

# TV 3326 for Alzheimer's dementia: a novel multimodal ChE and MAO inhibitors to mitigate Alzheimer's-like neuropathology

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## Keywords

Alzheimer's disease; cholinesterase inhibitor; ladostigil; monoamine oxidase inhibitor; TV 3326

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## Abstract

**Objectives** Alzheimer's disease (AD) is one of the most prevalent neurodegenerative disorders and a well-recognized cause of dementia with ageing. In this review, we have represented the ChE and MAO inhibitory potential of TV 3326 against AD based on current scientific evidence.

**Key findings** The aetiology of AD is quite complex and not completely understood. However, it has been observed that AD involves the deposition of abnormal amyloid beta (A $\beta$ ), along with hyperphosphorylation of tau, oxidative stress, low acetylcholine (ACh) level and biometal dyshomeostasis. Due to the complex nature of AD aetiology, active research is required in the areas of development of multi-target drugs with 2 or more complementary biological functions, as they might represent significant progress in the AD treatment. Interestingly, it has been found that TV 3326 (i.e. ladostigil) is regarded as a novel therapeutic agent since it has the potential to cause inhibition of monoamine oxidase (MAO) A and B, and acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) in the brain. Furthermore, it has the capacity to reverse memory impairments, which further suggests the ability of this drug to elevate cholinergic activity in the brain.

**Summary** TV 3326 can avert oxidative–nitritative stress and gliosis. It has also been confirmed that TV 3326 contains neuroprotective and anti-apoptotic properties. Therefore, this distinctive combined inhibition of ChE and MAO along with its neuroprotective property makes TV 3326 a useful drug in the treatment of AD.

## Introduction

Alzheimer's disease (AD) is a degenerative brain disease that is hard to treat, and the common characteristics include loss of memory and other cognitive deficits.<sup>[1–3]</sup> Because of the complex nature of AD, multiple hypotheses regarding the AD pathogenesis have been proposed in the past decades, including tau protein hypothesis,<sup>[4–6]</sup> oxidative stress (OS) hypothesis,<sup>[7–10]</sup> amyloid cascade hypothesis,<sup>[11–13]</sup> cholinergic hypothesis<sup>[14,15]</sup> and so on. It has been found that OS and

acetylcholine (ACh) have a significant contribution to AD pathogenesis.<sup>[16]</sup>

Restoration of acetylcholine (ACh) level is one of the probable ways of treating AD.<sup>[17]</sup> Thus, in this regard, reversible acetylcholinesterase (AChE) inhibitors can be used. So far, the United States Food and Drug Administration has approved multiple AChE inhibitors including galanthamine, rivastigmine, and donepezil as well as N-methyl-D-aspartate receptor antagonist memantine for clinical use and these drugs were found to be effective at improving memory in some AD individuals.<sup>[18–20]</sup> It has

been revealed by the study of crystal structures of AChE with inhibitors that there are 2 sites of binding, a catalytic active site (CAS) and a peripheral anionic site (PAS).<sup>[21–23]</sup> At the rim of the gorge, the PAS region of AChE is located and found to be associated with the interaction with A $\beta$  peptide, which might further trigger neurotoxic A $\beta$  fibril deposition.<sup>[24,25]</sup> It has been revealed by current studies that if both the peripheral and catalytic sites are blocked, AChE inhibitors not only can cause alleviation of the cognitive impairment of AD individuals via increasing the levels of ACh but also can play a role as a disease-modifying agent which can delay the formation of A $\beta$  plaque.<sup>[26,27]</sup> For instance, donepezil (i.e. a mixed-type inhibitor) exerts a minor effect on reducing the AChE-induced A $\beta$  aggregation (approximately 22%). The possible reason for this aforesaid result is the higher affinity of donepezil towards the active site than the PAS.<sup>[28]</sup>

In the central nervous system (CNS) and in peripheral tissues, monoamine oxidase (MAO)-A and MAO-B are supposed to have a significant contribution in terms of biogenic amine metabolism.<sup>[29]</sup> In addition to this, MAOs may also catalyse the oxidative deamination of vasoactive and neuroactive amines.<sup>[30,31]</sup> It has been observed in several studies that MAOs are involved with neurological and psychiatric disorders, including AD.<sup>[32,33]</sup> It is known that OS is involved in neurodegenerative disorders. Since MAO-B inhibitors (MAO-BIs) have the capacity to regulate neurotransmitters and to inhibit oxidative damage, therefore MAO-BIs might be considered as potential drugs to act against AD.<sup>[34]</sup>

To treat neurodegenerative diseases including AD, TV 3326 (ladostigil) is a novel neuroprotective agent which is being studied.<sup>[35]</sup> This agent plays a role as a reversible inhibitor of both butyrylcholinesterase (BuChE) and AChE and also acts as an irreversible inhibitor of MAO-B. In addition to this, the mode of action of TV 3326 is similar to the combined mechanisms of older agents including rasagiline and rivastigmine.<sup>[36,37]</sup> It has also been observed that the expression of neurotrophic factors including glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) can be increased by TV 3326. In addition to this, TV 3326 might also possess the ability to reverse the neurogenesis-induced damage which is observed in neurodegenerative disorders.<sup>[38]</sup>

The complex nature of AD in terms of its clinical, genetic and biological features has made the development of effective anti-AD agents more challenging.<sup>[39–41]</sup> The recent therapeutic technique involves designing drug candidates which will be able to act on multiple targets and will have a range of pharmacological activities. Certainly, this aforesaid phenomenon has encouraged the development of bifunctional drugs like TV 3326. Therefore, this review highlights the ChE and MAO inhibitory potential of TV 3326 against AD.

## Pharmacological Rationale of TV 3326

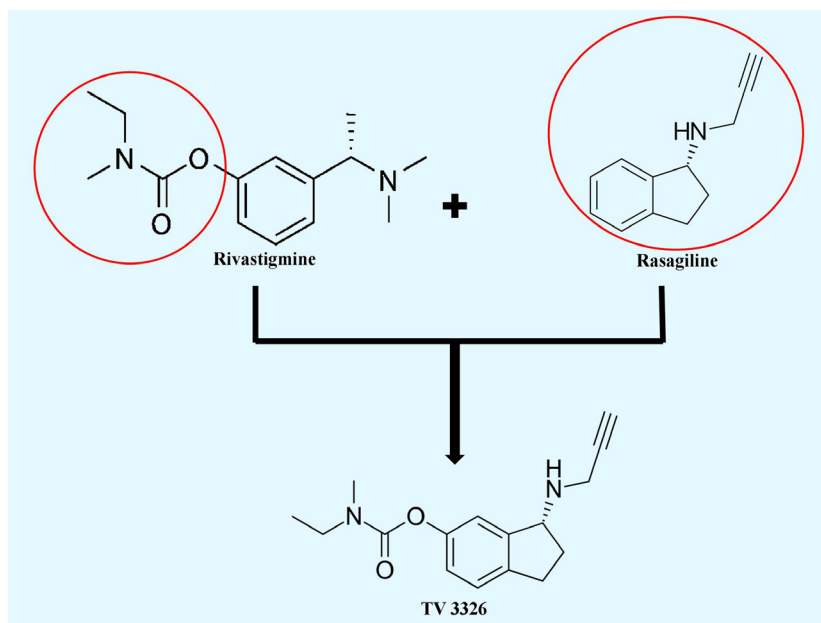
TV 3326 was developed to improve depression, elevate cholinergic transmission and avert the formation of reactive oxygen species (ROS) or their effects. TV 3326 is an aminoindan containing the propargylamine group found in the rasagiline and the carbamate moiety of rivastigmine as shown in Figure 1. Both BuChE and AChE can be inhibited by TV 3326, and it has a prolonged duration of action as compared to rivastigmine.

Furthermore, at doses that block MAO-A and MAO-B in the brain by more than eighty per cent, TV 3326 exerts minor or no action on the enzyme presents in the digestive tract as compared to other irreversible inhibitors of MAO-A. Thus, the cardiovascular action of orally administered tyramine is minimally potentiated by TV 3326. This selective inhibition might take place due to the formation of decarbamylated metabolites in the brain, which acts as a more effective MAO inhibitor as compared to TV 3326.<sup>[42]</sup>

## ChE Inhibitory Promise of TV 3326

Oral TV 3326 administration at the doses ranging from 12 to 35 mg/kg was found to cause inhibition in the brain ChE level by 25–40% and also antagonism of the scopolamine-triggered spatial memory impairments in rats.<sup>[42,43]</sup> In addition to this, in rats, TV 3326 at the doses of 12–26 mg/kg also exerted a dose-dependent antagonism of scopolamine-triggered impairments in working and reference memory and memory consolidation.<sup>[43]</sup> This aforesaid phenomenon signified that, in the synapse, the drug had the ability to adequately enhance the ACh levels to compete with the scopolamine for the muscarinic receptors linked to memory formation.<sup>[42,43]</sup> Symptoms that arise due to the excessive cholinergic stimulation in the periphery (e.g. muscle weakness, diarrhoea and salivation) or the brain (e.g. tremor, hypothermia or respiratory depression) were not seen, until doses over 139 mg/kg TV 3326 were administered, which suppressed the level of ChE by 50–60%<sup>[44]</sup>. This wide range of therapeutic activity might take place because of the relatively lower rate of increase in the inhibition of ChE with rising drug doses, found to take place with the dose of TV 3326 over the range of 17–69 mg/kg.<sup>[43,44]</sup> On the other hand, TV 3326 caused *in vitro* inhibition of both BuChE and AChE. In addition, as compared to BuChE, the inhibitory action was 100-fold more effective against AChE.<sup>[42,44]</sup>

In rats, at the dose of 52 mg/kg, chronic oral administration of TV 3326 for 3 weeks caused inhibition of striatal ChE activity by ~50%, along with inhibition of the hyperactivity behavioural syndrome triggered by L-3,4-dihydroxyphenylalanine (L-Dopa).<sup>[45]</sup> Like TV 3326, when



**Figure 1** Chemical structures of TV 3326 and its parent compounds [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

TV 3279 (i.e. the S-enantiomer of TV 3326) was orally administered chronically at the dose of 26 mg/kg for 3 weeks, it caused inhibition in the striatal ACh effect and attenuation of the hyperactivity syndrome response.<sup>[45]</sup> Indeed, the inhibitory actions of both TV3279 and TV 3326 on these hyperactivity syndromes were perhaps arbitrated by the increased functional levels of ACh and sustained the well-recognized inhibitory action of ACh on dopaminergic-induced stereotypy.<sup>[46,47]</sup> It was revealed by former *in vivo* study<sup>[48]</sup> that 50% of ChE inhibition was caused by ChE inhibitors (e.g. donepezil, metrifonate and rivastigmine), along with the following rise in the release of central ACh. Therefore, in rats, brain levels of ACh were found to be enhanced due to the use of TV 3326.<sup>[45]</sup>

From the previous results, it is known that cholinergic transmission, through muscarinic receptors, confirmed the correct processing mechanisms of cortical inputs forward from the cortex to projection cells; however, the contribution of ACh in the activity of striatal projection neurons is still not clear.<sup>[49,50]</sup> Therefore, the capacity of scopolamine to reverse the inhibitory actions of TV 3326 and TV3279 plus the non-selective inhibitor, tranlycypromine on L-dopa-triggered hyperactivity<sup>[45]</sup> is consistent with the hierarchical structure of the motor control system in the basal ganglia.<sup>[50]</sup>

### MAO Inhibitory Promise of TV 3326

It has been revealed by several studies that rather than the acute treatment, only chronic treatment with TV 3326 can

cause suppression of the activity of MAO-A and MAO-B in the brain.<sup>[43,51,52]</sup> This aforesaid phenomenon might take place due to the formation of active MAO inhibitory metabolites because of the hydrolysis of the carbamate moiety in TV 3326 via ChE, which has found to yield the 6-hydroxyrasagiline derivative.<sup>[53]</sup> Interestingly, 6-hydroxyrasagiline showed an increased affinity towards the effects of MAO-A and MAO-B.<sup>[51,52]</sup> Probably, sufficient concentration of 6-hydroxyrasagiline and other TV 3326 metabolites can cause considerable inhibition of MAO-A and MAO-B and elevation of the levels of striatal dopamine, noradrenaline and serotonin, which is responsible for the respective antidepressant and antiparkinsonian effects of TV 3326, confirmed in preclinical models.<sup>[54]</sup>

Therefore, in rats, administration of TV 3326 once daily at the dose of 26 mg/kg for 2 weeks caused inhibition of MAO-A and MAO-B by ~ 70%, with very slight or no action of these enzymes in the intestine.<sup>[43,55]</sup> Nevertheless, TV 3279 was found to have only akin ChE inhibitory effect; however, it lacked MAO inhibitory effect.<sup>[43]</sup> Furthermore, it has been confirmed in rats that TV 3326 can act like other antidepressants (e.g. moclobemide, amitriptyline and selective reversible inhibitors of MAO-A) in the forced swim test, which further suggests a potential for antidepressant-like effect.<sup>[35,42]</sup> In addition to this, this antidepressant-like effect of TV 3326 was observed along with the elevated levels of brain serotonin.<sup>[35]</sup> On the other hand, oral treatment with TV 3326 in rabbits at the dose of 26 mg/kg for 2 weeks caused inhibition of brain MAO by more than 90%; however, no inhibitory effect was observed

in case of intestinal MAO-A, which included ~80% of total intestinal MAO.<sup>[55]</sup> In addition to this, this brain selectivity causes nominal potentiation of the pressor response to oral tyramine.<sup>[42,55]</sup> Interestingly, in mice, chronic administration of TV 3326 at the dose of 52 mg/kg caused inhibition of both MAO-A and MAO-B in the hippocampus, striatum, brainstem and cerebral cortex; in addition, inhibition values of MAO-A and MAO-B reached 65% and 90%, respectively.<sup>[45,54]</sup> This aforesaid finding recommends that TV 3326 contains a higher selectivity for MAO-B than MAO-A in the mouse brain, and a similar trend was also observed in the case of rat and rabbit brain enzymes.<sup>[43,55,56]</sup> Inhibition of MAO via TV 3326, as like other propargylamines (e.g. rasagiline, selegiline and clorgyline), was also found to be irretrievable and slower to recuperate.<sup>[54]</sup>

## Neuroprotective Promise of TV 3326 against Alzheimer's Disease

Results of preclinical studies indicated that TV 3326 possesses a wide neuroprotective effect (Table 1) against a range of neurotoxins in neuronal cell cultures and *in vivo* models of neurodegeneration. Furthermore, it has been confirmed by the *in vivo* studies that at the dose of 26 mg/kg, chronic administration of TV 3326 once daily for 14 days caused prevention of the striatal dopamine reduction and decrease in the catabolites homovanillic acid and also 3,4-dihydroxyphenylacetic acid by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.<sup>[54]</sup> On the other hand, it has been confirmed that TV 3326 can ameliorate the cognitive impairments in aged monkeys.<sup>[57]</sup> Similarly, in rats, it can avert memory deficit triggered by intracerebroventricular injection of streptozotocin.<sup>[58,59]</sup> In rats, it has also been observed that TV 3326 can antagonize spatial memory impairment triggered by scopolamine.<sup>[43]</sup> Moreover, at the dose of 17 milligrams per kilogram, TV 3326 was also found to cause restoration of sodium azide (a cytochrome oxidase inhibitor)-triggered spatial memory impairments in rats<sup>[60]</sup> (Table 1). In addition to this, TV 3326 also considerably decreased the hippocampal cell damage produced by global ischaemia in gerbil and cerebral oedema triggered by a closed head injury in mice and also triggered the recovery of their memory abilities and motor skills.<sup>[43,44]</sup>

In rats, it has been confirmed by a genomic study that at the dose of 1 mg/kg, TV 3326 can considerably downregulate the hippocampi levels of the familial AD-related presenilin-1 (*PSEN1*) gene.<sup>[61]</sup> Interestingly, this action is useful in terms of reduction of A $\beta$  formation, as *PSEN1* is the main component of the  $\gamma$ -secretase complex, which has found to aid the A $\beta$  production through intramembraneous

proteolysis of amyloid precursor protein (APP).<sup>[62,63]</sup> In addition to this, TV 3326 at the dose of 1 mg/kg can also downregulate (~50% of control) the mRNA expression of casein kinase 1 delta mRNA, linked with pathological hallmarks in a number of neurodegenerative disorders in rat hippocampus.<sup>[61]</sup>

It has been confirmed by the *in vitro* studies that at the doses of 1–10  $\mu$ M, TV 3326 exhibits neuroprotective properties, for instance, inhibition of OS-induced ROS production, weakening of apoptotic cascades and prevention of the reduction of the mitochondrial membrane potential ( $\psi$ ).<sup>[43,56,64–66]</sup> Moreover, TV 3326 (1–10  $\mu$ M) can also exert substantial neuroprotective action, for instance, Bcl-2 induction and a decrease of *BAD* and *BAX* gene and protein expression and reduction of caspase-3 activation.<sup>[67]</sup> On the other hand, it has been confirmed by the previous results that propargyl moiety of rasagiline also promoted neuronal survival, arbitrated by mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK)- and protein kinase C (PKC)-dependent activation, connected with Bcl-2 family members<sup>[68]</sup> and mitochondrial membrane stabilization<sup>[64,67]</sup> and therefore might possess significant contribution in the neuroprotective effect of TV 3326. In addition to this, TV 3326 also has various stimulatory actions on MAPK and PKC cascades,<sup>[69]</sup> stimulating the phosphorylation of p42 and p44 MAPK, which was found to be obliterated by certain inhibitors of MAPK activation.<sup>[69]</sup> Then again, activation of ERK in the CNS has also been implied in mammalian learning and synaptic plasticity.<sup>[70]</sup> Therefore, the neuroprotective properties of TV 3326 may also take place due to the activation of ERK (Figure 2), which has also been observed in the case of compounds which contain propargylamine.<sup>[71]</sup>

As studies confirmed that neurotrophic factors have significant contribution in the function and development of neurons and might play roles as regulatory factors for memory, learning and synaptic transmission<sup>[72,73]</sup>; therefore, the neuroprotective property of TV 3326 may also be linked with its induction on mRNA expression levels of the neurotrophic factors, including GDNF and also BDNF (Figure 2), in neuronal cell cultures.<sup>[38]</sup> In addition to this, following ligand–receptor binding, GDNF and BDNF can cause induction of intracellular signalling mechanisms involved in survival and differentiation, including phosphatidylinositol 3-kinase, mitogen-activated protein kinase and phospholipase C- $\gamma$ .<sup>[72,74,75]</sup> In the old rat hippocampus, TV 3326 caused upregulation of the brain-specific isoform of the synaptotagmin (Syn) family, Syn IV.<sup>[61]</sup> Henceforth, TV 3326-mediated increment of neurotrophic factors, which perhaps trigger respective cell signalling cascades, may recommend an association of neurotrophic factors in the neuroprotective mode of action of TV 3326.

**Table 1** Studies reported neuroprotective promise of TV 3326 in experimental models of neurodegeneration

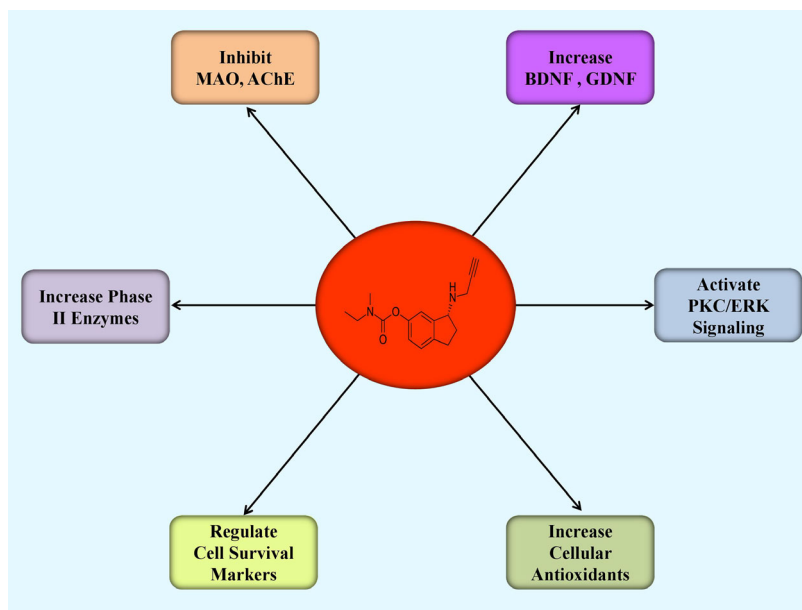
| Species/Studied Materials                                      | Mechanisms                                                                                                                                                                                                                                       | Effects                                                                                                                                                            | References |
|----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Young (8 months) and old (27 months) rats                      | Decreased ROS production as well as increase cell survival and synaptogenesis.                                                                                                                                                                   | Suppression of oxidative stress and mitochondrial dysfunction                                                                                                      | [61]       |
| Aged rats                                                      | Prevention of the astrocytes and microglia activation, as well as improved spatial memory                                                                                                                                                        | Antioxidant and anti-inflammatory effects                                                                                                                          | [115]      |
| Rats                                                           | Reduction of stereotyped motor behaviour and also preserve typical motor functions                                                                                                                                                               | Potential of aminergic and cholinergic transmission systems                                                                                                        | [116]      |
| Rats                                                           | Protection against spatial memory impairments                                                                                                                                                                                                    | Neuroprotective effects                                                                                                                                            | [43]       |
| Rats                                                           | Prevention of the choline acetyltransferase reduction, as well as the compensatory improvement in synaptic plasticity and the memory deficits                                                                                                    | Increase cytochrome oxidase activity                                                                                                                               | [60]       |
| Rats pheochromocytoma (PC12) cells                             | Reduction of the neuronal damage, microgliosis and memory impairments                                                                                                                                                                            | ChE inhibition and anti-apoptotic activity                                                                                                                         | [58]       |
| Rat PC12 and human SH-SY5Y neuroblastoma cells                 | Activation of MAPK, PKC and tyrosine kinase-dependent pathways                                                                                                                                                                                   | Anti-amyloidogenic effects                                                                                                                                         | [69]       |
| C57/BL mice                                                    | Regulation of APP processing and PKC-dependent mechanisms                                                                                                                                                                                        | Neuroprotective effects                                                                                                                                            | [83]       |
| SK-N-SH cells                                                  | Increased expression levels of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor                                                                                                                                 | Suppression of apoptosis and induce neurorescue                                                                                                                    | [38]       |
| Dopaminergic SH-SY5Y cells                                     | Stabilization of the mitochondrial membrane potential                                                                                                                                                                                            | Anti-apoptotic-neuroprotective effects                                                                                                                             | [64]       |
| Human neuroblastoma SK-N-SH cells                              | Reduction of cell death via inhibition of the cleavage and prevention of caspase-3 activation<br>Reduction of apoptotic-induced levels of holo-APP protein.<br>Stimulation of the release of the non-amyloidogenic $\alpha$ -secretase activity. | Neuroprotective effects, anti-amyloidogenic effects                                                                                                                | [67]       |
| Human neuroblastoma SH-SY5Y cells                              | Protection of cells from apoptosis.                                                                                                                                                                                                              | Neuroprotective effects                                                                                                                                            | [78]       |
| Human SH-SY5Y neuroblastoma cells                              | Decreased ROS production and increase activity of catalase and glutathione reductase.<br>Upregulated mRNA levels of catalase, peroxiredoxin 1 and quinone oxidoreductase 1                                                                       | Suppression of oxidative stress-induced cell apoptosis                                                                                                             | [56,66]    |
| Old rhesus monkeys                                             | Improvement of cognitive functions                                                                                                                                                                                                               | Neuroprotective effects                                                                                                                                            | [57]       |
| Adult patients (55 to 85 years) with mild cognitive impairment |                                                                                                                                                                                                                                                  | No significant effects on the neuropsychological test battery (NTB) composite, disability assessment in dementia (DAD) and geriatric depression scale (GDS) scores | [117]      |

As like TV 3326, it has been observed that TV 3279 also has neuroprotective property, which has been seen in multiple cell culture and animal models of neurodegeneration, including providing protection against ischaemia-triggered cytotoxicity in PC12 cells,<sup>[44]</sup> reduction of oedema, memory and motor function impairments following closed head injury in mice,<sup>[43,76]</sup> and antagonizing the scopolamine-induced spatial memory deficit in rats.<sup>[43]</sup> These aforesaid observations further suggesting that the neuroprotective property of TV 3326 is not dependent on the inhibition of MAO. There are also chances that multiple processes are

involved in the neuroprotective activities of TV 3326 might also be linked with various intrinsic pharmacological effects of its propargyl moiety, which is found to be similar in case of drugs containing propargylamine.<sup>[77–79]</sup>

### Controlling APP processing

It is known that A $\beta$  accumulation has a significant contribution in AD; therefore, numerous studies have concentrated on probable therapeutic intervention along the amyloid pathways involved in AD.<sup>[41,80–82]</sup> In case of



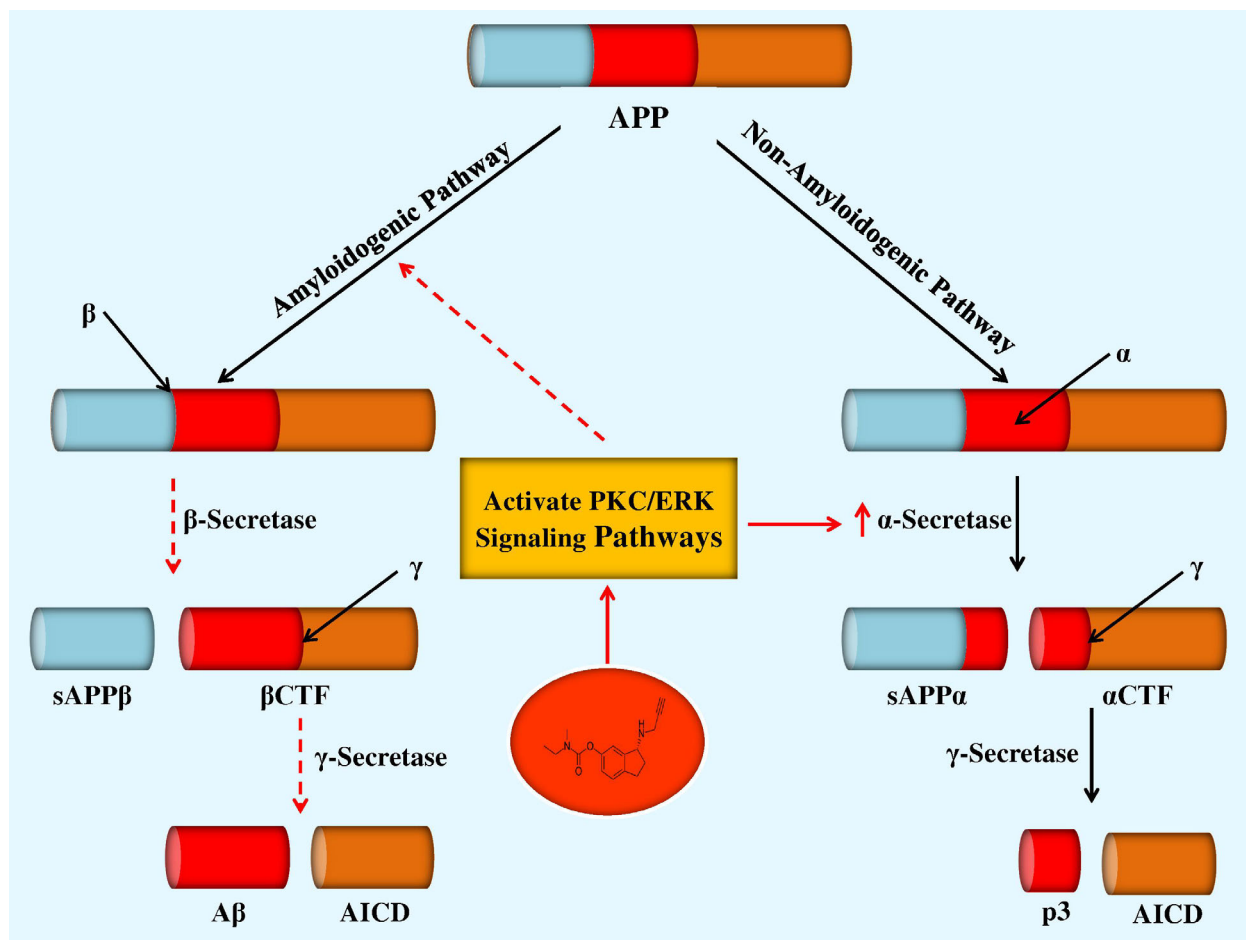
**Figure 2** Schematic outline of the neuroprotective and anti-Alzheimer's potentials of TV 3326 [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

various cellular model systems, current results confirmed that TV 3326 can cause significant elevation of soluble APP $\alpha$  (sAPP $\alpha$ ) and suppression of holo-APP protein levels, and this finding is found to be useful for accelerating the non-amyloidogenic APP processing pathway, thus decreasing the chance of the neurotoxic A $\beta$  generation.<sup>[67,69]</sup> In addition, it was also observed that in the mice hippocampus, cell-related holo-APP levels were found to be reduced due to the TV 3326 treatment, which further suggesting that expression of APP could also be controlled by TV 3326 *in vivo*.<sup>[83]</sup> On the other hand, levels of APP mRNA were not altered by TV 3326, which might recommend that the reduction in the levels of A $\beta$  and APP can lead to inhibition of APP translation, in analogy to another ChEI, phenserine.<sup>[84]</sup> Selective BuChE inhibitors have the capacity to decrease the levels of A $\beta$  and APP *in vivo* and *in vitro*.<sup>[85]</sup> Henceforth, the process underlying these actions might involve both non-cholinergic and cholinergic effects regulating APP processing and synthesis.

On the other hand, regulation of APP processing via TV 3326 was confirmed to involve MAPK- and PKC-dependent mechanisms<sup>[69]</sup> (Figure 3). Nevertheless, this neuroprotective action of TV 3326 does not take place solely due to its ChE inhibitory property, as propargylamine or rasagiline was also found to trigger the activation of ERK and PKC and also mediate the release of sAPP $\alpha$ .<sup>[86]</sup> In cell culture, the release of sAPP $\alpha$  was found to be elevated by a number of ChEIs including donepezil,<sup>[87]</sup> ganstigmine,<sup>[88]</sup> metrifonate,<sup>[89]</sup> physostigmine<sup>[90]</sup> and tacrine.<sup>[91]</sup>

Nevertheless, at high concentrations,<sup>[92]</sup> tacrine and phenserine<sup>[84]</sup> can reduce the release of sAPP $\alpha$ , which further recommends that the regulation of APP processing via ChEIs is not only connected to AChE inhibition. It is assumed that multiple processes are involved with this aforesaid phenomenon. For instance, in rats, ChEIs weakened the A $\beta$ -triggered downregulation of PKC<sup>[93]</sup> and elevated the levels of PKC *in vitro*.<sup>[94]</sup> Furthermore, donepezil has the ability to promote the trafficking of  $\alpha$ -secretase to the membrane; therefore, it has the capacity to increase the activity of  $\alpha$ -secretase.<sup>[87]</sup>

Like TV 3326, TV3279 acts as a ChEI; however, it does not have MAO inhibitory effect and also found to exert significant neuroprotective activity and APP processing, which further recommending that the mechanism of action is not dependent on inhibition of MAO.<sup>[67]</sup> These findings are in line with the previous results, which is providing strong proof that the neuroprotective actions of rasagiline (i.e. the irreversible MAO-B inhibitor) and TV 3326 are not dependent on MAO inhibition, rather linked with various intrinsic pharmacological effect of the propargyl moiety on the mitochondrial cell survival proteins.<sup>[78,79,95,96]</sup> It has been observed that propargylamine can cause inhibition of MAO activity considerably less than its abilities to induce anti-apoptotic and neuroprotective properties as well as regulating the processing of APP<sup>[68,79]</sup>. These aforesaid findings are further confirming that MAO inhibition is not essential to exert neuroprotective effect by TV 3326.



**Figure 3** Anti-Alzheimer's potentials of TV 3326 by regulating APP processing. In the amyloidogenic pathway, cleavages of APP by  $\beta$ - and  $\gamma$ -secretases lead to the genesis of A $\beta$  peptides and AICD. In the non-amyloidogenic pathway, cleavages of APP by  $\alpha$ - and  $\gamma$ -secretases lead to the genesis of p3 and AICD. TV 3326 enhances the non-amyloidogenic pathway of APP cleavage by enhancing  $\alpha$ -secretase activity and reduced A $\beta$  formation by reducing amyloidogenic pathway by means of activating PKC/ERK signalling pathways. APP, amyloid precursor protein; sAPP $\alpha$ , soluble APP alpha; sAPP $\beta$ , soluble APP beta;  $\alpha$ CTF, alpha C-terminal fragment;  $\beta$ CTF, beta C-terminal fragment; AICD, APP intracellular domain [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

### Antioxidant activity

In the pathogenesis of AD, OS is found to be arbitrated by ROS triggered by A $\beta$ .<sup>[97–99]</sup> It is known that hydrogen peroxide ( $H_2O_2$ ) is one of the major ROS which is generated during the redox reaction. In addition to this,  $H_2O_2$  is regarded as a messenger in case of intracellular signalling cascades, for instance, cellular proliferation and metabolism.<sup>[100,101]</sup> On the other hand,  $H_2O_2$  has the ability to cause DNA damage and lipid peroxidation, therefore, can trigger apoptosis in multiple cell types.<sup>[102–104]</sup> A number of proofs recommend that activation of BuChE and AChE may be associated with  $H_2O_2$ -associated apoptosis.<sup>[105,106]</sup> Indeed, in Alzheimer's, these aforesaid associations in between OS and the cholinergic signal can offer an additional therapeutic target for AChEIs. To protect the rat

PC12 cells against  $H_2O_2$  insult, the AChEIs including huperzine A<sup>[107]</sup> and tacrine<sup>[106]</sup> were found to be very effective. In SH-SY5Y cells, it was found that TV 3326 has noteworthy neuroprotective activity against the damage triggered by  $H_2O_2$ .<sup>[56]</sup> Furthermore, it has also been confirmed that TV 3326 exhibits its antioxidant effect via both direct free radical (i.e. generated in  $H_2O_2$ -treated cells) scavenging effects, and also indirect effect via inducing the expression and effect of cellular antioxidant enzymes including glutathione reductase and catalase, which further recommending their association in the cytoprotective effect of TV 3326.<sup>[56]</sup>

In the  $H_2O_2$ -treated SH-SY5Y cells, TV 3326 was found to increase the levels of mRNA expression of the antioxidant enzymes including quinone oxidoreductase 1 (NQO1), NADPH, peroxiredoxin 1 and catalase.<sup>[66]</sup> Then again, it has been observed from the study involving a high-density

cytotoxic model of SK-N-SH neuroblastoma cells, which is used in neuronal injury experiments as a potential ROS source,<sup>[108]</sup> that TV 3326 can trigger the levels of mRNA of the same antioxidant enzymes.<sup>[56]</sup> It has also been confirmed that NQO1 overexpression can induce neuroprotective effects against multiple toxins *in vitro*, which is also recommending its significant contribution in the CNS.<sup>[109,110]</sup> Moreover, the activity of NQO1 was found to be closely associated with the pathology of AD, which is suggesting that elevating the activity of NQO1 might offer a neuroprotective activity to treat neurodegenerative disorders.<sup>[111]</sup> Interestingly, it has been observed that TV 3326 has neuroprotective activity against multiple *in vitro* and *in vivo* injuries, for instance OS damage triggered either by glucose–oxygen deprivation<sup>[44]</sup> or by 3-morpholiniosydnonimine.<sup>[64,112]</sup>

In an *in vivo* study, the protective action of TV 3326 was found to be linked with its action against the OS. Therefore, chronic treatment with TV 3326 before and after the streptozotocin injection markedly decreased the changes in astrocytes and microglia and averted the rise in a marker of oxidative-OS, nitrotyrosine and the development of episodic memory impairments of rats.<sup>[59]</sup> This result also recommends that these effects are the result of the combination of effects of TV 3326 on glial and neuronal cells. On the other hand, the capacity to exert partial suppression of ChE can affect the episodic memory, as TV 3326 can cause inhibition of ChE activity in the cortex of the streptozotocin-induced AD rat model. In addition to this aforesaid finding, it was also observed that no inhibition of ChE activity was found to occur at the site of recognition, which was found to be dependent on cholinergic activity in the hippocampus. Therefore, due to the presence of distinct effects (i.e. for instance antioxidant effect), the memory impairments were found to be averted.<sup>[59]</sup> In addition to this, in rat, rasagiline was found to have the capacity to elevate the effects of antioxidant enzymes in the dopaminergic system of the brain.<sup>[113]</sup> The study on their peroxynitrite scavenging activity revealed that other propargylamine derivatives also have antioxidant effects.<sup>[114]</sup>

## Conclusions

TV 3326 possesses multiple functions, and its use might be beneficial to neurodegeneration with characteristics of

depression, dementia and extrapyramidal symptoms. Furthermore, it has the capacity to inhibit both BuChE and AChE. TV 3326 has also been found that as compared to rivastigmine, it exerts longer duration and slower onset of action. Its unique property to selectively suppress both MAO-A and MAO-B in the brain makes this agent an effective antidepressant. In mice, inhibition of MAO via TV 3326 can avert the neurotoxin MPTP-induced fall in the level of striatal dopamine, in addition, also can be helpful to maintain the levels of dopamine in individuals suffering from dementia. It is known that gliosis and OS are also observed in neurodegenerative disorders including AD. While studying with the rat model *in vivo*, the aforesaid features were found to be decreased. Therefore, the use of TV 3326 can be useful in decreasing neurodegeneration and in slowing down the progression of the disease. Collectively, it can easily be said that as TV 3326 contains various therapeutic effects, thus its use can be beneficial to treating Alzheimer's dementia.

## Declarations

### Conflict of Interests

The authors proclaim no conflict of interest.

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### Author Contributions

MSU conceived the original idea and designed the outlines of the study. MSU and MTK wrote the draft of the manuscript and prepared the figures for the manuscript. MMR, BM, MAS and GMA performed the literature review and aided in revising the manuscript.

## References

1. Hossain MF *et al.* Melatonin in Alzheimer's disease: a latent endogenous regulator of neurogenesis to mitigate Alzheimer's neuropathology. *Mol Neurobiol* 2019; 56: 8255–8276.
2. Uddin MS *et al.* APOE and Alzheimer's disease: evidence mounts that targeting APOE4 may combat Alzheimer's pathogenesis. *Mol Neurobiol* 2019; 56: 2450–2465.
3. Zaplatić E *et al.* Molecular mechanisms underlying protective role of quercetin in attenuating Alzheimer's disease. *Life Sci* 2019; 224: 109–119.
4. Lue L-F *et al.* Soluble amyloid  $\beta$  peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol* 1999; 155: 853–862.

5. Kamenetz F *et al.* APP processing and synaptic function. *Neuron* 2003; 37: 925–937.
6. Mamun AA *et al.* Toxic tau: structural origins of tau aggregation in Alzheimer's disease. *Neural Regen Res* 2020; 15: 1417–1420.
7. Walsh DM, Selkoe DJ. Abeta oligomers – a decade of discovery. *J Neurochem* 2007; 101: 1172–1184.
8. Walsh DM *et al.* Certain inhibitors of synthetic amyloid beta-peptide (Abeta) fibrillogenesis block oligomerization of natural Abeta and thereby rescue long-term potentiation. *J Neurosci* 2005; 25: 2455–2462.
9. Klyubin I *et al.* Amyloid beta protein dimer-containing human CSF disrupts synaptic plasticity: prevention by systemic passive immunization. *J Neurosci* 2008; 28: 4231–4237.
10. Klein WL *et al.* Targeting small A $\beta$  oligomers: the solution to an Alzheimer's disease conundrum? *Trends Neurosci* 2001; 24: 219–224.
11. Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell* 2005; 120: 545–555.
12. Uddin MS *et al.* Autophagic dysfunction in Alzheimer's disease: Cellular and molecular mechanistic approaches to halt Alzheimer's pathogenesis. *J Cell Physiol* 2019; 234: 8094–8112.
13. Harilal S *et al.* Advancements in nanotherapeutics for Alzheimer's disease: current perspectives. *J Pharm Pharmacol* 2019; 71: 1370–1383.
14. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid  $\beta$ -peptide. *Nat Rev Mol Cell Biol* 2007; 8: 101–112.
15. Busciglio J *et al.* Altered metabolism of the amyloid  $\beta$  precursor protein is associated with mitochondrial dysfunction in down's syndrome. *Neuron* 2002; 33: 677–688.
16. Uddin MS, Kabir MT. Oxidative stress in Alzheimer's disease: molecular hallmarks of underlying vulnerability. In: Ashraf G, Alexiou A, eds. *Biological, Diagnostic and Therapeutic Advances in Alzheimer's Disease*, 1st edn. Singapore: Springer, 2019: 91–115.
17. Kabir MT *et al.* Cholinesterase inhibitors for Alzheimer's disease: multitargeting strategy based on anti-Alzheimer's drugs repositioning. *Curr Pharm Des* 2019; 25: 1–17.
18. Colombres M *et al.* An overview of the current and novel drugs for Alzheimer's disease with particular reference to anti-cholinesterase compounds. *Curr Pharm Des* 2004; 10: 3121–3130.
19. Contestabile A. The history of the cholinergic hypothesis. *Behav Brain Res* 2011; 221: 334–340.
20. Kabir MT *et al.* NMDA receptor antagonists: repositioning of memantine as multitargeting agent for Alzheimer's therapy. *Curr Pharm Des* 2019; 25: 1–13.
21. Harel M *et al.* Quaternary ligand binding to aromatic residues in the active-site gorge of acetylcholinesterase. *Proc Natl Acad Sci* 1993; 90: 9031–9035.
22. Raves ML *et al.* Structure of acetylcholinesterase complexed with the nootropic alkaloid, (-)-huperzine A. *Nat Struct Biol* 1997; 4: 57–63.
23. Kryger G *et al.* Structure of acetylcholinesterase complexed with E2020 (Aricept®): implications for the design of new anti-Alzheimer drugs. *Structure* 1999; 7: 297–307.
24. Inestrosa NC *et al.* Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron* 1996; 16: 881–91.
25. Bourne Y *et al.* Structural insights into ligand interactions at the acetylcholinesterase peripheral anionic site. *EMBO J* 2003; 22: 1–12.
26. Piazzi L *et al.* 3-(4-[[Benzyl(methyl)amino]methyl]phenyl)-6,7-dimethoxy-2H-2-chromenone (AP2238) inhibits both acetylcholinesterase and acetylcholinesterase-induced beta-amyloid aggregation: a dual function lead for Alzheimer's disease therapy. *J Med Chem* 2003; 46: 2279–2282.
27. Carlier PR *et al.* Evaluation of short-tether bis-THA AChE inhibitors. A further test of the dual binding site hypothesis. *Bioorg Med Chem* 1999; 7: 351–357.
28. Bartolini M *et al.* beta-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. *Biochem Pharmacol* 2003; 65: 407–416.
29. Al Mamun A, Uddin MS. KDS2010: a potent highly selective and reversible MAO-B inhibitor to abate Alzheimer's disease. *Comb Chem High Throughput Screen* 2020; 23. <https://doi.org/10.2174/1386207323666200117103144>. (In Press)
30. Walker WH *et al.* The covalently-bound flavin of hepatic monoamine oxidase. 2. Identification and properties of cysteinyl riboflavin. *Eur J Biochem* 1971; 24: 328–331.
31. Chiba K *et al.* Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochem Biophys Res Commun* 1984; 120: 574–578.
32. Binda C *et al.* Structure of human monoamine oxidase B, a drug target for the treatment of neurological disorders. *Nat Struct Biol* 2002; 9: 22–26.
33. Edmondson DE *et al.* New insights into the structures and functions of human monoamine oxidases A and B. *J Neural Transm* 2007; 114: 703–705.
34. Carreiras MC, Marco JL. Recent approaches to novel anti-Alzheimer therapy. *Curr Pharm Des* 2004; 10: 3167–3175.
35. Weinstock M *et al.* TV3326, a novel neuroprotective drug with cholinesterase and monoamine oxidase inhibitory activities for the treatment of Alzheimer's disease. *J Neural Transm Suppl* 2000; 60: S157–S170.
36. Weinreb O *et al.* Multifunctional neuroprotective derivatives of rasagiline as anti-alzheimer's disease drugs. *Neurotherapeutics* 2009; 6: 163–174.
37. Weinstock M *et al.* Ladostigil, a novel multifunctional drug for the treatment of dementia co-morbid with depression. *J Neural Transm Suppl* 2006; 70: 443–446.

38. Weinreb O *et al.* Induction of neurotrophic factors GDNF and BDNF associated with the mechanism of neurorescue action of rasagiline and ladostigil: New insights and implications for therapy. *Ann New York Acad Sci*, Blackwell Publishing Inc., 2007; 1122: 155–168.
39. Uddin MS *et al.* Nootropic and anti-Alzheimer's actions of medicinal plants: molecular insight into therapeutic potential to alleviate Alzheimer's neuropathology. *Mol Neurobiol* 2019; 56: 4925–4944.
40. Bennett DA. Mixed pathologies and neural reserve: Implications of complexity for Alzheimer disease drug discovery. *PLoS Medicine* 2017; 14: e1002256.
41. Uddin MS *et al.* Emerging signal regulating potential of small molecule biflavonoids to combat neuropathological insults of Alzheimer's disease. *Sci Total Environ* 2020; 700: 1–11.
42. Weinstock M *et al.* A novel cholinesterase and brain-selective monoamine oxidase inhibitor for the treatment of dementia comorbid with depression and Parkinson's disease. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2003; 27: 555–561.
43. Weinstock M *et al.* TV3326, a novel neuroprotective drug with cholinesterase and monoamine oxidase inhibitory activities for the treatment of Alzheimer's disease. In: *Advances in Research on Neurodegeneration*. Vienna: Springer Vienna, 2000: 157–169. doi:10.1007/978-3-7091-6301-6\_10.
44. Weinstock M *et al.* Development of a novel neuroprotective drug (TV3326) for the treatment of Alzheimer's disease, with cholinesterase and monoamine oxidase inhibitory activities. *Drug Dev Res* 2000; 50: 216–222.
45. Sagi Y *et al.* The neurochemical and behavioral effects of the novel cholinesterase-monoamine oxidase inhibitor, ladostigil, in response to L-dopa and L-tryptophan, in rats. *Br J Pharmacol* 2005; 146: 553–560.
46. Davis KL, Rosenberg GS. Induction of behavioral supersensitivity to apomorphine by DFP treatment. *Life Sci* 1981; 28: 1953–1959. [https://doi.org/10.1016/0024-3205\(81\)90304-0](https://doi.org/10.1016/0024-3205(81)90304-0).
47. Rastogi SK *et al.* Effects of cholinergic and GABAergic drugs on apomorphine-induced gnawing behavior in rats. *Biol Psychiatry* 1982; 17: 937–946.
48. Scali C *et al.* Effect of subchronic administration of metrifonate, rivastigmine and donepezil on brain acetylcholine in aged F344 rats. *J Neural Transm* 2002; 109: 1067–1080.
49. Izzo PN, Bolam JP. Cholinergic synaptic input to different parts of spiny striatonigral neurons in the rat. *J Comp Neurol* 1988; 269: 219–234.
50. Calabresi P *et al.* Electrophysiology of dopamine in normal and denervated striatal neurons. *Trends Neurosci* 2000; 23(10 Suppl): S57–63.
51. Groner E *et al.* The kinetics of inhibition of human acetylcholinesterase and butyrylcholinesterase by two series of novel carbamates. *Mol Pharmacol* 2007; 71: 1610–1617.
52. Hubálek F *et al.* Inactivation of purified human recombinant monoamine oxidases A and B by Rasagiline and its analogues. *J Med Chem* 2004; 47: 1760–1766.
53. Sterling J *et al.* Novel Dual inhibitors of AChE and MAO derived from hydroxy aminoindan and phenethylamine as potential treatment for Alzheimer's disease. *J Med Chem* 2002; 45: 5260–5279.
54. Sagi Y *et al.* Attenuation of MPTP-induced dopaminergic neurotoxicity by TV3326, a cholinesterase-monoamine oxidase inhibitor. *J Neurochem* 2003; 86: 290–297.
55. Weinstock M *et al.* Limited potentiation of blood pressure response to oral tyramine by brain-selective monoamine oxidase A-B inhibitor, TV-3326 in conscious rabbits. *Neuropharmacology* 2002; 43: 999–1005.
56. Bar-Am O *et al.* The novel cholinesterase-monoamine oxidase inhibitor and antioxidant, ladostigil, confers neuroprotection in neuroblastoma cells and aged rats. *J Mol Neurosci* 2009; 37: 135–145.
57. Buccafusco JJ *et al.* Potential cognitive actions of (n-propargyl-(3R)-aminoindan-5-yl)-ethyl, methyl carbamate (tv3326), a novel neuroprotective agent, as assessed in old rhesus monkeys in their performance of versions of a delayed matching task. *Neuroscience* 2003; 119: 669–678.
58. Weinstock M *et al.* Neuroprotective effects of novel cholinesterase inhibitors derived from rasagiline as potential anti-Alzheimer drugs. *Ann N Y Acad Sci* 2006; 939: 148–161.
59. Shoham S *et al.* Ladostigil prevents gliosis, oxidative-nitrative stress and memory deficits induced by intracerebroventricular injection of streptozotocin in rats. *Neuropharmacology* 2007; 52: 836–843.
60. Luques L *et al.* Chronic brain cytochrome oxidase inhibition selectively alters hippocampal cholinergic innervation and impairs memory: Prevention by ladostigil. *Exp Neurol* 2007; 206: 209–219.
61. Weinreb O *et al.* The application of proteomics and genomics to the study of age-related neurodegeneration and neuroprotection. *Antioxidants Redox Signal* 2007; 9: 169–179.
62. Vetrivel KS, Thinakaran G. Amyloidogenic processing of beta-amyloid precursor protein in intracellular compartments. *Neurology* 2006; 66 (Issue 1, Supplement 1): S69–S73.
63. Kern A *et al.* Down-regulation of endogenous amyloid precursor protein processing due to cellular aging. *J Biol Chem* 2006; 281: 2405–2413.
64. Maruyama W *et al.* Anti-apoptotic action of anti-Alzheimer drug, TV3326 [(N-propargyl)-(3R)-aminoindan-5-yl]-ethyl methyl carbamate, a novel cholinesterase-monoamine oxidase inhibitor. *Neurosci Lett* 2003; 341: 233–236.
65. Wadia JS *et al.* Mitochondrial membrane potential and nuclear changes in apoptosis caused by serum and nerve growth factor withdrawal: time course and modification by (-)-deprenyl. *J Neurosci* 1998; 18: 932–947.
66. Weinreb O *et al.* The neuroprotective effect of ladostigil against hydrogen

- peroxide-mediated cytotoxicity. *Chem Biol Interact* 2008; 175: 318–326.
67. Yogev-Falach M *et al.* A multifunctional, neuroprotective drug, ladostigil (TV3326), regulates holo-APP translation and processing. *FASEB J* 2006; 20: 2177–2179.
  68. Weinreb O *et al.* Neuroprotection via pro-survival protein kinase C isoforms associated with Bcl-2 family members. *FASEB J* 2004; 18: 1471–1473.
  69. Yogev-Falach M *et al.* Involvement of MAP kinase in the regulation of amyloid precursor protein processing by novel cholinesterase inhibitors derived from rasagiline. *FASEB J* 2002; 16: 1674–1676.
  70. Sweatt JD. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J Neurochem* 2008; 76: 1–10.
  71. Weinreb O *et al.* Neuroprotective multifunctional iron chelators: from redox-sensitive process to novel therapeutic opportunities. *Antioxid Redox Signal* 2010; 13: 919–949.
  72. Siegel GJ, Chauhan NB. Neurotrophic factors in Alzheimer's and Parkinson's disease brain. *Brain Res Brain Res Rev* 2000; 33: 199–227.
  73. Shen L *et al.* Recent progress in studies of neurotrophic factors and their clinical implications. *J Mol Med (Berl)* 1997; 75: 637–644.
  74. Segal RA, Greenberg ME. Intracellular signaling pathways activated by neuropathic factors. *Annu Rev Neurosci* 1996; 19: 463–489.
  75. Murakami H *et al.* Enhanced phosphatidylinositol 3-kinase activity and high phosphorylation state of its downstream signalling molecules mediated by ret with the MEN 2B mutation. *Biochem Biophys Res Commun* 1999; 262: 68–75.
  76. Weinreb O *et al.* Ladostigil: a novel multimodal neuroprotective drug with cholinesterase and brain-selective monoamine oxidase inhibitory activities for Alzheimer's disease treatment. *Curr Drug Targets* 2012; 13: 483–494.
  77. Akao Y *et al.* Mitochondrial permeability transition mediates apoptosis induced by N-methyl(R)salsolinol, an endogenous neurotoxin, and is inhibited by Bcl-2 and rasagiline, N-propargyl-1(R)-aminoindan. *J Neurochem* 2002; 82: 913–923.
  78. Maruyama W *et al.* Neurotoxins induce apoptosis in dopamine neurons: Protection by N-propargylamine-1(R)- and (S)-aminoindan, rasagiline and TV1022. *J Neural Transm, Suppl.* 2000; 60: 171–186. [https://doi.org/10.1007/978-3-7091-6301-6\\_11](https://doi.org/10.1007/978-3-7091-6301-6_11).
  79. Bar-Am O *et al.* Regulation of Bcl-2 family proteins, neurotrophic factors, and APP processing in the neurorescue activity of propargylamine. *FASEB J* 2005; 19: 1899–1901.
  80. Pakaski M, Kasa P. Role of acetylcholinesterase inhibitors in the metabolism of amyloid precursor protein. *Curr Drug Targets CNS Neurol Disord* 2003; 2: 163–171.
  81. Rahman MA *et al.* Emerging potential of naturally occurring autophagy modulator against neurodegeneration. *Curr Pharm Des* 2020; 26. <https://doi.org/10.2174/1381612826666200107142541>.
  82. Uddin MS, Kabir MT. Emerging signal regulating potential of genistein against Alzheimer's disease: a promising molecule of interest. *Front Cell Dev Biol* 2019; 7: 1–12.
  83. Bar-Am O *et al.* Regulation of protein kinase C by the anti-Parkinson drug, MAO-B inhibitor, rasagiline and its derivatives, in vivo. *J Neurochem* 2004; 89: 1119–1125.
  84. Shaw KTY *et al.* Phenserine regulates translation of  $\alpha$ -amyloid precursor protein mRNA by a putative interleukin-1 responsive element, a target for drug development. *Proc Natl Acad Sci* 2001; 98: 7605–7610.
  85. Greig NH *et al.* A new therapeutic target in Alzheimer's disease treatment: attention to butyrylcholinesterase. *Curr Med Res Opin* 2001; 17: 159–165.
  86. Yogev-Falach M *et al.* The importance of propargylamine moiety in the anti-Parkinson drug rasagiline and its derivatives in MAPK-dependent amyloid precursor protein processing. *FASEB J* 2003; 17: 2325–2327.
  87. Zimmermann M *et al.* Acetylcholinesterase inhibitors increase ADAM10 activity by promoting its trafficking in neuroblastoma cell lines. *J Neurochem* 2004; 90: 1489–1499.
  88. Mazzucchelli M *et al.* Characterization of the effect of ganstigmine (CHF2819) on amyloid precursor protein metabolism in SH-SY5Y neuroblastoma cells. *J Neural Transm* 2003; 110: 935–947.
  89. Racchi M *et al.* Short- and long-term effect of acetylcholinesterase inhibition on the expression and metabolism of the amyloid precursor protein. *Mol Psychiatry* 2001; 6: 520–528.
  90. Lahiri DK *et al.* Effects of Cholinesterase Inhibitors on the Secretion of Beta-Amyloid Precursor Protein in Cell Cultures. *Ann N Y Acad Sci* 1997; 826(1 Cerebrovascul): 416–421.
  91. Chong YH, Suh Y-H. Amyloidogenic processing of Alzheimer's amyloid precursor protein in vitro and its modulation by metal ions and tacrine. *Life Sci* 1996; 59: 545–557.
  92. Lahiri DK, Farlow MR. Differential effect of tacrine and physostigmine on the secretion of the  $\beta$ -amyloid precursor protein in cell lines. *J Mol Neurosci* 1996; 7: 41–49.
  93. Zhang HY *et al.* Huperzine A enhances the level of secretory amyloid precursor protein and protein kinase C- $\alpha$  in intracerebroventricular  $\beta$ -amyloid-(1–40) infused rats and human embryonic kidney 293 Swedish mutant cells. *Neurosci Lett* 2004; 360: 21–24.
  94. Pakaski M *et al.* Reversible and irreversible acetylcholinesterase inhibitors cause changes in neuronal amyloid precursor protein processing and protein kinase C level in vitro. *Neurochem Int* 2001; 38: 219–226.
  95. Maruyama W *et al.* N-Propargyl-1(R)-aminoindan, rasagiline, increases glial cell line-derived neurotrophic

- factor (GDNF) in neuroblastoma SH-SY5Y cells through activation of NF-kappaB transcription factor. *Neurochem Int* 2004; 44: 393–400.
96. Akao Y *et al.* An anti-Parkinson's disease drug, N-propargyl-1(R)-aminoindan (rasagiline), enhances expression of anti-apoptotic bcl-2 in human dopaminergic SH-SY5Y cells. *Neurosci Lett* 2002; 326: 105–108.
  97. Mamelak M. Alzheimer's disease, oxidative stress and gammahydroxybutyrate. *Neurobiol Aging* 2007; 28: 1340–1360.
  98. Nunomura A *et al.* Oxidative damage is the earliest event in Alzheimer disease. *J Neuropathol Exp Neurol* 2001; 60: 759–767.
  99. Uddin MS, Upaganlawar AB. *Oxidative Stress and Antioxidant Defense: Biomedical Value in Health and Diseases*. USA: Nova Science Publishers, 2019.
  100. Rhee SG *et al.* antioxidant defense mechanisms: a new thiol-specific antioxidant enzyme. *Ann N Y Acad Sci* 2006; 738: 86–92.
  101. Stone JR, Yang S. Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal* 2006; 8: 243–270.
  102. Halliwell B. Reactive Oxygen Species and the Central Nervous System. *J Neurochem* 1992; 59: 1609–1623.
  103. Halliwell B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging* 2001; 18: 685–716.
  104. Zhang L *et al.* Protective effects of salidroside on hydrogen peroxide-induced apoptosis in SH-SY5Y human neuroblastoma cells. *Eur J Pharmacol* 2007; 564: 18–25.
  105. Schallreuter KU, Elwary S. Hydrogen peroxide regulates the cholinergic signal in a concentration dependent manner. *Life Sci* 2007; 80: 2221–2226.
  106. Xiao XQ *et al.* Bis(7)-tacrine, a promising anti-Alzheimer's agent, reduces hydrogen peroxide-induced injury in rat pheochromocytoma cells: comparison with tacrine. *Neurosci Lett* 2000; 290: 197–200.
  107. Xiao XQ *et al.* Huperzine A protects rat pheochromocytoma cells against hydrogen peroxide-induced injury. *Neurosci Lett* 1999; 275: 73–76.
  108. Ba F *et al.* The establishment of a reliable cytotoxic system with SK-N-SH neuroblastoma cell culture. *J Neurosci Methods* 2003; 123: 11–22.
  109. Stringer JL *et al.* Presence and induction of the enzyme NAD(P)H: Quinone oxidoreductase 1 in the central nervous system. *J Comp Neurol* 2004; 471: 289–297.
  110. Zafar KS *et al.* Overexpression of NQO1 protects human SK-N-MC neuroblastoma cells against dopamine-induced cell death. *Toxicol Lett* 2006; 166: 261–267.
  111. SantaCruz KS *et al.* Regional NAD(P)H:quinone oxidoreductase activity in Alzheimer's disease. *Neurobiol Aging* 2004; 25: 63–69.
  112. Maruyama W, Naoi M. Neuroprotection by (-)-deprenyl and related compounds. *Mech Ageing Dev* 1999; 111: 189–200.
  113. Carrillo MC *et al.* Enhancing effect of rasagiline on superoxide dismutase and catalase activities in the dopaminergic system in the rat. *Life Sci* 2000; 67: 577–585.
  114. Dragoni S *et al.* Antioxidant properties of propargylamine derivatives: assessment of their ability to scavenge peroxynitrite. *J Pharm Pharmacol* 2006; 58: 561–565.
  115. Weinstock M *et al.* Ladostigil prevents age-related glial activation and spatial memory deficits in rats. *Neurobiol Aging* 2011; 32: 1069–1078.
  116. Sagi Y *et al.* The neurochemical and behavioral effects of the novel cholinesterase- monoamine oxidase inhibitor, ladostigil, in response to L-dopa and L-tryptophan, in rats. *Br J Pharmacol* 2005; 146: 553–560.
  117. Schneider LS *et al.* Low-dose ladostigil for mild cognitive impairment: A phase 2 placebo-controlled clinical trial. *Neurology* 2019; 93: e1474–e1484.