMentis, Inc./Astellas Pharma, Inc. I (AD)</td>
</tr>
<tr>
<td>Davunetide</td>
<td>NAPVSIPQ</td>
<td>Tau deposition inhibitor</td>
<td>Allon Therapeutics, Inc. I/II (AD, PD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microtubule stabilizer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amyloid protein deposition inhibitor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PARP stimulator</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GSK3 inhibitor</td>
<td></td>
</tr>
<tr>
<td>Deferiprone</td>
<td><img src="image" alt="Structure" /></td>
<td>Iron chelator</td>
<td>University Hospital, Lille IVIII (PD)</td>
</tr>
</tbody>
</table>

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

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<tbody>
<tr>
<td>Dexpramipexole (KNS-760704)</td>
<td><img src="image1" alt="Structure" /></td>
<td>Mitochondria stabilizer</td>
<td>Biogen Idec, Inc./ Knopp Neurosciences, Inc.</td>
<td>III (ALS)</td>
</tr>
<tr>
<td>Dimebon (latrepirdine)</td>
<td><img src="image2" alt="Structure" /></td>
<td>Pleiotropic neurotransmitter signaling actions</td>
<td>Pfizer, Inc./ Medivation, Inc.</td>
<td>III (AD)*</td>
</tr>
<tr>
<td>DSP-8658</td>
<td>NA</td>
<td>PPARα/γ modulator /β-amyloid deposition inhibitor</td>
<td>Dainippon Sumitomo Pharma Co. Ltd</td>
<td>I (AD)</td>
</tr>
<tr>
<td>E-2012</td>
<td><img src="image3" alt="Structure" /></td>
<td>γ-secretase modulator</td>
<td>Eisai Co. Ltd</td>
<td>I (AD)*</td>
</tr>
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<tbody>
<tr>
<td>E-2212* (presumed structure)</td>
<td></td>
<td>γ-secretase modulator</td>
<td>Eisai Co. Ltd</td>
<td>I (AD)</td>
</tr>
<tr>
<td>ELND-005 (AZD-103, cycl-inositol)</td>
<td></td>
<td>Aβ oligomer accumulation inhibitor</td>
<td>Elan Corp plc/Transition Therapeutics, Inc.</td>
<td>II (AD)</td>
</tr>
<tr>
<td>ELND-006</td>
<td>NA</td>
<td>γ-secretase inhibitor</td>
<td>Elan Corp plc</td>
<td>I (AD)*</td>
</tr>
<tr>
<td>Etazolate (EHT-0202)</td>
<td></td>
<td>GABA&lt;sub&gt;A&lt;/sub&gt; receptor modulator</td>
<td>Exonhit SA</td>
<td>II (AD)</td>
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<tr>
<td>EVP-0334</td>
<td>NA</td>
<td>Histone deacetylase inhibitor</td>
<td>EnVivo Pharmaceuticals, Inc.</td>
<td>I (AD, PD)</td>
</tr>
<tr>
<td>EVP-0962</td>
<td>NA</td>
<td>γ-secretase modulator</td>
<td>EnVivo Pharmaceuticals, Inc.</td>
<td>I (AD)</td>
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<tr>
<td>EVP-6124</td>
<td><img src="image1" alt="Structure" /></td>
<td>$\alpha_7nAChR$ partial agonist 5-HT$_3$ receptor antagonist</td>
<td>EnVivo Pharmaceuticals, Inc.</td>
<td>II (AD)</td>
</tr>
<tr>
<td>Exebryl-1</td>
<td>NA</td>
<td>$\beta$-amyloid formation/accumulation inhibitor Amyloid plaque deposition inhibitor</td>
<td>ProteoTech, Inc./Tasly Pharmaceutical Co. Ltd</td>
<td>I (AD)</td>
</tr>
<tr>
<td>GM1-ganglioside</td>
<td><img src="image2" alt="Structure" /></td>
<td>Neuronal plasma membrane constituent</td>
<td>Thomas Jefferson University</td>
<td>II (PD)</td>
</tr>
</tbody>
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</thead>
<tbody>
<tr>
<td>HF-0220</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Neuroprotective/cytoprotective (activation of 7-hydroxysteroid-driven neuroprotection pathways)</td>
<td>Newron Pharmaceuticals SpA</td>
<td>II (AD)</td>
</tr>
<tr>
<td>HPP-854</td>
<td>NA</td>
<td>BACE1 inhibitor</td>
<td>High Point Pharmaceuticals LLC</td>
<td>I (AD)</td>
</tr>
<tr>
<td>Ispronicline (TC-1734, AZD-3480)</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>(\alpha_4\beta_2) nAChR partial agonist</td>
<td>AstraZeneca plc</td>
<td>II (AD)</td>
</tr>
<tr>
<td>Ladostigil (TV-3326)</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>Acetylcholinesterase inhibitor / MAO-B inhibitor</td>
<td>Avraham Pharmaceuticals Ltd</td>
<td>II (AD)</td>
</tr>
<tr>
<td>MEM1414</td>
<td>NA</td>
<td>PDE4 inhibitor</td>
<td>Memory Pharmaceuticals Corp</td>
<td>II (AD)*</td>
</tr>
<tr>
<td>MEM3454 (RG3487, RO5313534)</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>(\alpha_7) nAChR partial agonist / 5-HT_3 receptor antagonist</td>
<td>Memory Pharmaceuticals Corp/ F. Hoffmann – La Roche Ltd</td>
<td>II (AD)*</td>
</tr>
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<tbody>
<tr>
<td>MEM-63908</td>
<td>NA</td>
<td>$\alpha_2$AChR partial agonist</td>
<td>Memory Pharmaceuticals Corp/F. Hoffmann-La Roche Ltd</td>
<td>I (AD)*</td>
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<tr>
<td>MK0249</td>
<td>NA</td>
<td>H$_3$ receptor inverse agonist</td>
<td>Merck &amp; Co., Inc.</td>
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<tr>
<td>MK0752</td>
<td><img src="image.png" alt="Structure" /></td>
<td>$\gamma$-secretase inhibitor</td>
<td>Merck &amp; Co., Inc.</td>
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<tr>
<td>MK0952</td>
<td><img src="image.png" alt="Structure" /></td>
<td>PDE$_4$ inhibitor</td>
<td>Merck &amp; Co., Inc.</td>
<td>II (AD)</td>
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<tbody>
<tr>
<td>Nilvadipine</td>
<td><img src="image1" alt="Nilvadipine Structure" /></td>
<td>Amyloid protein deposition inhibitor Calcium channel blocker</td>
<td>Archer Pharmaceuticals, Inc.</td>
<td>III (AD)</td>
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<tr>
<td>(ARC-029)</td>
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<td>NP-12 (tideglusib)</td>
<td><img src="image2" alt="NP-12 Structure" /></td>
<td>GSK3 inhibitor</td>
<td>Noscira SA</td>
<td>II (AD)</td>
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<tbody>
<tr>
<td>NP-61</td>
<td><img src="image" alt="NP-61" /></td>
<td>β-amyloid modulator Acetylcholinesterase inhibitor</td>
<td>Noscira SA</td>
<td>I (AD)</td>
</tr>
<tr>
<td>NRM-8499</td>
<td>NA</td>
<td>Amyloid protein deposition inhibitor</td>
<td>Bellus Health, Inc.</td>
<td>I (AD)</td>
</tr>
<tr>
<td>Pardoprunox (SLV-308)</td>
<td><img src="image" alt="Pardoprunox" /></td>
<td>D₂ and D₃ receptor partial agonist 5-HT₁A receptor full agonist</td>
<td>Abbott Laboratories</td>
<td>III (PD)</td>
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</tbody>
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<tbody>
<tr>
<td>PBT2</td>
<td>NA</td>
<td>Metal-protein attenuator</td>
<td>Prana Biotechnology Ltd</td>
<td>II (AD, HD)</td>
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<tr>
<td></td>
<td></td>
<td>Tau protein modulator</td>
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<tr>
<td></td>
<td></td>
<td>Amyloid protein deposition inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posiphen</td>
<td></td>
<td>APP/tau/α-synuclein synthesis inhibitor</td>
<td>QR Pharma, Inc.</td>
<td>I (AD)</td>
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<tr>
<td>PQ-912</td>
<td>NA</td>
<td>Glutaminyl cyclase inhibitor</td>
<td>Probiodrug AG</td>
<td>I (AD)</td>
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<tr>
<td>Preladenant</td>
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<td>Adenosine A2a receptor antagonist</td>
<td>Merck &amp; Co., Inc.</td>
<td>III (PD)</td>
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<tr>
<td>PU-H71</td>
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<td>Hsp90 inhibitor</td>
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<td>I (AD)</td>
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<tbody>
<tr>
<td>PYM50018</td>
<td>NA</td>
<td>Unspecified target</td>
<td>Phytopharm plc</td>
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<td>PYM50028</td>
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<td>GDNF/BDNF activator</td>
<td>Phytopharm plc</td>
<td>II (AD, PD)</td>
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<td>RG-1577</td>
<td>NA</td>
<td>MAO-B inhibitor</td>
<td>F. Hoffmann – La Roche Ltd</td>
<td>I (AD)</td>
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<tr>
<td>RG-7129</td>
<td>NA</td>
<td>β-amyloid synthesis inhibitor/ BACE1 inhibitor</td>
<td>F. Hoffmann – La Roche Ltd</td>
<td>I (AD)</td>
</tr>
<tr>
<td>RQ-00000009</td>
<td>NA</td>
<td>5-HT₄ partial agonist/ Ampakine/ BDNF modulator</td>
<td>RaQualia Pharma, Inc. Servier</td>
<td>I (AD)</td>
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<tr>
<td>S47445 (CX-1632)</td>
<td>NA</td>
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<tr>
<td>Safinamide</td>
<td><img src="image1" alt="Structure" /></td>
<td>MAO-B inhibitor Glutamate release inhibitor Dopamine reuptake inhibitor Sodium and calcium channel blocker</td>
<td>Merck &amp; Co., Inc.</td>
<td>III (PD)</td>
</tr>
<tr>
<td>Selisistat (SEN00141196/EX-527)</td>
<td><img src="image2" alt="Structure" /></td>
<td>SIRT1 inhibitor</td>
<td>Siena Biotech SpA</td>
<td>II (HD)</td>
</tr>
<tr>
<td>SK-PC-B70M</td>
<td><img src="image3" alt="Structure" /></td>
<td>β-amyloid modulator</td>
<td>SK Chemicals Life Science</td>
<td>III (AD)</td>
</tr>
</tbody>
</table>

*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).

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Mechanism of action</th>
<th>Investigator</th>
<th>Phase (indication)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKL-PD (YKP10461)</td>
<td>NA</td>
<td>MAO-B inhibitor (selective and reversible)</td>
<td>SK Biopharmaceuticals Co. Ltd</td>
<td>I (PD)</td>
</tr>
<tr>
<td>ST-101 (ZSET-1446)</td>
<td></td>
<td>Cholinergic modulator APP processing modulator</td>
<td>Sonexa Therapeutics, Inc./Zenyaku Kogyo Co. Ltd</td>
<td>II (AD)</td>
</tr>
<tr>
<td>SYN115 (tozadenant)</td>
<td><img src="https://via.placeholder.com/150" alt="" /></td>
<td>A2A receptor inhibitor</td>
<td>Biotie Therapies Corp/UCB Pharma</td>
<td>II (PD)</td>
</tr>
<tr>
<td>T-817MA</td>
<td><img src="https://via.placeholder.com/150" alt="" /></td>
<td>Neurotrophic modulator</td>
<td>Toyama Chemical Co. Ltd</td>
<td>II (AD)</td>
</tr>
</tbody>
</table>

*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).

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<th>Investigator</th>
<th>Phase (indication)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC5619</td>
<td><img src="TC5619" alt="Image" /></td>
<td>(\alpha_7 nAChR) agonist</td>
<td>Targecept, Inc.</td>
<td>II (AD)</td>
</tr>
<tr>
<td>Thalidomide</td>
<td><img src="Thalidomide" alt="Image" /></td>
<td>Immunomodulator Angiogenesis inhibitor</td>
<td>Celgene Corp</td>
<td>II (AD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNF-(\alpha /VEGF/ FGF-2) inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRO19622</td>
<td><img src="TRO19622" alt="Image" /></td>
<td>Mitochondrial pore modulator</td>
<td>Trophos SA</td>
<td>III (ALS)</td>
</tr>
<tr>
<td>(olesoxime)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT-301</td>
<td>NA</td>
<td>Anti-inflammatory (inhibits overproduction/ release of (pro) inflammatory cytokines (e.g., IL-1(\beta, TNF-(\alpha)) Macrophage and glial cell activation suppressor</td>
<td>Transition Therapeutics, Inc.</td>
<td>I (AD)</td>
</tr>
</tbody>
</table>

*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 Aβ1-42 [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 Aβ1-42 levels and plaque

Table 2. Selected investigational drugs with putative neuroprotective effect for Alzheimer’s disease/Parkinson’s disease/Huntington’s disease/amyotrophic lateral sclerosis (continued).
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-HT7, 5-HT6, a1A, 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].

Table 2A. Neuroprotective agents in AD.

<table>
<thead>
<tr>
<th>Neuropathology</th>
<th>Target</th>
<th>Investigational agents (exemplary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP processing/Aβ production, accumulation of toxic Aβ aggregates</td>
<td>α-secretase stimulation</td>
<td>etazolate, M1 receptor agonists (AF2678, talsacidine)</td>
</tr>
<tr>
<td>Neurotransmitter/receptor signaling dysfunction</td>
<td>Cholinesterase inhibitors</td>
<td>ACI-91, LY2886721, HPP854, CTS-2116</td>
</tr>
<tr>
<td>Abnormal tau hyperphosphorylation, aggregation (Neuro)inflammation</td>
<td>Tau kinase inhibitors (e.g., GSK3β)</td>
<td>semagacestat, BMS708163, ELND006/007</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>ROS scavengers, (dietary) antioxidants</td>
<td>EVP0962, tarenflurbil, CHF-5074, E2212, E2012, begacestat</td>
</tr>
<tr>
<td>Trophic factor deficiency</td>
<td>Nerve growth factor (NGF) delivery, NGF agonists</td>
<td>ELND005, NRM8499, tramiprosate, colostrinin, NP-61</td>
</tr>
<tr>
<td>Transcriptional dysfunctions</td>
<td>Histone deacetylase inhibitors</td>
<td>Clioquinol, PBT2</td>
</tr>
<tr>
<td>Excitotoxicity, calcium homeostasis dysregulation</td>
<td>NMDA receptor modulators, Ca2+ channel blockers</td>
<td>Bapineuzumab, solanezumab, ACC001, CAD106, LY2062430, AAB002, AN1792, IVIg, gantenerumab, MABTS102A</td>
</tr>
<tr>
<td>Miscellaneous Aβ-related targets (APP translation inhibitors, 3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme inhibitors, RAGE modulators)</td>
<td></td>
<td>Posiphen, statins, nilvadipine</td>
</tr>
<tr>
<td>Neurotransmitter/receptor signaling dysfunction</td>
<td>Nicotinic receptor agonists (α4β2/α2β2γδnAChR, α7nAChR)</td>
<td>Dimebon, huperzine A</td>
</tr>
<tr>
<td></td>
<td>Serotonin receptor modulators (5-HT2 agonists, 5-HT1A antagonists, 5-HT7 antagonists)</td>
<td>Ispronicline, RG3487, EVP-6124</td>
</tr>
<tr>
<td></td>
<td>H3 receptor inverse agonists</td>
<td>PRX03140, RQ-00000009, TD-5108, velusetrag</td>
</tr>
<tr>
<td></td>
<td>MAO-B inhibitors</td>
<td>MK0249</td>
</tr>
<tr>
<td></td>
<td>Anti-inflammatory agents, immunomodulators</td>
<td>EVP-302</td>
</tr>
<tr>
<td></td>
<td>PPARγ agonists</td>
<td>NP-12</td>
</tr>
<tr>
<td></td>
<td>ROS scavengers, (dietary) antioxidants</td>
<td>Methylioninium, davunetide</td>
</tr>
<tr>
<td></td>
<td>Anti-inflammatory agents, immunomodulators</td>
<td>NSAIIDs, TT301/302</td>
</tr>
<tr>
<td></td>
<td>PSAR4 agonists</td>
<td>Rosiglitazone, DSP8658</td>
</tr>
<tr>
<td></td>
<td>Vitamin E, coenzyme Q10</td>
<td>Mitoquinone, melatonin</td>
</tr>
<tr>
<td></td>
<td>CERE 110, PYM50028</td>
<td>Coenzyme Q10</td>
</tr>
<tr>
<td></td>
<td>Histone deacetylase inhibitors</td>
<td>Ampakines</td>
</tr>
<tr>
<td></td>
<td>Nerve growth factor (NGF) delivery, NGF agonists</td>
<td>EVP0334</td>
</tr>
<tr>
<td></td>
<td>BDNF expression activation</td>
<td>Nimodipine</td>
</tr>
<tr>
<td></td>
<td>Histone deacetylase inhibitors</td>
<td>Amantadine</td>
</tr>
<tr>
<td></td>
<td>Histone deacetylase inhibitors</td>
<td>Methylioninium, davunetide</td>
</tr>
</tbody>
</table>

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2.1.2 Phase II trials

Agents affecting the neuronal nicotinic acetylcholine (ACh) receptors (nAChRs) 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 (α7, α4β4) 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-amylodogenic pathway, lowered Aβ production, increased neuroprotective sAPPα formation. α7-nAChRs 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 α7nAChR 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-HT3A antagonist effect as well as showing partial agonism at α7-nAChRs that can be beneficial in potential nicotinic agonist side effects [20]. Following oral administration, EVP-6124 showed good brain penetration. **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 proognitive effect could be blocked by a selective α7-nAChR 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 proognitive 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 α7-nAChR partial agonist/5-HT3A antagonist profile with EVP-6124, with no activity at α4β4-nAChRs 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 proognitive in vivo effects – presumably mediated via α7-nACh 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 α7-nAChR partial agonist with no 5-HT3 antagonism. 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 α7-nAChR agonism and no activity on 5-HT3 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 stage). 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 α6β4/α6β3nAChR agonist (devoid of α7 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 α4β4-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 Aβ1-40 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 Aβ1-40 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 Ia 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 Aβ1-40 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
Clinical utility of neuroprotective agents in neurodegenerative diseases

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 Aβ42 levels but no correlation was found between biomarkers (Aβ40, Aβ42, 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, glialosis 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 repurposing. The parent compound of ACI-91, pirenzipine – a muscarinic receptor antagonist – is approved for the treatment of gastriuc 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 pro-cognitive 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 sAPPβ 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) leakage, 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].

H3 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]. H3 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 noradrenaline. In AD-related actions, activation of postsynaptic H3R antagonists (e.g., b-carboline) might be of relevance as well. Several H3R antagonists have advanced to clinical phases, for treatment of ADHD, AD, cognitive deficits in schizophrenia or sleep disorders [58].

MK0249 is a histamine H3R 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]. PDE4 inhibitors demonstrated neuroprotective, neuroregenerative and anti-inflammatory effects in a series of in vivo experiments. Indeed, the prototype PDE4 inhibitor rolipram demonstrated several beneficial cognitive effects in preclinical models, particularly reversing Aβ40 induced memory impairment and it was also neuroprotective in an MPTP-induced toxicity PD model [62,63]. Function, targeting of PDE4 subpopulations is still the subject of ongoing research (see e.g., [64]). Of note, tumor therapy may be a novel direction for PDE4 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 PDE4 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 anxiolytic 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, proognitive sAPPα [67], in addition to presumably suppressing Aβ-induced neuronal overexcitability. Proognitive effects were verified in an age-related cognitive deficit rat model (spatial learning and memory) [68]. Etazolate has a PDE4 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 AChE inhibitor [68]. A trend toward cognitive improvement was shown and the response was dependent on the apolipoprotein (Apo)E4 status.

HF0220 (7b-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-PGJ2) 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 aminooindane from rasagiline, the ladostigil metabolite hydroxymethylindane 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) \textit{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 \textbf{Phase I trials}

\textbf{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 Aβ40/Aβ42 levels and was able to reverse cognitive deficits (contextual fear-conditioning). Dose-dependent transient reductions of plasma Aβ40 levels (less intensive effect observed on plasma Aβ1-42) were verified in healthy human volunteers and AD patients following single doses, with later rebounds [79]. Further development was discontinued in 2010. \textit{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. \textit{E2012}, an orally active cinemamide (with a non-NSAID structure)-derived GSM entered the clinic in 2006. In rat cortical neuron culture, E2012 reduced Aβ40/42 and increased Aβ38 (less prone to aggregation) without changing total Aβ levels and causing APP-carboxy terminal fragments (CTF) accumulation. Aβ40/42 levels were decreased following oral treatment \textit{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 \textit{E2212} has recently entered Phase I trials [NCT01221259]. It has improved \textit{in vitro/in vivo} activity and a better safety profile compared to E2012. \textit{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 Aβ1-42 levels, amyloid plaques, brain inflammation and reversing behavioral deficits in AD transgenic mice [86]. \textit{CHF5074} is a flurbiprofen analog GSM, devoid of cyclooxygenase (COX) activity and interactions with Notch signaling. \textit{In vitro} CHF5074 showed dose-dependent inhibition of Aβ42 secretion. In mice AD model, CHF5074 dose-dependently reduced plasma Aβ levels and the Aβ42/Aβ40 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].

\textbf{AAD-2004}, a drug candidate for AD, displays Aβ lowering action and antioxidative properties (reducing free radicals
and PGE₂) as well as anti-inflammatory effects. The 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, antioxidant 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 antioxidant 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 Aβ_{40/42} levels and plaque load presumably via equilibrium shift. Central and peripheral Aβ_{42} 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 [NCT01428013].

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 Aβ_{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 AChE inhibitor, 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 hyperphosphorylation, 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-HT₄ receptor agonist – is in Phase II for GI motility disorders. Regarding AD applications, 5-HT₄ 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-HT₄ agonist, PRX-03140 was reported to improve cognitive functions in a Phase IIa AD clinical trial. Velusetrag enhanced cognitive functions in a scopolamine-induced...
Clinical utility of neuroprotective agents in neurodegenerative diseases

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-HT4 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 fibril 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 (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 Marrazzo 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

A2A 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]. A2A 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]. A2A receptors are colocalized in the striatum with dopamine D2 receptors on GABAAergic 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 A2A antagonists. Investigational agents indeed provided motor benefits in preclinical models, and several representatives of the class have already entered the clinic. A selective A2A antagonist, istradsfylline (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 A3, A2B and A3 subtypes), competitive non-xanthine A2A 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 A2A agonist treatment, L-Dopa induced contralateral rotation potentiation in unilaterally 6-OHDA-lesioned rats, attenuation of D3 antagonist induced catalepsy, inhibition of L-Dopa-induced behavioral sensitization) – which point toward a compensating effect for loss of D3 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 D2/D3 receptor partial agonist (expected to result in DA receptor activation without motor complications, the action depending on the dopaminergic tone) and 5-HT1A 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 H2O2 (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, proteosomal 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 (Ferrirprox) is an oral, brain permeable bidentate iron chelating agent, used in the treatment of peripheral iron load...
disorders, as thalassasemia major. In in vivo studies, deferiprone was neuro/cytotoxic against AD and PD relevant insults (ferric iron, H2O2, Aβ1-40, 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, Friedrich’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 Ca2+ channels (without affecting peripheral L-type Ca2+ 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). Antidysskinetic 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 III trials
SYN115 (tozadenant) is a (non-xanthine) adenosine A2A 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 Ib 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

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Table 2B. Neuroprotective agents in PD.

<table>
<thead>
<tr>
<th>Neurotransmitter/receptor signaling dysfunction</th>
<th>Target</th>
<th>Investigational agents (exemplary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine A2A antagonists</td>
<td>MAO-B inhibitors, metal ion chelators, metal protein attenuators, kynurenine pathways modulators</td>
<td>Istradefylline, preladenant, SYN-115, vipadenant</td>
</tr>
<tr>
<td>Neurotrophic factor deficiency</td>
<td>Neurotrophic factor based therapies</td>
<td>SAFINAMIDE, Deferiprone</td>
</tr>
<tr>
<td>Neurotransmitter/receptor signaling dysfunction</td>
<td>Neurotransmitter/receptor signaling dysfunction</td>
<td>Neurotransmitter/receptor signaling dysfunction</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Dietary antioxidants, mitochondrial function modulators</td>
<td>Coenzyme Q10, creatine, green tea polyphenols</td>
</tr>
<tr>
<td>Mitochondrial dysfunction</td>
<td>Ca2+ channel blockers</td>
<td>Isradipine</td>
</tr>
</tbody>
</table>

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Clinical utility of neuroprotective agents in neurodegenerative diseases
M₁-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 pathologies. A mechanism of action of GM1 involves restoration of damaged but viable DAergic neurons, stimulation of sprouting of neurites, enhanced dopamine synthesis and interaction with neuromodulatory 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 and animal models [159].

**AV-101** (L-4-chlorokynurenine) is an orally available prodrug (to enhance brain delivery) of 7-chlorokynurenine acid, a kynurenine 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 kynurenine 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]. Arimoclo
tol 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 prami-
pxole, unrelated to its dopamine agonist activity (reviewed in,
E.g., [171]). Dexpramipexole demonstrated antioxidan
t, 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 cur-
rents 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, edara-
vone 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 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), affect-
ing 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 reac-
tion [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, pharmacokinet-
ic properties 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
metabolism [177].

**PYM50018** (Myogane), a sapogenin-type small molecule related to
PYM50028 has demonstrated neuroprotective effects in several preclinical models, via induction of neuro-
protective 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 inclu-
sions, 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 NFkB pathway activity and
expression of the prostaglandin synthesizing enzyme cas-
cade, as well as suppression of JNK activation might contrib-
ute 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, fol-
lowed 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 III trials

The role of sirtuins in neuroprotection and in neurodegen-
erative diseases and consequently the relevance of their phar-
macological modulation are at best controversial. In Drosophila
HD models the Sir2 ortholog Sir2 demonstrated distinct
effects on neuronal survival and lifespan, where reducing
Sir2 improved the former [183]. Sir1 inhibition exerted neuro-
protection against oxidative stress in cultured neurons and
Sir2 inhibition suppressing neuronal degeneration has been
observed in other Drosophila models as well. HD is character-
ized by a (mutant Htt-induced) transcriptional dysregula-
tion that might be restored by HDAC and particularly
Sir2/SIRT1 inhibition (acting via histones and other targets
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-HT3 ligand activity, H3 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 1. Examples of natural products studied as therapeutic agents for neurodegenerative diseases.
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 memooquin – an agent derived from the AChEI – M₃ 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. HL20A 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 A₂A antagonism [206] or antioxidant effect (ROS/RNS scavenging, neuronal NOS inhibition). Istradefylline (KW-6002) can be considered as the prototype MAO-B/A₂A 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⁺/Ca²⁺ 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 tetrahydroaquoquinoline 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, insensitivity 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.
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.

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.

Declaration of interest

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

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