

Review

Multi-target Natural and Nature-Inspired Compounds against Neurodegeneration: A Focus on Dual Cholinesterase and Phosphodiesterase Inhibitors

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Abstract: Alzheimer's disease is a memory-related neurodegenerative condition leading to cognitive impairment. Cholinergic deficit, together with other underlying mechanisms, leads to the onset and progression of the disease. Consequently, acetylcholinesterase inhibitors are used for the symptomatic treatment of dementia, even if limited efficacy is observed. More recently, some specific phosphodiesterase isoforms emerged as promising, alternative targets for developing inhibitors to contrast neurodegeneration. Phosphodiesterase isoforms 4,5 and 9 were found to be expressed in brain regions that are relevant for cognition. Given the complex nature of Alzheimer's disease and the combination of involved biochemical mechanisms, the development of polypharmacological agents acting on more than one pathway is desirable. This review provides an overview of recent reports focused on natural and Nature-inspired small molecules, or plant extracts, acting as dual cholinesterase and phosphodiesterase inhibitors. In the context of the multi-target directed ligand approach, such molecules would pave the way for the development of novel agents against neurodegeneration. More precisely, according to the literature data, xanthines, other alkaloids, flavonoids, coumarins and polyphenolic acids represent promising scaffolds for future optimization.

Keywords: Alzheimer's disease; acetylcholinesterase; phosphodiesterase; multi-target directed ligand; polypharmacology; natural compounds

Citation: Ribaudo, G.; Memo, M.; Gianoncelli, A. Multi-target Natural and Nature-Inspired Compounds against Neurodegeneration: A Focus on Dual Cholinesterase and Phosphodiesterase Inhibitors. *Appl. Sci.* **2021**, *11*, 5044. <https://doi.org/10.3390/app11115044>

Academic Editors: Jan Hošek, Gregorio Peron

Received: 10 May 2021

Accepted: 27 May 2021

Published: 29 May 2021

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder linked to deficits of neurotransmission [1]. From the clinical point of view, AD is a memory-related disease that is characterized by a progressive decline leading to cognitive impairment. Malfunctioning of cholinergic transmission and glycation, formation of amyloid deposits and oxidative stress have been proposed to be involved in pathogenesis and progression of the disease [2,3]. In this connection, drugs sustaining the cholinergic tone have been developed to contrast the progressive cognitive decline that characterizes AD. In particular, acetylcholinesterase (AChE) inhibitors such as donepezil (Figure 1a) are used for the symptomatic treatment of dementia, even if only moderate efficacy is observed in AD patients [4,5].

On the other hand, phosphodiesterases (PDEs) are emerging as promising targets for developing inhibitors to contrast neurodegeneration [6–8]. In particular, selective small molecules targeting PDE4, PDE5 and PDE9 isoforms are being studied to explore alternative strategies against AD in light of their brain localization and of their role, to different extents, in cognitive processes [7,9–11]. More specifically, PDE4D is expressed in the frontal cortex and it hydrolyzes cyclic adenosine monophosphate (cAMP) to its corresponding linear metabolite. It is involved in memory consolidation, and in vivo

studies demonstrated that the use of the selective inhibitor rolipram ameliorates cognition [12,13]. In addition, compound MK-0952, another PDE4 inhibitor, was also studied in clinical trials for the improvement of cognitive impairment [14]. On the other hand, PDE5A, a cyclic guanosine monophosphate (cGMP)-selective isoform, is upregulated in the brains of AD patients, and its functioning influences neuronal plasticity. Several PDE5 inhibitors, such as tadalafil (Figure 1b), are being studied for their applications against dementia in the context of drug repurposing [8,15].

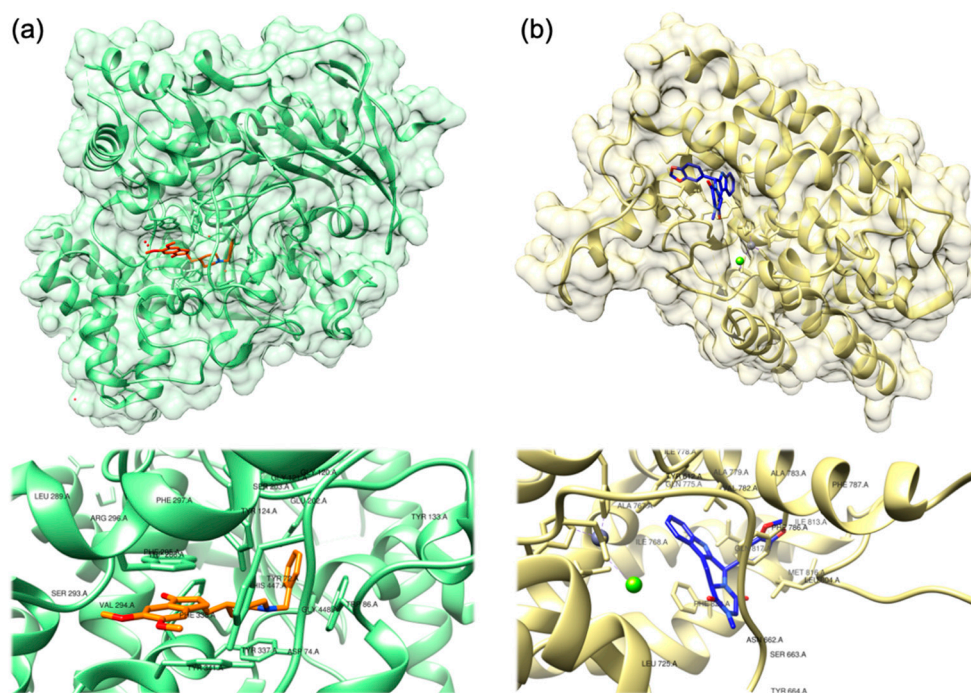


Figure 1. 3D structures of AChE in complex with donepezil (**a**, PDB ID: 4EY7) and of PDE5 in complex with tadalafil (**b**, PDB ID: 1UDU). In the artworks, the residues interacting with the ligands (<5 Å) have been labeled.

Eventually, PDE9A, which selectively hydrolyzes cGMP, is expressed in brain regions that are relevant for cognition and, thus, is recently receiving more attention [16,17]. The efficacy of selective PDE9 inhibitors, such as BAY 736691, PF-04447943 and BI 409306, was proved in preclinical and clinical studies [18–20]. Thus, since PDE4, PDE5 and PDE9 isoforms are involved in the nitric oxide (NO) signaling cascade, which includes NO-sensitive guanylyl cyclase and protein kinase G, in improving neuronal plasticity via cAMP responsive element binding (CREB) protein activation, in maintaining the efficiency of neuronal vascularization and in glutamate release, the action of selective PDE inhibitors could represent an alternative and complementary approach to contrast dementia [7,8].

As anticipated, given the complex nature of AD and the combination of multiple biochemical mechanisms underlying onset and progression of this disease, the development of polyfunctional therapeutic agents acting on more than one pathway is desirable, and natural compounds are historically endowed with polypharmacological features [3,21,22]. In medicinal chemistry, the multi-target directed ligand (MTDL) approach is being pursued with the same aim [15]. In this context, this review is focused on natural and Nature-inspired small molecules, or plant extracts, acting at the same time as AChE and PDE inhibitors, and thus with a potential application in contrasting neurodegeneration.

More than 70 research papers and reviews were screened for the preparation of the current article. Scientific contributions were retrieved by searching PubMed

(www.ncbi.nlm.nih.gov/pubmed/ (accessed on 25 March 2021)) and Scopus (www.scopus.com (accessed on 25 March 2021)) databases using keywords such as “phosphodiesterase”, “PDE4”, “PDE5”, “PDE9”, “acetylcholinesterase”, “butyrylcholinesterase”, “natural compounds”, “neurodegeneration”, “Alzheimer’s disease” and their combinations. Papers published in the 2000–2021 timeframe were considered. The 3D models of the studied protein and complexes were retrieved from the Protein Data Bank (PDB, www.rcsb.org (accessed on 25 March 2021)) and UCSF Chimera software was used to prepare the artworks [23].

2. Multi-Target Natural and Nature-Inspired Compounds

Clinical practice with AChE inhibitors highlights their limited efficacy and the onset of tolerance that occurs after long-term use [24]. Thus, the search for alternative strategies and combined therapies is wide open. An overview of the chemical classes of the currently studied Nature-inspired dual cholinesterase-PDE inhibitors will be presented in the following sections of the manuscript.

2.1. Xanthines and Other Alkaloids

Xanthines, including caffeine and caffeine-derived compounds, such as synthetic derivatives, represent outstanding examples of alkaloids modulating many different biochemical pathways in humans (Figure 2) [25,26]. Caffeine itself, found in *Coffea arabica* and *C. canephora*, has been referred to as a multi-target compound for decades, in light of its polypharmacological effects [26]. In fact, besides its primary activity on adenosine receptors, caffeine interferes with other neurotransmission systems, including the pathways mediated by acetylcholine, epinephrine, serotonin, dopamine and glutamate [27–29]. More specifically, in the context of the cholinergic system, caffeine acts as a non-competitive AChE inhibitor ($K_i = 175 \mu\text{M}$). This mechanism may be involved in its neuroprotective and anti-inflammatory effects [26]. On the other hand, it has also been reported that caffeine can interfere with intracellular cAMP and cGMP levels by acting as a weak, non-specific reversible PDE inhibitor ($\text{IC}_{50} = 500\text{--}1000 \mu\text{M}$) [25,30]. On this basis, caffeine is defined as a “cognitive enhancer”, and a role for this alkaloid in the modulation of cognitive decline in AD has been proposed. Nevertheless, it must be pointed out that the anti-inflammatory effect on neuro-inflammation conditions may also play a role [31–33]. Moreover, it has been reported that caffeine has an effect on delaying onset and progression of Parkinson’s disease (PD), while it is interestingly negatively implicated in Huntington’s disease (HD) [34,35]. On the side of synthetic derivatives of xanthines being studied as potential tools against neurodegeneration, propentofylline (Figure 2) received great attention. More specifically, this compound was reported to improve cognition and dementia severity in mild-to-moderate AD in clinical trials. Propentofylline is known to target biochemical pathways mediated by PDEs, but other mechanisms have also been proposed. In particular, regulation of genes involved in the onset and progression of oxidative stress, lipid homeostasis, which may interfere with neuronal function, appears to be involved [36,37]. Moreover, propentofylline inhibits AChE in the μM range ($\text{IC}_{50} = 6.40 \mu\text{M}$) [38]. Pyrazolopyrimidinones, which are structurally related to naturally occurring xanthines, were also studied as selective PDE9 inhibitors for the discovery of novel compounds to contrast dementia. In these studies, compound showing inhibitory activities against PDE9 in the nM range were identified (IC_{50} values $< 200 \text{ nM}$) [39,40]. On a similar basis, 3-isobutyl-1-methylxanthine (IBMX) was previously highlighted as a non-selective inhibitor targeting PDE9 as well as other isoforms in the μM range ($\text{IC}_{50} = 230 \mu\text{M}$ for PDE9) [41]. Pyrazolopyridine is another xanthine-inspired scaffold that has been recently considered for the development of dual cholinesterase-PDE inhibitors since such compounds are known to target peripheral anionic binding sites (PAS) of AChE. Pan et al. reported the synthesis of tacrine-pyrazolopyridine hybrid derivatives targeting AChE, butyrylcholinesterase (BuChE), another enzyme involved in sustaining cholinergic tone, and PDE4D. The compounds demonstrated μM and sub- μM inhibitory activity on

cholinesterases ($IC_{50} = 0.125\text{--}0.412\ \mu\text{M}$ for AChE and $IC_{50} = 0.245\text{--}1.283\ \mu\text{M}$ for BuChE) and even better activity on the PDE ($IC_{50} = 0.041\text{--}1.307\ \mu\text{M}$) [10].

Other alkaloids of natural origin have also been reported as dual inhibitors targeting AChE and PDEs. In particular, natural compounds from camel artemisia (*Peganum nigellastrum*) and their synthetic derivatives were tested in this context. Zhou et al. studied beta-carboline and vasicinone (Figure 2) as lead compounds using in silico tools and prepared indoline-2,3-dione and quinazoline derivatives that were then tested in vitro against both AChE and PDE5. The authors identified a small set of inhibitors acting on the two enzymes in the nM and μM range. In particular, compounds inhibited AChE with IC_{50} values between 44 and 298 nM, and PDE5 with IC_{50} values between 17 and 746 nM. Moreover, no obvious toxicity was observed on A549 cells treated with these molecules, suggesting that the compounds should be well-tolerated [15].

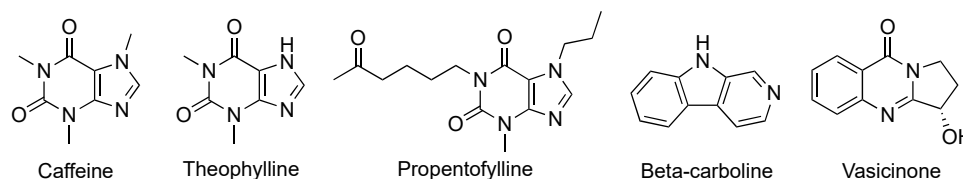


Figure 2. Chemical structures of representative alkaloids and derivatives considered in the cited studies: caffeine, theophylline, propentofylline, beta-carboline and vasicinone.

2.2. Flavonoids and Coumarins

Flavonoids are known for possessing a wide range of biological activities. Through the years, the compounds belonging to this class have been studied as antioxidants, anti-inflammatory agents and also as AChE inhibitors and, more in general, as promising scaffolds for developing compounds to contrast neurodegeneration through different mechanisms [42,43]. Singh et al. synthesized a set of drug-like flavonoid derivatives, based on the chromen-4-one scaffold, inhibiting AChE at nM concentrations ($IC_{50} = 5.87\ \text{nM}$ for the best performing compound) and endowed with antioxidant properties. Besides in vitro results, the authors also observed that one of the synthesized compounds was effective in restoring memory in vivo in a mouse model of scopolamine-induced amnesia [3]. On the other hand, the activity of flavonoids as non-specific or specific PDE inhibitors is well-documented. Icarin, icarisid II (Figures 3 and 4) and sophoflavescenol are cGMP-specific inhibitors and isoflavones have been studied as PDE4, PDE5 and PDE9 inhibitors. In particular, icariin inhibits PDE5 with an IC_{50} value of $5.9\ \mu\text{M}$ [44–48]. Naringenin (Figure 4), dioclein, epigallocatechin-3-gallate represent some other examples of compounds targeting several PDE isoforms [11,49,50].

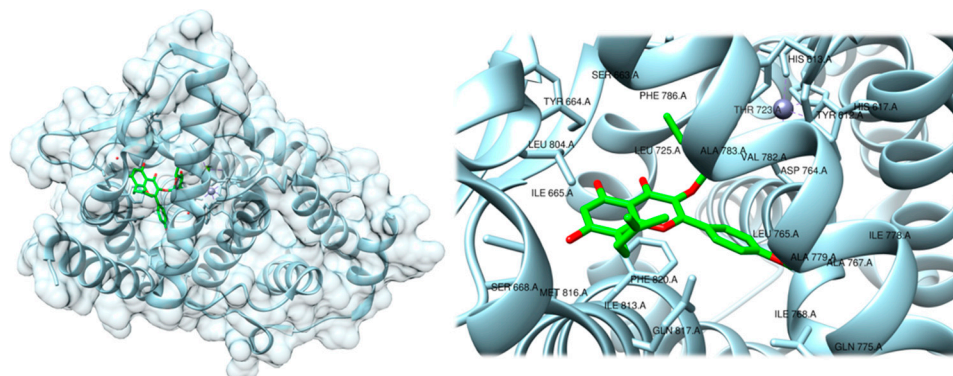


Figure 3. 3D structure of PDE5 in complex with icarisid II, a natural inhibitor from *Epimedium brevicornium* (PDB ID: 2H44). In the artwork, the residues interacting with the ligand (<5 Å) have been labeled.

Besides the several available examples of multi-target compounds from this class, it must be pointed out that recent contributions are starting to investigate closely the dual AChE-PDE inhibitory activity of flavonoids. More specifically, Adefegha et al. reported that rutin and its aglycone quercetin (Figure 4) efficiently reduce AChE activity in rat tissues (25 and 50 mg/kg) and, at the same time, inhibit PDE5. In particular, a more marked inhibitory activity on PDE5 was observed for rutin over quercetin. Moreover, the compounds showed antioxidant activity, as expected. Importantly, even if the focus of the cited article was on the treatment of erectile dysfunction (ED), the results could be translated into the field of central nervous system (CNS) diseases, for which the management of oxidative status is of primary relevance. In neurodegeneration, in particular, antioxidant activity often parallels AChE and PDE inhibition [51–54].

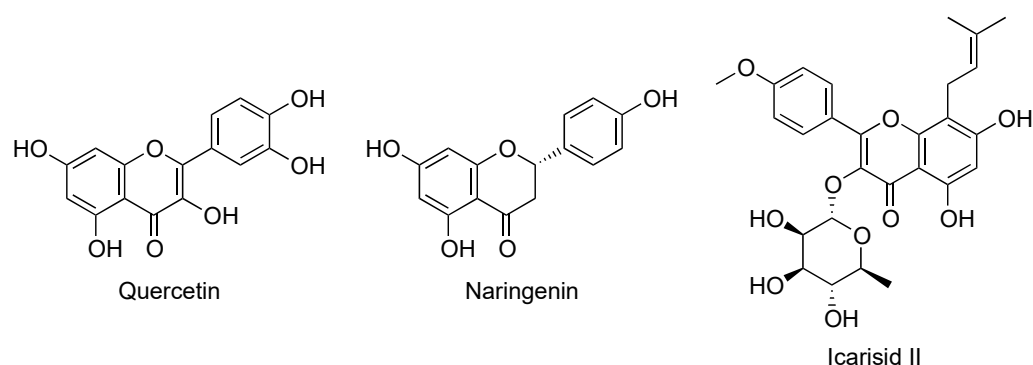


Figure 4. Chemical structures of quercetin, naringenin and icaricidin, representative flavonoids studied in the cited research works.

Coumarins are a family of naturally occurring small molecules with a wide range of biological activities, including a role in contrasting CNS diseases. This is likely due to their reported effect on enzymes such as AChE and monoamine oxidases (MAOs) [55,56]. Compounds from this class were also studied both as PDE inhibitors and anti-AD agents [55,57]. Nevertheless, their dual inhibitory activity on cholinesterases and PDEs, as well as their isoform selectivity, has not been investigated in detail to date. On the other hand, Jiang et al. synthesized a set of drug-like, blood-brain barrier (BBB)-permeable coumarin-dithiocarbamate hybrids that act through a combination of mechanisms. In particular, the authors studied the activity of these compounds on AChE ($IC_{50} = 0.21\text{--}36.85\ \mu\text{M}$), BuChE ($6.21\text{--}37.24\%$ enzyme inhibition) and A β aggregation [58].

2.3. Natural Extracts Containing Polyphenolic Acids

Several recent reports investigated the issue of dual cholinesterase-PDE inhibition from a slightly different perspective [59–61]. Many authors, indeed, described the activity on such enzymes, and in particular on AChE and on the PDE5 isoform, of whole aqueous or ethanolic extracts of plants or plant parts. In such research works, these tests are generally paired by analytical profiling, by means of HPLC or other chromatographic techniques, providing a description of the content of the studied extracts. In this context, Oboh et al. reported the *in vitro* activity of Nigerian plantain (*Musa sapientum*) extracts. In particular, the authors prepared aqueous extracts of unripe and ripe plantain peels using distilled water. The extracts were analyzed using HPLC-DAD, showing that rutin, caffeic acid and quercetin were the most abundant components of phenolic fraction of ripe peel extract, while unripe peel extract was rich in rutin, chlorogenic acid and catechin. Unripe peel extract was found to be more efficient in inhibiting AChE ($IC_{50} = 6.30\ \mu\text{g/mL}$) and PDE5 ($IC_{50} = 3.10\ \mu\text{g/mL}$) isolated from rats [59]. Similarly, the same research group presented a study focused on spice extracts. In particular, alligator pepper (*Aframomum melegueta*) and basted melegueta (*Aframomum danieli*) were considered. In this case, the authors performed the extraction of alkaloids using a 10% acetic acid solution in ethanol.

The composition of such extracts was investigated by GC-FID and revealed that, among other alkaloids, alligator pepper is particularly rich in theophylline (Figure 2), lupanine and emetine. On the other hand, high concentrations of ellipicine, gingerdione and seneccionine were detected in bastered melegueta extracts. Interestingly, alligator pepper extract inhibited AChE more efficiently ($IC_{50} = 5.42 \mu\text{g/mL}$), while bastered melegueta extract performed better towards PDE5 ($IC_{50} = 7.24 \mu\text{g/mL}$) [62]. African walnut (*Tetracarpidium conophorum*) is another source of natural dual inhibitors. Aqueous extracts of pulverized walnuts, with and without shell, were analyzed by HPLC-DAD, showing the presence of gallic acid, caffeic acid (Figure 5) and quercetin (Figure 4) in similar concentrations. Nevertheless, overall higher concentrations of bioactive components were observed in the first extract, which was also the most efficient in inhibiting AChE ($IC_{50} = 0.87 \mu\text{g/mL}$) and PDE5 ($IC_{50} = 8.59 \mu\text{g/mL}$) [63]. Similarly, Dada et al. reported that aqueous extracts of pulverized almond (*Terminalia catappa*) leaf and stem bark modulate the activity of AChE and PDE5 in the cardiac tissue of rats (100–200 mg/kg) [61]. These parts of the plant are rich in polyphenols and organic acids, such as ferulic, caffeic, coumaric (Figure 5) and 2-prenylated benzoic acid, but also of catechin and ellagic acid derivatives [64]. Ojo et al. characterized the leaves extract of *Ocimum gratissimum*, a perennial medicinal plant endowed with several pharmacological properties. Aqueous extracts of *O. gratissimum* dose-dependently inhibited AChE ($IC_{50} = 43.19\text{--}44.67 \mu\text{g/mL}$) and PDE5 ($IC_{50} = 44.23\text{--}53.99 \mu\text{g/mL}$) isolated from rat. Moreover, the extracts were tested for radical scavenging activity showing an antioxidant effect [65]. The authors connected the observed biological effects with the phenolic content of the extract, that includes rutin, kaempferol, rosmarinic acid, caffeic acid and cichoric acid [65,66].

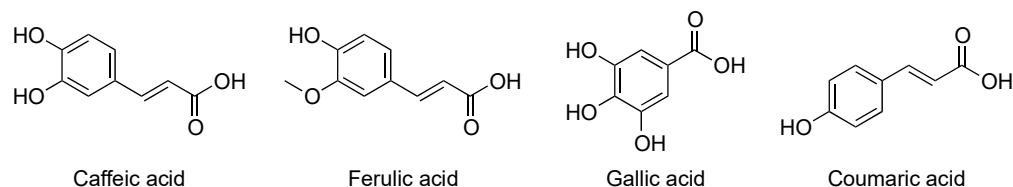


Figure 5. Chemical structures of representative polyphenolic acids detected in the investigated extracts.

Taken together, the observations that can be retrieved from the reports overviewed in this section confirm the potential of flavonoids and alkaloids as dual inhibitors of AChE and PDEs, since these compounds are the most represented in the considered extracts. Similarly, the role of polyphenolic acids cannot be ruled out. Moreover, it must be considered that, in several contributions, the authors primarily studied these extracts as remedies against ED, for which PDE5 traditionally represents the most studied pharmacological target. Nevertheless, the information concerning dual AChE-PDE5 inhibition are valuable also outside of this specific context and should be kept into account when translating these results into the field of drug development against neurodegeneration.

3. Conclusions and Perspectives

AD is the most common single cause of dementia and has become a worldwide health concern [67]. Thus, increasing efficacy and limiting side effects in managing AD is a priority. Complex pathogenesis prompted medicinal chemists to search for dual or multiple target drugs with the aim of generating a synergistic therapeutic effect, going beyond conventional AChE inhibitors [9]. Compounds acting at the same time on other targets or mechanisms, such as A β aggregation, BuChE and oxidative stress, were studied through the years. Most importantly, reports in the literature demonstrated that the combined use of cholinesterase and PDE inhibitors, in particular, could turn out to have a different, complementary effect on early and late long-term potentiation (LTP) of

cognitive function [7]. This strategy is also recently being pursued in the field of synthetic medicinal chemistry, with the development of drug-like small molecules targeting PDE5 and AChE [9] and in the context of drug repurposing, as in the case of the well-known drug tadalafil [8,68,69]. The field of dual cholinesterase-PDE inhibitors attracts great interest in developing novel tools against dementia and has been previously investigated from the point of view of the underlying biochemical mechanisms and synergistic effects promoted by the inhibition of these two enzymes. More specifically, Prickaerts et al. reported that AChE and PDE5 inhibitors affect memory processes at different extents. In particular, PDE5 inhibitors improve consolidation of object information, while AChE inhibitors induce a different outcome by improving processes of acquisition of object information [70]. Moreover, PDE5 inhibitors can stimulate the neurogenesis and, thus, dual inhibitors potentially produce synergistic effects on AD [71].

From a chemical point of view, compounds acting on these two targets must be endowed with some specific features which may be deduced from the available structures of AChE and PDEs and of their complexes with ligands. As depicted in Figure 1b, tadalafil interacts with PDE5 through a pool of hydrophobic residues (Ala767, Ile778, Ala779, Ala783, Phe787, Phe786, Ile813, Phe820), but also via some polar amino acids, such as Gln775 and Gln817. Interestingly, a similar interaction pattern with PDE5 is shared by icaridisid II, which binds the identical region of PDE5, mainly through the same hydrophobic residues (Leu725, Ala783, Phe786, Phe820; Figure 3). Thus, the presence of an aromatic and hydrophobic portion in the small molecule appears to be mandatory to target this enzyme. Similarly, in the structure of the donepezil-AChE complex (Figure 1a), the ligand interacts with the target via hydrophobic amino acids (Phe295, Phe297, Phe338). Nevertheless, a more polar region can be also identified in the binding site (Tyr72, Asp74, Trp86, Glu202, His447). Thus, even if the two binding sites share some structural similarities, AChE also requires the presence of polar moieties. These features must be taken into account in the design and optimization of dual inhibitors.

In the field of natural and Nature-inspired compounds, polypharmacology and synergistic effects are two established milestones. This especially holds true when plant extracts are considered, in which the entourage effect of different chemicals on several biological targets and biochemical pathways often lays the basis for the observed therapeutic outcome. Nevertheless, in the context of dementia, it must be pointed out that the macromolecular targets of such small molecules are located in the CNS, beyond the BBB. This highlights the primary relevance of the pharmacokinetic properties of the studied natural and Nature-inspired compounds since drug-likeness requirements are even stricter in this case. Thus, opportune physico-chemical features of such molecules are required to proceed from a promising *in vitro* lead to an effective drug candidate for preclinical and clinical studies. This is where rational synthetic optimization of the compounds may have its role, e.g., with opportune derivations or the preparation of prodrugs.

Taken together, the information retrieved from the current literature suggests that alkaloids, and xanthines in particular, flavonoids, coumarins and polyphenolic acids represent promising scaffolds for developing dual cholinesterase-PDE inhibitors.

Author Contributions: Conceptualization, G.R. and A.G.; software, G.R.; investigation, G.R. and A.G.; writing—original draft preparation, G.R. and A.G.; writing—review and editing, A.G. and M.M.; funding acquisition, G.R. and A.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by University of Brescia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable. No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Castellani, R.J.; Rolston, R.K.; Smith, M.A. Alzheimer Disease. *Disease* **2010**, *56*, 484–546, doi:10.1016/j.disamonth.2010.06.001.
- Singh, M.; Kaur, M.; Chadha, N.; Silakari, O. Hybrids: A new paradigm to treat Alzheimer's disease. *Mol. Divers.* **2015**, *20*, 271–297, doi:10.1007/s11030-015-9628-9.
- Singh, M.; Kaur, M.; Singh, N.; Silakari, O. Exploration of multi-target potential of chromen-4-one based compounds in Alzheimer's disease: Design, synthesis and biological evaluations. *Bioorganic Med. Chem.* **2017**, *25*, 6273–6285, doi:10.1016/j.bmc.2017.09.012.
- Kaduszkiewicz, H.; Zimmermann, T.; Beck-Bornholdt, H.-P.; Bussche, H.V.D. Cholinesterase inhibitors for patients with Alzheimer's disease: Systematic review of randomised clinical trials. *BMJ* **2005**, *331*, 321–327, doi:10.1136/bmj.331.7512.321.
- Tsuno, N. Donepezil in the treatment of patients with Alzheimer's disease. *Expert Rev. Neurother.* **2009**, *9*, 591–598, doi:10.1586/ern.09.23.
- Schmidt, C.J. Phosphodiesterase inhibitors as potential cognition enhancing agents. *Curr. Top. Med. Chem.* **2010**, *10*, 222–230, doi:10.2174/156802610790411009.
- Kroker, K.S.; Rast, G.; Giovannini, R.; Marti, A.; Dorner-Ciossek, C.; Rosenbrock, H. Inhibition of acetylcholinesterase and phosphodiesterase-9A has differential effects on hippocampal early and late LTP. *Neuropharmacology* **2012**, *62*, 1964–1974, doi:10.1016/j.neuropharm.2011.12.021.
- Ribaud, G.; Ongaro, A.; Zagotto, G.; Memo, M.; Gianoncelli, A. Therapeutic potential of phosphodiesterase inhibitors against neurodegeneration: The Perspective of the medicinal chemist. *ACS Chem. Neurosci.* **2020**, *11*, 1726–1739, doi:10.1021/acscchemneuro.0c00244.
- Mao, F.; Wang, H.; Ni, W.; Zheng, X.; Wang, M.; Bao, K.; Ling, D.; Li, X.; Xu, Y.; Zhang, H.; et al. Design, synthesis, and biological evaluation of orally available first-generation dual-target selective inhibitors of acetylcholinesterase (AChE) and phosphodiesterase 5 (PDE5) for the treatment of Alzheimer's disease. *ACS Chem. Neurosci.* **2018**, *9*, 328–345, doi:10.1021/acscchemneuro.7b00345.
- Pan, T.; Xie, S.; Zhou, Y.; Hu, J.; Luo, H.; Li, X.; Huang, L. Dual functional cholinesterase and PDE4D inhibitors for the treatment of Alzheimer's disease: Design, synthesis and evaluation of tacrine-pyrazolo[3,4-b]pyridine hybrids. *Bioorganic Med. Chem. Lett.* **2019**, *29*, 2150–2152, doi:10.1016/j.bmcl.2019.06.056.
- Ribaud, G.; Memo, M.; Gianoncelli, A. A Perspective on natural and nature-inspired small molecules targeting phosphodiesterase 9 (PDE9): Chances and challenges against neurodegeneration. *Pharmaceuticals* **2021**, *14*, 58, doi:10.3390/ph14010058.
- Lakics, V.; Karran, E.H.; Boess, F.G. Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. *Neuropharmacology* **2010**, *59*, 367–374, doi:10.1016/j.neuropharm.2010.05.004.
- Ji, H.-F.; Shen, L. Berberine: A potential multipotent natural product to combat Alzheimer's disease. *Molecules* **2011**, *16*, 6732–6740, doi:10.3390/molecules16086732.
- Gallant, M.; Aspiotis, R.; Day, S.; Dias, R.; Dube, D.; Dubé, L.; Friesen, R.W.; Girard, M.; Guay, D.; Hamel, P.; et al. Discovery of MK-0952, a selective PDE4 inhibitor for the treatment of long-term memory loss and mild cognitive impairment. *Bioorganic Med. Chem. Lett.* **2010**, *20*, 6387–6393, doi:10.1016/j.bmcl.2010.09.087.
- Zhou, L.-Y.; Zhu, Y.; Jiang, Y.-R.; Zhao, X.-J.; Guo, D. Design, synthesis and biological evaluation of dual acetylcholinesterase and phosphodiesterase 5A inhibitors in treatment for Alzheimer's disease. *Bioorganic Med. Chem. Lett.* **2017**, *27*, 4180–4184, doi:10.1016/j.bmcl.2017.07.013.
- Hutson, P.; Finger, E.; Magliaro, B.; Smith, S.; Converso, A.; Sanderson, P.; Mullins, D.; Hyde, L.; Eschle, B.; Turnbull, Z.; et al. The selective phosphodiesterase 9 (PDE9) inhibitor PF-04447943 (6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) enhances synaptic plasticity and cognitive function in rodents. *Neuropharmacology* **2011**, *61*, 665–676, doi:10.1016/j.neuropharm.2011.05.009.
- Patel, N.S.; Klett, J.; Pilarzyk, K.; Lee, D.I.; Kass, D.; Menniti, F.S.; Kelly, M.P. Identification of new PDE9A isoforms and how their expression and subcellular compartmentalization in the brain change across the life span. *Neurobiol. Aging* **2018**, *65*, 217–234, doi:10.1016/j.neurobiolaging.2018.01.019.
- Van der Staay, F.J.; Rutten, K.; Bärfacker, L.; DeVry, J.; Erb, C.; Heckroth, H.; Karthaus, D.; Tersteegen, A.; van Kampen, M.; Blokland, A.; et al. The novel selective PDE9 inhibitor BAY 73-6691 improves learning and memory in rodents. *Neuropharmacology* **2008**, *55*, 908–918, doi:10.1016/j.neuropharm.2008.07.005.
- Schwam, E.M.; Nicholas, T.; Chew, R.; Billing, C.B.; Davidson, W.; Ambrose, D.; Altstiel, L.D. A multicenter, double-blind, placebo-controlled trial of the PDE9A inhibitor, PF-04447943, in Alzheimer's disease. *Curr. Alzheimer Res.* **2014**, *11*, 413–421, doi:10.2174/1567205011666140505100858.
- Moschetti, V.; Boland, K.; Feifel, U.; Hoch, A.; Zimdahl-Gelling, H.; Sand, M. First-in-human study assessing safety, tolerability and pharmacokinetics of BI 409306, a selective phosphodiesterase 9A inhibitor, in healthy males. *Br. J. Clin. Pharmacol.* **2016**, *82*, 1315–1324, doi:10.1111/bcp.13060.

21. Zanforlin, E.; Zagotto, G.; Ribaudo, G. The medicinal chemistry of natural and semisynthetic compounds against Parkinson's and Huntington's diseases. *ACS Chem. Neurosci.* **2017**, *8*, 2356–2368, doi:10.1021/acscchemneuro.7b00283.
22. Zagotto, G.; Ribaudo, G. An overview of new possible treatments of Alzheimer's disease, based on natural products and semi-synthetic compounds. *Curr. Med. Chem.* **2017**, *24*, 3749–3773, doi:10.2174/0929867324666170712161829.
23. Pettersen, E.F.; Goddard, T.D.; Huang, C.C.; Couch, G.S.; Greenblatt, D.M.; Meng, E.C.; Ferrin, T.E. UCSF Chimera—A visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, *25*, 1605–1612, doi:10.1002/jcc.20084.
24. Diniz, B.S.; Pinto, J.A.; Gonzaga, M.L.C.; Guimarães, F.M.; Gattaz, W.F.; Forlenza, O.V. To treat or not to treat? A meta-analysis of the use of cholinesterase inhibitors in mild cognitive impairment for delaying progression to Alzheimer's disease. *Eur. Arch. Psychiatry Clin. Neurosci.* **2009**, *259*, 248–256, doi:10.1007/s00406-008-0864-1.
25. Ongaro, A.; Zagotto, G.; Memo, M.; Gianoncelli, A.; Ribaudo, G. Natural phosphodiesterase 5 (PDE5) inhibitors: A computational approach. *Nat. Prod. Res.* **2019**, *35*, 1648–1653, doi:10.1080/14786419.2019.1619726.
26. Pohanka, M. The perspective of caffeine and caffeine derived compounds in therapy. *Bratisl. Lek. List.* **2015**, *116*, 520–530, doi:10.4149/bll_2015_106.
27. Szadujkis-Szadurska, K.; Grzesk, G.; Szadujkis-Szadurski, L.; Gajdus, M.; Matusiak, G. Role of acetylcholine and calcium ions in three vascular contraction models: Angiotensin II, phenylephrine and caffeine. *Exp. Ther. Med.* **2012**, *4*, 329–333, doi:10.3892/etm.2012.573.
28. Cummings, K.J.; Commons, K.G.; Trachtenberg, F.L.; Li, A.; Kinney, H.C.; Nattie, E.E. Caffeine improves the ability of serotonin-deficient (Pet-1^{-/-}) mice to survive episodic asphyxia. *Pediatr. Res.* **2013**, *73*, 38–45, doi:10.1038/pr.2012.142.
29. Gołębiewska, K.; Dziubina, A. The effect of adenosine A2A receptor antagonists on hydroxyl radical, dopamine, and glutamate in the striatum of rats with altered function of VMAT. *Neurotox. Res.* **2012**, *22*, 150–157, doi:10.1007/s12640-012-9316-9.
30. Herman, A.P. Caffeine's mechanisms of action and its cosmetic use. *Ski. Pharmacol. Physiol.* **2013**, *26*, 8–14, doi:10.1159/000343174.
31. Nehlig, A. Is caffeine a cognitive enhancer? *J. Alzheimer's Dis.* **2010**, *20*, S85–S94, doi:10.3233/jad-2010-091315.
32. Pohanka, M. Alzheimer's disease and oxidative stress: A review. *Curr. Med. Chem.* **2013**, *21*, 356–364, doi:10.2174/09298673113206660258.
33. Rivera-Oliver, M.; Díaz-Ríos, M. Using caffeine and other adenosine receptor antagonists and agonists as therapeutic tools against neurodegenerative diseases: A review. *Life Sci.* **2014**, *101*, 1–9, doi:10.1016/j.lfs.2014.01.083.
34. Qi, H.; Li, S. Dose-response meta-analysis on coffee, tea and caffeine consumption with risk of Parkinson's disease. *Geriatr. Gerontol. Int.* **2014**, *14*, 430–439, doi:10.1111/ggi.12123.
35. Kobal, J.; Melik, Z.; Cankar, K.; Strucl, M. Cognitive and autonomic dysfunction in presymptomatic and early Huntington's disease. *J. Neurol.* **2014**, *261*, 1119–1125, doi:10.1007/s00415-014-7319-6.
36. Sanders, O.; Rajagopal, L. Phosphodiesterase inhibitors for Alzheimer's Disease: A systematic review of clinical trials and epidemiology with a mechanistic rationale. *J. Alzheimer's Dis. Rep.* **2020**, *4*, 185–215, doi:10.3233/adr-200191.
37. Janitschke, D.; Lauer, A.A.; Bachmann, C.M.; Seyfried, M.; Grimm, H.S.; Hartmann, T.; Grimm, M.O.W. Unique role of caffeine compared to other methylxanthines (theobromine, theophylline, pentoxifylline, propentofylline) in regulation of AD relevant genes in neuroblastoma SH-SY5Y wild type cells. *Int. J. Mol. Sci.* **2020**, *21*, 9015, doi:10.3390/ijms21239015.
38. Mohamed, T.; Osman, W.; Tin, G.; Rao, P.P. Selective inhibition of human acetylcholinesterase by xanthine derivatives: In vitro inhibition and molecular modeling investigations. *Bioorganic Med. Chem. Lett.* **2013**, *23*, 4336–4341, doi:10.1016/j.bmcl.2013.05.092.
39. Singh, N.; Saravanan, P.; Thakur, M.; Patra, S.; Parameswaran, S. Development of xanthine based inhibitors targeting phosphodiesterase 9A. *Lett. Drug Des. Discov.* **2017**, *14*, 1122–1137, doi:10.2174/1570180813666161102125423.
40. Huang, M.; Shao, Y.; Hou, J.; Cui, W.; Liang, B.; Huang, Y.; Li, Z.; Wu, Y.; Zhu, X.; Liu, P.; et al. Structural asymmetry of phosphodiesterase-9A and a unique pocket for selective binding of a potent enantiomeric inhibitor. *Mol. Pharmacol.* **2015**, *88*, 836–845, doi:10.1124/mol.115.099747.
41. Huai, Q.; Wang, H.; Zhang, W.; Colman, R.W.; Robinson, H.; Ke, H. Crystal structure of phosphodiesterase 9 shows orientation variation of inhibitor 3-isobutyl-1-methylxanthine binding. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9624–9629, doi:10.1073/pnas.0401120101.
42. Ribaudo, G.; Coghi, P.; Zanforlin, E.; Law, B.Y.K.; Wu, Y.Y.J.; Han, Y.; Qiu, A.C.; Qu, Y.Q.; Zagotto, G.; Wong, V.K.W.; et al. Semi-synthetic isoflavones as BACE-1 inhibitors against Alzheimer's disease. *Bioorganic Chem.* **2019**, *87*, 474–483, doi:10.1016/j.bioorg.2019.03.034.
43. Singh, M.; Kaur, M.; Silakari, O. Flavones: An important scaffold for medicinal chemistry. *Eur. J. Med. Chem.* **2014**, *84*, 206–239, doi:10.1016/j.ejmech.2014.07.013.
44. Ribaudo, G.; Pagano, M.A.; Pavan, V.; Redaelli, M.; Zorzan, M.; Pezzani, R.; Mucignat-Caretta, C.; Vendrame, T.; Bova, S.; Zagotto, G. Semi-synthetic derivatives of natural isoflavones from *Maclura pomifera* as a novel class of PDE-5A inhibitors. *Fitoterapia* **2015**, *105*, 132–138, doi:10.1016/j.fitote.2015.06.020.
45. Ribaudo, G.; Vendrame, T.; Bova, S. Isoflavones from *Maclura pomifera*: Structural elucidation and in silico evaluation of their interaction with PDE5. *Nat. Prod. Res.* **2017**, *31*, 1988–1994, doi:10.1080/14786419.2016.1269101.
46. Ribaudo, G.; Ongaro, A.; Zagotto, G. 5-Hydroxy-3-(4-hydroxyphenyl)-8,8-dimethyl-6-(3-methylbut-2-enyl)pyrano[2,3-h]chromen-4-one. *Molbank* **2018**, *2018*, M1004, doi:10.3390/m1004.
47. Gianoncelli, A.; Ongaro, A.; Zagotto, G.; Memo, M.; Ribaudo, G. 2-(3,4-Dihydroxyphenyl)-4-(2-(4-nitrophenyl)hydrazono)-4H-chromene-3,5,7-triol. *Molbank* **2020**, *2020*, M1144, doi:10.3390/M1144.

48. Oselladore, E.; Ongaro, A.; Zagotto, G.; Memo, M.; Ribaudo, G.; Gianoncelli, A. Combinatorial library generation, molecular docking and molecular dynamics simulations for enhancing the isoflavone scaffold in phosphodiesterase inhibition. *N. J. Chem.* **2020**, *44*, 19472–19488, doi:10.1039/d0nj02537b.
49. Orallo, F.; Camiña, M.; Álvarez, E.; Basaran, H.; Lugnier, C. Implication of cyclic nucleotide phosphodiesterase inhibition in the vasorelaxant activity of the citrus-fruits flavonoid ($\pm$)-naringenin. *Planta Med.* **2005**, *71*, 99–107, doi:10.1055/s-2005-837774.
50. Gonçalves, R.L.; Lugnier, C.; Keravis, T.; Lopes, M.J.; Fantini, F.A.; Schmitt, M.; Cortes, S.F.; Lemos, V.S. The flavonoid dioclein is a selective inhibitor of cyclic nucleotide phosphodiesterase type 1 (PDE1) and a cGMP-dependent protein kinase (PKG) vasorelaxant in human vascular tissue. *Eur. J. Pharmacol.* **2009**, *620*, 78–83, doi:10.1016/j.ejphar.2009.08.008.
51. Adefegha, S.A.; Oyeleye, S.I.; Dada, F.A.; Olasehinde, T.A.; Oboh, G. Modulatory effect of quercetin and its glycosylated form on key enzymes and antioxidant status in rats penile tissue of paroxetine-induced erectile dysfunction. *Biomed. Pharmacother.* **2018**, *107*, 1473–1479, doi:10.1016/j.biopha.2018.08.128.
52. Bortoli, M.; Tiezza, M.D.; Muraro, C.; Pavan, C.; Ribaudo, G.; Rodighiero, A.; Tubaro, C.; Zagotto, G.; Orian, L. Psychiatric disorders and oxidative injury: Antioxidant effects of zolpidem therapy disclosed in silico. *Comput. Struct. Biotechnol. J.* **2019**, *17*, 311–318, doi:10.1016/j.csbj.2019.02.004.
53. Muraro, C.; Tiezza, M.D.; Pavan, C.; Ribaudo, G.; Zagotto, G.; Orian, L. Major depressive disorder and oxidative stress: In Silico investigation of fluoxetine activity against ROS. *Appl. Sci.* **2019**, *9*, 3631, doi:10.3390/app9173631.
54. Ribaudo, G.; Bortoli, M.; Pavan, C.; Zagotto, G.; Orian, L. Antioxidant potential of psychotropic drugs: From clinical evidence to in vitro and in vivo assessment and toward a new challenge for in silico molecular design. *Antioxidants* **2020**, *9*, 714, doi:10.3390/antiox9080714.
55. Xie, S.-S.; Wang, X.; Jiang, N.; Yu, W.; Wang, K.D.; Lan, J.-S.; Li, Z.-R.; Kong, L.-Y. Multi-target tacrine-coumarin hybrids: Cholinesterase and monoamine oxidase B inhibition properties against Alzheimer's disease. *Eur. J. Med. Chem.* **2015**, *95*, 153–165, doi:10.1016/j.ejmech.2015.03.040.
56. Stefanachi, A.; Leonetti, F.; Pisani, L.; Catto, M.; Carotti, A. Coumarin: A natural, privileged and versatile scaffold for bioactive compounds. *Molecules* **2018**, *23*, 250, doi:10.3390/molecules23020250.
57. Kumbar, M.N.; Kamble, R.R.; Kamble, A.A.; Salian, S.R.; Kumari, S.; Nair, R.; Kalthur, G.; Adiga, S.K.; Prasad, D.J. Design and microwave assisted synthesis of coumarin derivatives as PDE inhibitors. *Int. J. Med. Chem.* **2016**, *2016*, 1–16, doi:10.1155/2016/9890630.
58. Jiang, N.; Huang, Q.; Liu, J.; Liang, N.; Li, Q.; Li, Q.; Xie, S.-S. Design, synthesis and biological evaluation of new coumarin-dithiocarbamate hybrids as multifunctional agents for the treatment of Alzheimer's disease. *Eur. J. Med. Chem.* **2018**, *146*, 287–298, doi:10.1016/j.ejmech.2018.01.055.
59. Oboh, G.; Ademiluyi, A.O.; Oyeleye, S.I.; Olasehinde, T.A.; Boligon, A.A. Modulation of some markers of erectile dysfunction and malonaldehyde levels in isolated rat penile tissue with unripe and ripe plantain peels: Identification of the constituents of the plants using HPLC. *Pharm. Biol.* **2017**, *55*, 1920–1926, doi:10.1080/13880209.2017.1340966.
60. Ajiboye, B.O.; Ojo, O.A.; Fatoba, B.; Afolabi, O.B.; Olayide, I.; Okesola, M.A.; Oyinloye, B.E. In vitro antioxidant and enzyme inhibitory properties of the n-butanol fraction of Senna podocarpa (Guill. and Perr.) leaf. *J. Basic Clin. Physiol. Pharmacol.* **2020**, *31*, doi:10.1515/jbcpp-2019-0123.
61. Dada, F.A.; Oyeleye, S.I.; Adefegha, S.A.; Oboh, G. Extracts from almond (*Terminalia catappa*) leaf and stem bark mitigate the activities of crucial enzymes and oxidative stress associated with hypertension in cyclosporine A-stressed rats. *J. Food Biochem.* **2021**, *45*, doi:10.1111/jfbc.13435.
62. Adefegha, S.A.; Oboh, G.; Okeke, B.M.; Oyeleye, S.I. Comparative Effects of alkaloid extracts from Aframomum melegueta (Alligator Pepper) and Aframomum danielli (Bastered Melegueta) on enzymes relevant to erectile dysfunction. *J. Diet. Suppl.* **2017**, *14*, 542–552, doi:10.1080/19390211.2016.1272661.
63. Ademiluyi, A.O.; Oyeleye, S.I.; Ogunsuyi, O.B.; Oboh, G. Phenolic analysis and erectogenic function of African Walnut (*Tetradlepidium conophorum*) seeds: The impact of the seed shell on biological activity. *J. Food Biochem.* **2019**, *43*, e12815, doi:10.1111/jfbc.12815.
64. Baratelli, T.D.G.; Gomes, A.C.C.; Wessjohann, L.A.; Kuster, R.M.; Simas, N.K. Phytochemical and allelopathic studies of Terminalia catappa L. (Combretaceae). *Biochem. Syst. Ecol.* **2012**, *41*, 119–125, doi:10.1016/j.bse.2011.12.008.
65. Ojo, O.A.; Ojo, A.B.; Oyinloye, B.E.; Ajiboye, B.O.; Anifowose, O.O.; Akawa, A.; Olaiya, O.E.; Olasehinde, O.R.; Kappo, A.P. Ocimum gratissimum Linn. Leaves reduce the key enzymes activities relevant to erectile dysfunction in isolated penile and testicular tissues of rats. *BMC Complement. Altern. Med.* **2019**, *19*, 71, doi:10.1186/s12906-019-2481-0.
66. Salawu, S.O.; Bester, M.; Duodu, K.G. Phenolic composition and bioactive properties of cell wall preparations and whole grains of selected cereals and legumes. *J. Food Biochem.* **2014**, *38*, 62–72, doi:10.1111/jfbc.12026.
67. Zemek, F.; Drtinova, L.; Nepovimova, E.; Sepsova, V.; Korabecny, J.; Klimes, J.; Kuca, K. Outcomes of Alzheimer's disease therapy with acetylcholinesterase inhibitors and memantine. *Expert Opin. Drug Saf.* **2014**, *13*, 759–774.
68. García-Barroso, C.; Ricobaraza, A.; Pascual-Lucas, M.; Unceta, N.; Rico, A.J.; Goicolea, M.A.; Sallés, J.; Lanciego, J.L.; Oyarzabal, J.; Franco, R.; et al. Tadalafil crosses the blood-brain barrier and reverses cognitive dysfunction in a mouse model of AD. *Neuropharmacology* **2013**, *64*, 114–123, doi:10.1016/j.neuropharm.2012.06.052.
69. Ribaudo, G.; Pagano, M.A.; Bova, S.; Zagotto, G. New therapeutic applications of phosphodiesterase 5 inhibitors (PDE5-Is). *Curr. Med. Chem.* **2016**, *23*, 1239–1249, doi:10.2174/0929867323666160428110059.

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70. Prickaerts, J.; Şık, A.; Van Der Staay, F.J.; De Vente, J.; Blokland, A. Dissociable effects of acetylcholinesterase inhibitors and phosphodiesterase type 5 inhibitors on object recognition memory: Acquisition versus consolidation. *Psychopharmacology* **2005**, *177*, 381–390, doi:10.1007/s00213-004-1967-7.
 71. Beato, A.; Gori, A.; Boucherle, B.; Peuchmaur, M.; Haudecoeur, R. β -Carboline as a privileged scaffold for multitarget strategies in Alzheimer's disease therapy. *J. Med. Chem.* **2021**, *64*, 1392–1422, doi:10.1021/acs.jmedchem.0c01887.