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Curcumin Analogues as Novel Anti-Alzheimer's Candidates: Synthesis Development Strategy, *In vitro*, Cell-Based and *In vivo* Studies

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ABSTRACT

Alzheimer's disease (AD) is a neurological illness that is known to cause a wide range of cognitive symptoms linked to amyloid plaque deposition, neurofibrillary tangles, oxidative stress, and neuronal death in the whole brain. Curcumin has shown promising efficacy in preclinical studies for AD treatment. However, it failed to exhibit expected clinical outcomes in clinical studies. Besides, this molecule was found to have low stability, solubility, and bioavailability properties. Hence, scientists have synthesized several curcumin analogues in order to improve their bioavailability and biological activity. In this narrative review, we aim to discuss the development of curcumin analogue synthesis published in 2016–2021 and its efficacy as an anti-Alzheimer's candidate through in vitro, cell-based, and in vivo studies. PubMed and Scopus database were searched using the keywords "curcumin" AND "analogues" OR "analogs" AND "Alzheimer's" to find relevant studies. In our review, we included 16 eligible journal articles to discuss. In total, 15 curcumin analogues exhibited promising efficacy in preclinical studies and are found suitable for anti-Alzheimer's candidates. Further studies should explore curcumin analogues' effectiveness in other AD sub-pathologies as well as its pharmacokinetic profile and ability to achieve target action in the brain.

Keywords: Curcumin analogues, anti-Alzheimer's candidates, Aβ biosynthesis, cell-based assay, transgenic mice

INTRODUCTION

Alzheimer's disease (AD) is a known disorder that is often characterized by the progressive loss of nerve cells in the brain, generally a common form of dementia (Rogan & Lippa, 2002; Thies & Bleiler, 2013). According to GBD 2019 Dementia Forecasting Collaborators (2022), the global prevalence of this disorder was reported to be 57.5 million cases in 2019 and is projected to rise to 152.5 million cases in 2050. Patients with AD often suffer from a diminished ability to think and talk, mood and behavior changes, memory loss, and several cognitive symptoms (Atri, 2019; Voulgaropoulou *et al*., 2019). Those symptoms are primarily related to the accumulation of senile plaques (SPs) and neurofibrillary tangles (NFTs) in the brain (Barry *et al*., 2011; Song *et al*., 2020). SPs are generated due to excess extracellular amyloid-β (Aβ), whereas NFTs are formed by abnormal deposition and hyperphosphorylation of Tau protein. Aβ is known to decrease the rate of new nerve cell formation or neurogenesis (Donovan *et al*., 2006; Zhou *et al*., 2011). Because the mortality rate is faster than the required regeneration of new nerve cells in these conditions, the number and function of nerve cells

will then decline (Lazarov & Marr, 2010; Vasic *et al*., 2019). Neuroinflammation, oxidative stress, genetics, and environmental aspects are other pathologies associated with neurodegradation (Andrade *et al*., 2019; Sung *et al*., 2020). The precise cause of AD is yet to be elucidated. Therefore, future studies into other pathogenesis of AD and effective drug therapy development are required.

Scientists, together with venture capital industry, the federal government, foundations, and philanthropy, need mega-funding as it can take decades to find a novel drug for AD treatment (Cummings *et al*., 2018). Since the memantine approval in 2003 (Soria Lopez *et al*., 2019), it was only aducanumab (an anti-Aβ monoclonal antibody) has gained the Food and Drugs Administration approval for AD treatment in 2021 (Tampi *et al*., 2021). Previously, Yiannopoulou *et al*. (2019) stated that roughly 200 clinical trials for Alzheimer's therapy had failed or ended in the last 10 years. Some of them are anti-amyloid (avagacestat, tarenflurbil, atabecestat, lanabecestat, verubecestat, bapineuzumab, solanezumab, and gammagard liquid) and anti-tau (LMTM). The authors also explain some reasons for the clinical trial failure. For one, AD has complex pathogenesis. The concept of drugs with one target is the first reason for the deterioration in its clinical study. Other factors include delays in initiating therapy, inaccuracies in determining therapeutic targets, and improper drug doses. However, this problem only keeps scientists motivated to discover novel drug for AD treatment. Further, anti-Alzheimer's candidates should have a multiple target effect, including repairing damaged nerve cells, regenerating nerve cells, and stimulating neurogenesis.

Based on recent developments, Cumming *et al*. (2020) reported that there were 29 anti-Alzheimer's candidates that have successfully entered the phase 3 clinical trial. These candidates were anti-Aβ monoclonal antibody (aducanumab, gantenerumab, solanezumab, and BAN2401), synaptic vesicle modulator 2A or SV2A (AGB101), anti-inflammatory (cromolyn + ibuprofen), sigma-1 receptor agonist (blarcamesine, AVP-786, AXS-05), receptor for advance glycation and end product/RAGE antagonist (azeliragon), glutamate modulator (troriluzole), D2 receptor partial agonist (brexpiprazole), and bacterial protease inhibitor (COR338). Other candidates are selective serotonin reuptake inhibitor (escitalopram), antioxidant from plant extracts (*Ginkgo biloba*), alpha-2 adrenergic agonist (guanfacine), neuroprotector (icosapent ethyl), vascular risk reduction (losartan + amlodipine + atorvastatin), tyrosine kinase inhibitor (masitinib), insulin sensitizer (metformin), dopamine reuptake inhibitor (methylphenidate), adrenergic alpha-1 antagonist (mirtazapine), acetylcholinesterase inhibitor (octahedron-aminocridine succinate), ketone body stimulant (tricaprilin), tau protein aggregation inhibitor (TRx0237), and modulator of GABA-A receptor (zolpidem and zopiclone).

Various research papers have widely reported the potential of curcumin and its analogues as drug candidates for AD treatment. Curcumin, which is a natural compound from *Curcuma longa* L., has been investigated intensively for various disease treatments (Hewlings & Kalman, 2017). The anti-inflammatory, antioxidant, neuroprotective, chemoprotective, antiviral, antifungal, antibacterial, antinociceptive, antineurodegenerative, antiproliferative, and antiatherosclerotic activities of curcumin are well documented (Noureddin *et al*., 2019; Voulgaropoulou *et al*., 2019). Some preclinical studies reported that curcumin can cross the blood-brain barrier (Hamada *et al*., 2020; Yan *et al*., 2021). Hence, curcumin has a chance to be developed as a drug candidate for central nervous system diseases, such as AD, Parkinson's disease, and multiple sclerosis (Benameur *et al*., 2021; Yavarpour-Bali *et al*., 2019). For AD treatment, curcumin can effectively inhibit Aβ aggregation and trigger Aβ degradation in vitro. Curcumin injection (500 μM in 200 μL PBS) into the carotid artery inhibits Aβ formation by 43% in transgenic amyloid precursor protein mice (APPsw Tg2576 mice). Meanwhile, high doses of curcumin bind to β-amyloid and prevent its aggregation (Yang *et al*., 2005). Hydroxy and methoxy group substitution on two aromatic rings improves the ability of the curcumin linker region to attach to the Aβ peptide (Bukhari & Jantan, 2015). Meanwhile, Chainoglou *et al.* (2020) stated that the keto-enol moiety tautomers have a crucial role in amplifying curcumin-Aβ peptide interaction.

Curcumin is also known to have good safety properties (Hewlings & Kalman, 2017), making it suitable as a drug candidate for treating diseases that require long-term therapy, such as neurodegenerative diseases. However, this compound has low bioavailability, solubility, absorption, rapid metabolism, and excretion (Dei Cas & Ghidoni, 2019; Prasad *et al*., 2014).

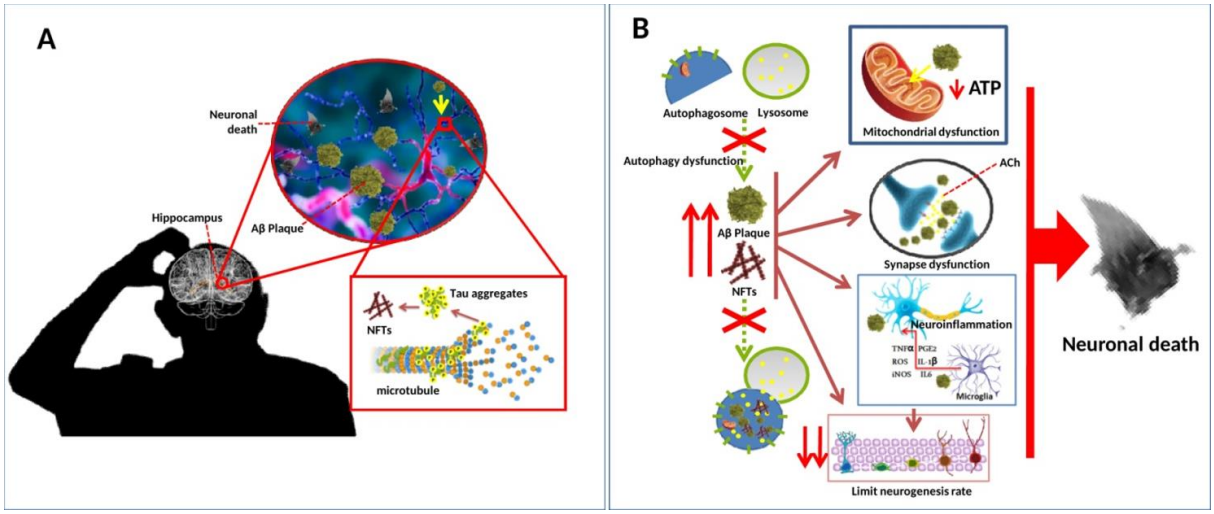

Figure 1. Pathophysiology of Alzheimer's disease. (A) Aβ plaque and NFT accumulation in hippocampus AD patients. (B) Autophagy dysfunction contributes to escalated Aβ and NFT levels. These two AD biomarkers further trigger mitochondrial and synaptic dysfunction, neuroinflammation and limited hippocampal neurogenesis, leading to neuronal loss and death.

Therefore, many scientists worldwide have synthesized curcumin analogues with better pharmacology and pharmacokinetic profile. This review article discusses AD pathophysiology, the development of curcumin analogue synthesis published in 2016–2021, and its efficacy that reveal its effect as an anti-Alzheimer's candidate through *in vitro*, cell-based, and *in vivo* studies.

PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

Alzheimer's disease is a neurodegenerative disorder commonly affecting the elderly; it is often related to diminished cognitive impairment and memory loss. Patients with AD may have trouble deciding, communicating, and creating judgments. Besides, they may experience psychological and behavioral changes (Sharma, 2019; Voulgaropoulou *et al*., 2019), like disorientation, anxiety, irritability, depression, sleep disorders, getting lost, and stop attending to hygiene and health (Koenig *et al*., 2016). These symptoms are seen to get more severe with age. Eventually, patients cannot conduct routine daily tasks and thus become dependent on others, contributing to the high cost of care (Mantzavinos and Alexiou, 2017). Pathologically, dysfunction and loss of cholinergic nerves in the hippocampus and cortex can result in diverse symptoms in AD patients (Zheng *et al*., 2016) (Figure 1).

Enormous toxic oligomer Aβ biosynthesis and abnormal phosphorylation of Tau protein generated diverse symptoms that are hallmarks of AD (Wu *et al*., 2021). Aβ (Aβ$_{38}$, Aβ$_{40}$, and Aβ$_{42}$), a degradation product of amyloid precursor protein (APP), has a crucial role in neurotransmitter transmission. But, during APP breakdown in AD patients, several anomalies were noted to develop, one of which is Aβ misfolding that generates toxic oligomers Aβ (Aβ plaque). This plaque has been found to prevent neurotransmitter transmission within the neural circuit; as a result, CNS enhances the biosynthesis of a beta-secretase to reinforce synaptic communication, driving more APP breakdown into Aβ (Hillen, 2019). Otherwise, Aβ plaque provokes the Tau protein hyperphosphorylation and improves NFT formation (Weller and Budson, 2018). Next, NFTs suppress the normal function of many other intracellular proteins in nerve cells, which could lead to neuronal death. Several published reports explained that increased NFTs might improve Aβ toxicity via a positive feedback loop mechanism, multiplying the resultant neurotoxic effect (Briggs *et al*., 2016).

Toxic oligomers Aβ and NFTs induce a chronic inflammatory response in the nerve cell membrane before neuronal death, particularly in the cortex and hippocampus (Sung *et al*., 2020). Aβ and NFTs simultaneously activate microglia as an innate immune response and decrease mitochondrial function. Monocytes, macrophages, neutrophils, and natural killer cells migrate from

the blood vessels into the CNS to protect affected neurons by accelerating Aβ clearance. However, the protective effect of microglia decreases in the end stage of AD, which may lead to an increase in inflammatory responses, such as interleukin (IL)-1β and tumor necrosis factor (TNF-α). Meanwhile, the interaction of Aβ with mitochondria diminishes ATP production, which is the primary energy source for nerve cells in the CNS. The ultimate result of this condition is a quicker rate of nerve cell damage and death (Wu *et al*., 2021). Another view of complicated AD pathogenesis explains that Aβ deposition, microglial activation, and neuro-inflammation limit neurogenesis rate (Karimipour *et al*., 2019) and synapse loss (Zheng *et al*., 2019) and that impaired neurogenesis cannot restore neuronal loss. In addition, the new nerve cells produced are noted to be fragile (Lazarov and Marr, 2010).

Previous authors show that autophagy dysfunction as a housekeeping system is involved in AD pathogenesis (Funderburk *et al*., 2010). Under normal physiology, autophagosome captures excess Aβ and Tau proteins and fuse with lysosomes. Lysosome peptidase and the ubiquitin-proteasome system will then degrade Aβ and Tau oligomers and remove them from the CNS (Di Meco *et al*., 2020). Yet, several mutations occurred in genes involved in the autophagosome-lysosomal pathway, such as mutations in the presenilin 1 (PSEN 1) and PSEN 2 genes. As a result, these mutations result in lysosomal downregulation and inhibition of autophagosome-lysosomal fusion (Zhang *et al*., 2022).

DEVELOPMENT STRATEGY OF CURCUMIN ANALOGUE SYNTHESIS FOR ANTI-ALZHEIMER'S CANDIDATE

Although it has a broad spectrum of pharmacological action, curcumin **(cur)** has been determined to be a polyphenol compound with poor stability, solubility, and bioavailability (Kumar *et al*., 2017; Maiti & Dunbar, 2018; Voulgaropoulou *et al*., 2019). Besides, curcumin also did not provide satisfactory outcomes in various previous clinical trials (Noureddin *et al*., 2019; Voulgaropoulou *et al*., 2019). Therefore, scientists have developed various curcumin analogues to improve their bioavailability and biological activity (Figure 2). According to Gupta *et al.* (2017), the methoxy group attached to the two aromatic rings contributes to curcumin's lipophilic properties. Furthermore, the keto-enol group in the central part of the curcumin structure is the cause of the low stability of curcumin in the digestive tract. Overall, that liability will then generate low bioavailability and a high variability effect (Chainoglou *et al*., 2020). Methoxy group modification attached in an aromatic ring with a hydroxy group enhances the solubility of curcumin. Venkateswarlu *et al*. (2005) synthesized four polyhydroxy curcumins **(3a–3d)** with better antioxidant properties than curcumin (IC_{50}: 4.6–14.6 μM). Polyhydroxy curcumin also shows an anti-inflammatory and neuroprotective effect (Khanna *et al*., 2009) and is the lead compound for drug discovery for AD (Chen *et al*., 2006). Hydroxy phenolic groups at the curcumin aromatic ring can also be replaced with palmitic acid, a long-chain fatty acid, through an ester bond. Qi *et al*. (2017) succeeded in synthesizing palmitic curcumin or p-curcumin **(7)**, a curcumin ester, and then examined its effect as a neuroprotector against the Aβ toxic effects. Since the palmitic acid can penetrate the non-polar parts of the cell membrane, palmitic moiety in the curcumin structure intends to improve the strength and duration of curcumin interaction with SH-SY5Y cells. As per the result, it was found that p-curcumin enhanced the protection of SH-SY5Y cells against Aβ-induced neurotoxicity.

Curcumin is a potent metal ion chelator (Prasad *et al*., 2021; Heger *et al*., 2014). Some literature has reported that curcumin interaction with copper ions (copper) plays a significant role in the pathology of several diseases associated with oxidative stress, such as neurodegenerative diseases (Atwood *et al*., 1998; Barik *et al*., 2005). The keto-enol part at the center of the curcumin structure was found to be responsible for chelating the metal ion (Priyadarsini, 2014). Ferrari *et al*. (2011) succeeded in synthesizing four curcumin analogues (K2T series) by inserting a short-chain alkyl group at the keto-enol moiety **(4a–4d)**. Those analogues have good stability in physiological solutions. Therefore, modification in the curcumin keto-enol group was determined to improve its biological activity as an anti-Alzheimer's candidate (Ferrari *et al*., 2017). Several other studies have also concluded that keto-enol group modification with the pyrazole group will enhance the permeability of the curcumin analogue into the brain (Liu *et al*., 2008).

These curcumin analogues have shown neuroprotective and anti-inflammatory effects by inhibiting the nuclear factor kappaB (NF-κB) and p38 mitogen-activated protein kinase (MAPK) pathways and improving memory function.

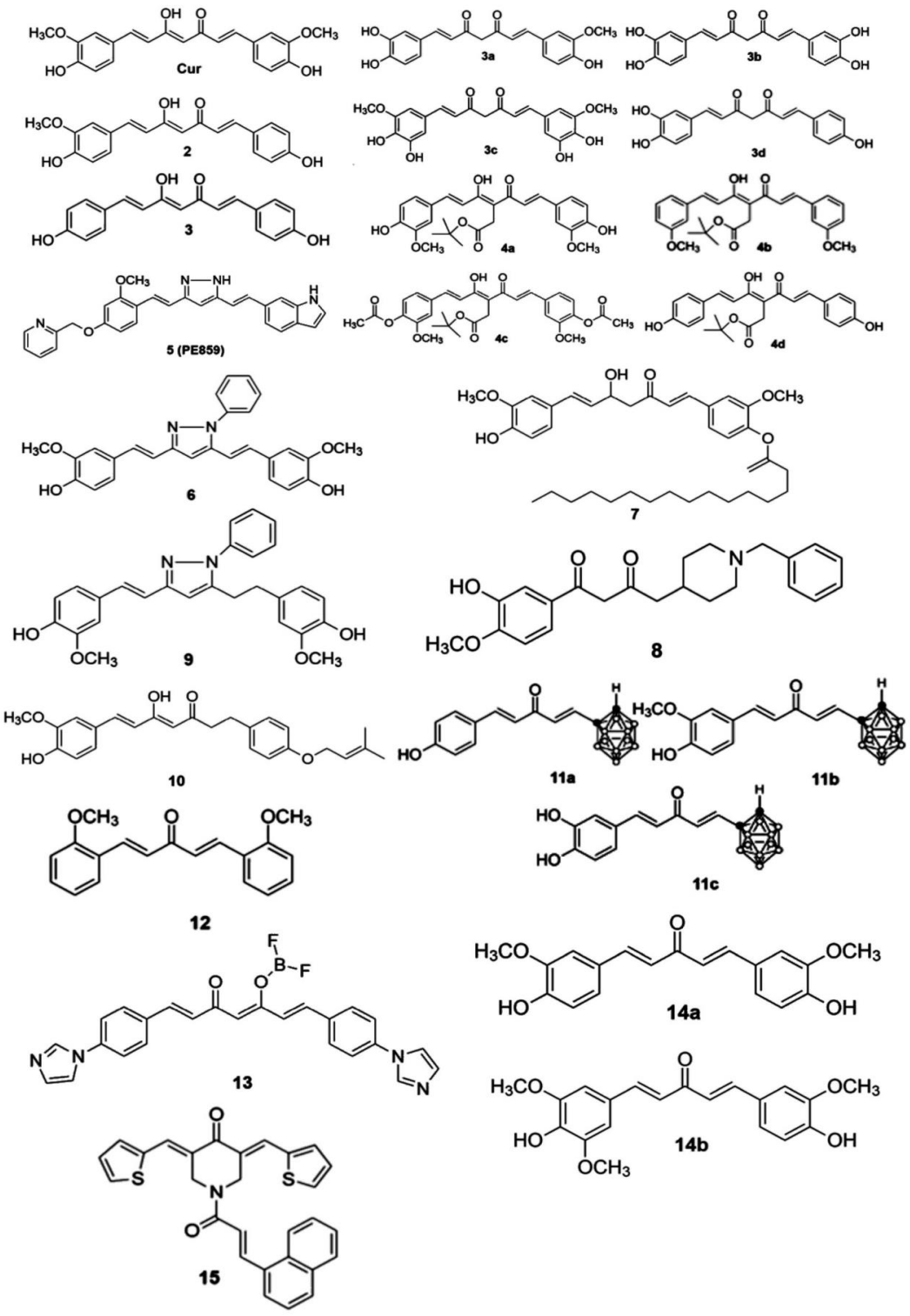

Figure 2. Chemical structure of curcumin (cur), curcuminoids (2–3), and its analogues (4–15) for anti-Alzheimer's candidates

Song *et al*. (2020) modified the keto-enol part of curcumin and produced 18 mono-carbonyl curcumin analogues. Analog C1 **(12)** determines as a transcription factor EB (TFEB) activator on three types of transgenic Alzheimer's mice. Furthermore, analogue C1 can develop for the prevention and treatment of AD. Mono-carbonyl curcumin has better stability than curcumin, with the same biological activity and pharmacokinetic profile even better than curcumin (Azzi *et al*., 2019). Lastly, Xu *et al*. (2019) reported that a mono-carbonyl curcumin analogue **(14a)** has a neuroprotective effect on PC1 2 cell-induced Aβ through its activities to increase the superoxide dismutase/catalase expression.

Curcumin structure modification has been accomplished simultaneously on both the aromatic ring and the keto-enol group. Okuda *et al*. (2016) have synthesized an asymmetric curcumin analogue PE859 **(5)** with an inhibitory effect on Aβ and Tau protein aggregation in vitro. Substitution of another cyclic compound at the aromatic ring of curcumin is noted to improve the curcumin analogue action to Tau protein aggregation. Meanwhile, modification of the keto-enol group with pyrazole was found to improve the pharmacokinetic profile, biological activity, and penetration of curcumin analogues into the brain (Kotani *et al*., 2017; Okuda *et al*., 2016). Bisceglia *et al*. (2019) synthesized four curcumin analogues by substituting ortho-hydroxy-methoxy groups on the aromatic ring with isoprene and mono-carbonyl. Analogue 1 **(10)** has been recognized as a multipotent compound anti-Alzheimer's candidate.

In recent years, curcumin analogues were developed by combining the curcumin structure with other pharmacophore substances such as donepezil and cinnamic acid. Yan *et al*. (2018) has synthesized 18 hybrid curcumin analogues, wherein analogue 11b **(8)** was found to be an anti-Alzheimer's candidate with a multi-target effect. Chainoglou *et al*. (2020) have also succeeded in synthesizing 18 hybrid analogues of curcumin with cinnamic acid, wherein analogue 3e **(15)** appears to be a potential lead compound for anti-Alzheimer's discovery. One of the latest structural modifications of curcumin to enhance its therapeutic effect, stability, and pharmacokinetic profile is to include non-endogenous elements such as boron into the curcumin structure (Azzi *et al*., 2019). In this context, they have successfully synthesized six boronated mono-carbonyl curcumin analogue compounds (BMAC). The BMAC 6a–6c **(11a–11c)** decreases Aβ fibril synthesis so that they have the potential to be developed as an anti-Alzheimer's candidate. According to another report, Wan *et al*. (2019) have replaced the keto-enol group of the curcumin structure with a difluoroboranyloxy group. They have substituted an imidazole group for the hydroxy group on curcumin's aromatic side chain. These modifications have resulted in 58 novel curcumin analogues known as CRANAD. CRANAD-17 **(13)**, known as curcumin-like compound 17 (CLC-17), is a curcumin analogue with promising anti-amyloid properties for AD treatment.

IN VITRO STUDIES

In vitro study is a preferred method for screening anti-Alzheimer's candidates (Table I). There were two applied methods, particularly *in vitro* acetylcholinesterase-butyrylcholinesterase (AChE-BChE) and thioflavin (ThT) fluorescence assay. The last method employed is capillary electrophoresis (CE) and transmission electron microscopy (TEM).

Curcumin analogues inhibit AChE and BChE activity

Acetylcholine is a major brain neurotransmitter that is known to play a crucial role in memory and learning (Solari & Hangya, 2018). In AD patients, acetylcholine declines because of cholinergic nerve damage in the basal forebrain (Francis, 2005). Moreover, acetylcholine degradation also appears to accelerate due to the high expression of AChE and BChE in the AD synaptic cholinergic nerve patient (Nyakas *et al*., 2011; Yan *et al*., 2017). To date, drugs used in AD therapy, such as donepezil and galantamine, work by inhibiting both AChE and BChE activities (Jung *et al*., 2009). Curcumin and its analogues are known for inhibiting AChE and BChE activities. Bisdemethoxycurcumin **(3)**, one of the curcuminoids present in turmeric, inhibits both AChE and BChE activities in vitro with IC_{50}: 2.14 ± 0.78 μM and 67.2 ± 0.6 μM, respectively (Kalaycıoğlu *et al*., 2017). Yan *et al*. (2017) have synthesized and examined 18 curcumin analogues. Among those, compound 11b **(8)** was determined as the most potent AChE (IC_{50}: 0.187 ± 0.015 μM) and BChE (IC_{50}: 12.4 ± 1.03 μM) inhibitor, with a BChE/AChE selectivity ratio of 66.3. However, the selectivity of this compound is lower than that of donepezil (BChE/AChE selectivity ratio: 85.4).

Table I. Summary of *in vitro* studies of curcumin analogues for AD Treatment

Curcumin Analogues	Method	Result	Ref.
Curcuminoid	*In vitro* inhibition of AChE and BChE	Bisdemehoxycurcumin **(3)** shows AChE and BChE activity inhibition better than curcumin and demethoxycurcumin	Kalaycıoğlu *et al*., 2017
Hybrid analogues of curcumin and donepezil	*In vitro* inhibition of AChE and BChE	Compound 11b **(8)** is a potent and selective AChE inhibitor	Yan *et al*., 2017
	In vitro ThT fluorescence assay	Compound 11b **(8)** had a moderate ability to inhibit Aβ aggregation	
Asymmetric curcumin analogues	*In vitro* ThT fluorescence assay	Compound 4 or PE859 **(5)** is the best Aβ and Tau protein aggregation inhibitor, better than curcumin	Okuda *et al*., 2016
K2T curcumin analogues	*In vitro* ThT fluorescence assay	K2T curcumin analogues **(4a, 4b and 4c)** inhibit Aβ fibrillation in a concentration-dependent manner.	Ferrary *et al*., 2017
Boronated monocarbonyl analogues curcumin (BMAC)	*In vitro* ThT fluorescence assay	BMAC 6c **(11c)** suppress fibril Aβ aggregation over 100% better than curcumin	Azzy *et al*., 2019
Prenylated curcumin analogues	CE and TEM	Analogue 1 **(10)** inhibits fibril $A\beta_{1-42}$ aggregation for up to 21 days in a concentration-dependent manner. This Analogue also inhibits Aβ oligomers formation.	Bisceglia *et al*., 2019

Curcumin analogues inhibit fibrillation and oligomerization of Aβ

Aβ plaque is an insoluble peptide that accumulates in the brain, which could result in cognitive deficits among AD patients (Voulgaropoulou *et al*., 2019). This plaque can develop due to Aβ fibrillation and oligomerization, which is toxic because it can promote oxidative stress and significantly contribute to nerve cell degeneration (Viola & Klein, 2015). Treatment that inhibits Aβ aggregation and increases Aβ degradation could improve and restore cognitive deficits in AD patients (Okuda *et al*., 2016). Aβ aggregation can be examined in vitro by thioflavin (ThT) fluorescence assay. ThT is a fluorescent compound that can bind specifically to the β sheet area in the Aβ structure. The instrument identifies Aβ aggregation through enhanced ThT fluorescence intensity (Wetzel *et al.*, 2018). Using this method, curcumin and its analogues bind and break β sheet Aβ (Okuda *et al*., 2016). Ferrari *et al*. (2017) revealed that K2T curcumin analogues **(4a, 4b, and 4c)** prevent the formation of fibrils $A\beta_{1-40}$ with 12.5±0.9 μM, 8.1±0.9 μM, and 7.5±0.9 μM of IC_{50}, respectively. These analogues provoke changes in the fibrils $A\beta_{1-40}$ morphology and further diminish their numbers. The aggregates are recognized in the form of short fibrils and globular aggregates. In total, 21 curcumin analogues synthesized by Okuda *et al*. (2016) reported inhibiting Aβ aggregation better than curcumin. In this study, Compound 4, also known as PE859 **(5)**, was inhibiting Aβ aggregation with an IC_{50} of 1.2 μM, better than curcumin (IC_{50}: 5.6 μM). Yan *et al*. (2017) also reported that three curcumin analogues (compounds 11b, 11d, and 11e) inhibit Aβ aggregation using the ThT assay. Compound 11b **(8)** has shown the most potent inhibitory effect (45.3%), although lower than curcumin (54.9%). Furthermore, BMAC 6c **(4c)** synthesized by Azzi *et al*. (2019) also can suppress fibril Aβ aggregation over 100% better than curcumin. Most recently, analogue 1 **(10)**, as synthesized by Bisceglia *et al*. (2019), was reported to inhibit fibril $A\beta_{1-42}$ aggregation up to 21 days after incubation in a concentration-dependent manner.

Inhibition of Aβ aggregation with ThT assay has limitations because it only estimates Aβ fibrillation. Consequently, the inhibition of Aβ oligomer formation for an anti-amyloid activity requires a specific method, such as CE. This technique can snapshot Aβ elongation when

forming toxic soluble oligomers Aβ (Lee *et al*., 2017). Analogue 1 **(10)** has been demonstrated to suppress toxic oligomers $A\beta_{1-42}$ formation better than curcumin. Based on these studies, curcumin analogues not only inhibit Aβ fibril formation, but also inhibit Aβ oligomer formation (Bisceglia *et al*., 2019).

CELL-BASED ASSAY STUDIES

Several pathways for developing anti-Alzheimer's candidates are Aβ biosynthesis inhibition, accelerated Aβ degradation, and neuroinflammation suppression as targeted action (Table II). There is mounting evidence reporting on the cell-based assay to learn drug candidate effect for AD treatment. Cell lines express a wide variety of biochemicals naturally or under cytotoxic molecule exposure (Ceitin *et al*., 2022). Several cell lines employed for anti-Alzheimer's candidate discovery are SH-SY5Y primary cell line, Chinese hamster ovary (CHO) cell line, human neuroblastoma SK-N-SH cell line, primary cultured rat microglia, and peripheral blood mononuclear cell (PBMC) from AD patients.

SH-SY5Y cell line

SH-SY5Y primary culture cells are human-derived cells that have been widely used in various in vitro experiments, including anti-Alzheimer's discovery (Strother *et al*., 2021). Since these are derived from human cells, SH-SY5Y cells express human-specific protein and protein isoforms. These proteins are often undetected in rodent primary cell culture (Kovalevich & Langford, 2013). One of the proteins expressed by the SH-SY5Y cell is neprilysin (NEP), which is an ectoenzyme involved in Aβ degradation (Grimm *et al*., 2013). Four curcumin analogues have been reported to increase NEP activity in the SH-SY5Y cell line. Conversely, curcumin did not increase NEP activity. Based on Western blot analysis, monohydroxylated demethoxycurcumin **(3a)** and dihydroxylated bisdemethoxycurcumin **(3b)** increased the expression of NEP in the membrane fraction of the undifferentiated SH-SY5Y cell line (Chen *et al*., 2016).

SH-SY5Y cells are known to express human amyloid-β protein precursor (AβPP). Furthermore, β- and γ-secretase separate the AβPP to compose Aβ (Thinakaran & Koo, 2008). Kotani *et al*. (2017) published a curcumin analogue known as CU6 **(6)**, showing an inhibitory effect on $A\beta_{1-40}$ and $A\beta_{1-42}$ generation in SH-SY5Y cells. Observation with immunoblotting analysis confirmed an increase in immature AβPP levels without affecting the expression of mRNA AβPP. Therefore, they concluded that CU6 **(6)** that increases the AβPP levels lies in the post-translational state. CU6 activity has been estimated through inhibition of AβPP cleavage to generate $A\beta_{1-40}$ and not due to repression in the synthesis of AβPP.

Accumulation of Aβ in brains induce neuronal loss through its ability to generate reactive oxygen species (ROS), such as hydroxyl radicals, superoxide, and hydrogen peroxide. ROS will trigger oxidation stress and nerve cell apoptosis (Yu *et al*., 2011). Aβ is known to be toxic to the SH-SY5Y cell line, wherein Aβ significantly diminishes cell viability (Wang *et al*., 2010). Qi *et al*. (2017) reported that a curcumin analogue known as p-curcumin **(7)** has a neuroprotective effect equal to curcumin in shielding the SH-SY5Y cell line against the toxic effects of oligomers and fibrillar Aβ. Based on the Fenton reaction, p-curcumin and curcumin also can scavenge ROS and significantly decrease ROS generation in SH-SY5Y cells induced by Aβ. The potency of p-curcumin as ROS scavenger and ROS generation inhibitor is equal to curcumin. Further, p-curcumin and curcumin significantly decrease Aβ-induced lipid peroxidation. Meanwhile, Bisceglia *et al*. (2019) reported that a curcumin analogue **(10)** suppressed intracellular ROS generation in H_2O_2-induced SH-SY5Y cells.

CHO cell line

An Aβ expression inhibitory effect of a compound can also be examined using the CHO cell line. These cell cultures express human AβPP751 (CHO-AβPP) (Koo & Squazzo, 1994). Kotani *et al*. (2017) concluded that four synthetic curcumin analogues (CU3, CU5, CU6, and CU7) showed an inhibitory effect on the $A\beta_{1-40}$ production CHO cell line without producing a toxic effect. Their results proved that CU6 **(6)** was the most potent inhibitor against $A\beta_{1-40}$ biosynthesis in the CHO cell line. Further, pretreatment CHO cell line with CU6 for 72 hours attenuate $A\beta_{1-40}$ and $A\beta_{1-42}$ synthesis and enhance AβPP level. However, CU6 only inhibits $A\beta_{1-40}$ and $A\beta_{1-42}$ biosynthesis in the early stages of its secretion pathway. The authors firmly believe this inhibition is not due to the increased expression of β- and γ-secretase.

Human neuroblastoma SK-N-SH cell line

Research evidence has reported that Aβ induces the release of pro-inflammatory cytokines through the NF-κB signaling pathway and upregulated inflammatory cytokines such as interleukin-6 (IL6), tumor necrosis factor-α (TNF-α), inducible NO synthase (iNOS), and cyclooxygenase-2 (COX-2) (Díaz *et al*., 2014).

Table II. Summary of cell-based assay results of the curcumin analogue for AD treatment

Curcumin Analogues	Cell line	Methods	Result	Ref.
Polyhydroxy curcuminoid	SH-SY5Y primary cell line	NEP activity assay and western-blot	Monohydroxylated demethoxycurcumin **(3a)** and dihidroxylated bisdemethoxycurcumin **(3b)** enhance NEP activity and NEP expression	Chen *et al*., 2016
Curcumin derivative	SH-SY5Y primary cell line	ELISA, immunoblotting analysis, RT-PCR	CU6 **(6)** inhibit $A\beta_{1-40}$ and $A\beta_{1-42}$ production and increase immature AβPP level but does not affect AβPP mRNA expression	Kotani *et al*., 2017
	CHO cell line	ELISA, immunoblotting analysis	CU6 **(6)** suppressed $A\beta_{1-40}$ secretion while elevating full-length AβPP levels in the CHO cell line. CU6 **(6)** only inhibits $A\beta_{1-40}$ and $A\beta_{1-42}$ biosynthesis in the early stages of its secretion pathway.	
Palmitic acid curcumin ester (P-curcumin)	SH-SY5Y primary cell line	MTT assay, cell staining, morphology analysis, Fenton-like reaction and lipid peroxidation assay	Despite having a specific affinity for some lipid components on the cell surface, P-curcumin **(7)** has an equal neuroprotective effect, scavenging ROS, declining ROS generation and lipid peroxidation with curcumin in Aβ-induced SH-SY5Y toxicity.	Qi *et al*.,2017
Prenylated curcumin analogues	SH-SY5Y primary cell line	2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) oxidation assay	Curcumin analogue **(10)** suppressed intracellular ROS generation in H_2O_2-induced SH-SY5Y cells better than curcumin.	Bisceglia *et al*., 2019
Synthesized curcuminoid	Human neuroblastoma SK-N-SH cell line	DCFH-DA oxydation assay, western blot, nitroblue tetrazolium method, immunocystochemistry	Di-O-demethylcurcumin **(3)** decreases ROS formation, NO and iNOS expression, inhibits NF-κB translocation into the nucleus and enhances Nrf2 expressions via translocation from the cytoplasm into the cell nucleus. Besides, di-O-demethylcurcumin increases SOD activity, an endogenous antioxidant enzyme in SK-N-SH cells induced by $A\beta_{25-35}$.	Pinkaew *et al*., 2016
Synthetic pyrazole derivate of curcumin	Primary cultured rat microglia	Colorimetric method, western blot, ORAC and HORAC activity assay kit, immunofluorescence staining	CNB-001 **(9)** decreased LPS-induced NO release, LPS-induced iNOS expression, LPS-induced NF-κB p65 translocation into the nucleus, and phosphorylated p38 MAPK expression	Akaishi & Abe, 2017
Prenylated curcumin analogues	Primary cultured rat microglia	ELISA	Analog 1 **(10)** inhibits the release of LPS-induced IL-1β and TNF-α from primary cultured rat microglia.	Bisceglia *et al*., 2019
	SH-SY5Y primary cell line	western blot	Analog 1 **(10)** triggers Nrf2 translocation into the nucleus	
Curcuminoid	PBMC from AD patient	Illumination MiSequencer and AlphaLISA	Bisdemethoxycurcumin **(3)** suppressed BACE-1, NF-κB expression and $A\beta_{1-42}$ biosynthesis and enhanced MGAT3, VDR mRNA expression, and MGAT3 gene expression	Gagliardi *et al*., 2018

These pro-inflammatory cytokines are involved in the nerve cell damage and progression of neurodegenerative diseases (Smith *et al*., 2012). Therefore, antioxidant and anti-inflammatory agents protect nerve cells through their neuroprotector effect. The neuroprotection effect of anti-Alzheimer's candidates can be examined using the human neuroblastoma SK-N-SH cell line (Ba *et al*., 2003). Pinkaew *et al*. (2016) examined the neuroprotective effect of di-*O*-demethylcurcumin **(3)** using SK-N-SH cells induced by AB_{25-35}. In that study, di-*O*-demethylcurcumin (2, 4, and 8 μM) has significantly decreased the formation of ROS, nitric oxide (NO), and iNOS expression in a concentration-dependent manner, as well as NF-κB translocation into the nucleus was inhibited. Moreover, di-*O*-demethylcurcumin enhances nuclear factor erythroid 2-related factor 2 (Nrf2) expressions via translocation from the cytoplasm into the cell nucleus. Furthermore, Nrf-2 binds to DNA and then initiates transcription genetic for antioxidants endogen syntheses, such as heme oxygenase (HO-1), glutamate-cysteine ligase catalytic (γ-GCLC), quinine oxidoreductase-1(NQO-1), and superoxide dismutase (SOD). The results also stated that di-*O*-demethylcurcumin inhibited the ROS formation in the human neuroblastoma SK-N-SH cell line induced by AB_{25-35} through the NF-κB pathway and inhibited translocation of the p65 subunit NF-κB into the nucleus and further reduced iNOS expression and production NO. Another significant finding showed that di-O-demethylcurcumin increases SOD activity, which is an endogenous antioxidant enzyme in SK-N-SH cells induced by $A\beta_{25-35}$.

Primary cultured rat microglia

Microglial cells release pro-inflammatory mediators in response to nerve cell damage in the brain (Harry & Kraft, 2008). However, an excessive microglia response will cause severe nerve cell loss, as seen in AD patients (Bachiller *et al*., 2018). Primary cultured rat microglia is a cell-based assay used to examine the neuroprotective effect of a drug candidate. Pretreatment of lipopolysaccharide (LPS) in these cell cultures will trigger the release of NO radicals, increase iNOS expression, and then generate an inflammatory reaction at the nerve cell membrane (Chen *et al*., 2017). Furthermore, examining the expression of transcription factors of NF-κB, MAPK, extracellular signal-regulated kinase (ERK), c-jun terminal kinase (JNK), and p38 MAPK can investigate the inflammatory response (Wang *et al*., 2014). Akaishi and Abe (2018) have reported that CNB-001 **(9)**, which is a curcumin analogue, can significantly decrease LPS-induced NO release in primary cultured rat microglia. In this context, 10 μM of CNB-001 was found to be more potent than 20 μM of curcumin. Based on Western blot analysis, 10 μM of CNB-001 significantly decreases LPS-induced iNOS expression (almost 100%) in primary cultured rat microglia. On the other hand, based on immunofluorescence staining analysis, CNB-001 seems to inhibit LPS-induced NF-κB p65 translocation into the nucleus of primary cultured rat microglia. CNB-001 significantly declined phosphorylated p38 MAPK expression, although there was no evidence that CNB-001 can suppress the phosphorylated ERK and phosphorylated JNK expression. Using a similar approach, Bisceglia *et al*. (2019) have examined the neuroprotective effect of curcumin analogues. In conclusion, analogue 1 **(10)** can inhibit LPS-induced interleukin-1β (IL-1β) and TNF-α release from primary cultured rat microglia. Western blot analysis concluded that curcumin and analogue 1 **(10)** 1–5 μM triggered Nrf2 translocation into the nucleus, which could explain the neuroprotection effect of curcumin and analogue 1 **(10)**.

Peripheral blood mononuclear cell (PBMC) from an Alzheimer's patient

Various researches have documented the neuroprotective activity of drug candidates investigated utilizing cell lines and animal models. Those models have different physiological conditions from brain AD patients. Conversely, only a few studies have reported employing cell-based experimental models from Alzheimer's patients (Gagliardi *et al*., 2012). PBMCs from AD patients are known to be the cells that can be used as a better model to investigate drug efficacy in inhibiting neurodegenerative processes. Gagliardi *et al*. (2018) reported that PMBCs from AD patients overexpress the beta-site of APP cleaving enzyme 1 (BACE1) and NF-κB. BACE1 is involved in Aβ formation, while NF-κB initiates an inflammatory response at the nerve cell membrane (Chami *et al*., 2012). Chen *et al*. (2012) reported that both proteins increased significantly in AD patients' brains. Otherwise, beta-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase (MGAT3) and 1,25-dihydroxyvitamin *D3 receptor* (VDR) protein decreased significantly, and this condition would then increase Aβ levels around the nerve cells of AD patients (Fiala *et al*., 2007). Aggarwal and Harikumar (2009) explained that MGAT3 would increase macrophage viability and enhance Aβ clearance.

Table III. Summary of curcumin analogue activity on animal models of AD

Curcumin Analogue	Animal model	Preparation	Dose and duration	Route	Result	Ref.
Polyhydroxy curcuminoid	5-month old-normal B6Ce mice	Compound in mixture of PEG 600 and Chremophor EL (v/v 2:1)	10 mg/kg for 7 days	oral	Compounds 7 and 8 **(3a and 3b)** enhance mRNA NEP levels in B6Ce mice brain tissue.	Chen *et al*., 2016
	3 month-The double-transgenic APPswe/PS1DE9 mice	Compound mixed with feed	10 mg/kg for six times a week 6.5-8.0 months	oral	Compound 7 **(3a)** enhances mRNA NEP levels and decimated $A\beta_{1-40}$ and $A\beta_{1-42}$ accumulation in mice cortex and hippocampus compared to mice receiving vehicles. Meanwhile, compound 8 **(3b)** effectively decreases amyloid plaque burden in the brain, especially in the hippocampus.	
Asymmetric curcumin analogue	8-month-Transgenic JNPL3 human Tau P301 mice	NA	20 mg/kg per day for four weeks	oral	Compound 4/PE859 **(5)** has a better pharmacokinetic profile than curcumin. Treatment with compound 4/PE859 **(5)**, 20 mg/kg per day for four weeks, significantly reduces insoluble Tau sarcosyl protein levels in transgenic JNPL3 human Tau P301 mice brain.	Okuda *et al*., 2016
Curcumin analog	One month-Transgenic P301S mice	Mixing with regular feed	5 and 10 mg/kg daily for three months	oral	Transgenic JNPL3 human Tau P301 mice treated with curcumin analogue C1 **(12)** can last longer on increased speed rotarod (4-40 rpm). This compound **(12)** also deteriorated Tau protein level (AT8 and PHF1), conformation-specific Tau (MC1), and total Tau protein in the soluble-sarcosyl fraction of P301S mice brain. Analogue C1 **(12)** also reduced AT8 protein in P301S mice cortex and hippocampus based on immunofluorescence staining.	Song *et al*., 2020
	Two mounth-5xFAD transgenic mice	Suspended in 1% CMC-Na	(10 mg/kg) daily for three months	oral	Analogue C1 **(12)** passes the blood-brain barrier with 261.7±55.3 ng/g brain level and enhanced memory deficit better than curcumin in 5xFAD mice. Treatment with C1 reduced APP and Aβ levels, accompanied by TFEB activation, increased autophagy, and lysosome activity in the cortex and hippocampus. This curcumin analogue also decreases Aβ load in the cortex and hippocampus of 5xFAD mice, reducing $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in an entire brain.	
	Six month female-3xTG mice	Suspended in 1% CMC-Na	5 and 10 mg/kg daily for seven months		Analogue C1 **(12)** decreases freezing time and increases 3xTg mice exploration around the open-field arena. The 3xTG mice treated with analogue C1 travelled a shorter distance to reach the hidden platform on the Morris water maze task.	

Meanwhile, the activation of VDR by vitamin D3 will trigger an immunostimulant effect in inducing phagocytosis and Aβ degradation by macrophages and monocytes. Gagliardi *et al*. (2018) have reported the neuroprotective effects of curcumin analogues using PMBCs in Alzheimer's patients. Observations with the Illumination MiSequencer concluded that bisdemethoxy-curcumin **(3)** significantly suppressed BACE-1 and NF-κB expression of PBMCs in Alzheimer's patients (6 and 8 times) compared to PBMCs in healthy volunteers. Conversely, bisdemethoxycurcumin increased MGAT3 and VDR mRNA expression in PBMCs in AD patients (5 and 6 times) compared to PBMCs in healthy volunteers. In their report, MGAT3 gene expression has also increased significantly. Based on immunofluorescence techniques, bisdemethoxycurcumin suppressed $A\beta_{1\text{-}42}$ synthesis in PBMCs of AD patients and did not affect PBMCs in healthy volunteers.

IN VIVO STUDIES

Drummond and Wisniewski (2017) reported 15 transgenic mice models to assess anti-Alzheimer's candidate activity in vivo. Transgenic mice exhibit mutations in many genes involved in APP biosynthesis, as well as mutant Tau protein and overexpression of human Aβ plague and NFTs. Four transgenic mouse models have been used to evaluate the anti-Alzheimer's effect of curcumin analogues (Table III).

Normal mice B6Ce

NEP is known to be involved in Aβ degradation, and several studies have reported decreased NEP expression in the brains of AD patients. This condition has led to Aβ accumulation and was associated with memory deficit (Hellström-Lindahl *et al*., 2008). NEP level also declines in normal mice with aging (Caccamo *et al*., 2005). Therefore, a treatment approach with a target action of increasing NEP expression in the brain is required to diminish Aβ accumulation and restore memory function. Chen *et al*. (2016) examined the efficacy of curcumin and its analogues 7 and 8 **(3a and 3b)** in normal B6Ce mice. Mice were treated with curcumin and its analogues (10 mg/kg) daily for 7 days. Mice were sacrificed 1 hour after the last dose, and the cortex and hippocampus were isolated from the rest of the brain tissue. Expression of mRNA NEP in mice brain was measured using real time-PCR. The results concluded that curcumin analogues significantly enhance mRNA NEP expression in the brain tissue of B6Ce mice. Conversely, curcumin did not show the same effect.

Double-transgenic APP_{swe}/PS_1DE_9 mice

Chen *et al*. (2016) also examined compound 7 **(3a)** on double-transgenic APP_{swe}/PS_1DE_9 mice, also known as double-transgenic mice APP/PS1, and found the same result. These mice had a genetic mutation in the genes that code for the APP and NEP biosynthesis.

Mice will show Aβ deposits at 4 months of age for the first time. In studies that develop innovative therapeutic approaches specifically targeted against amyloid plaque and associated neuroinflammation, these transgenic mice provide a valuable tool (Malm *et al*., 2011). The double-transgenic APP_{swe}/PS_1DE_9 mice were treated with compound 7 (10 mg/kg) six times a week for 6.5 months. As per the result, it was found that compound 7 significantly enhances mRNA NEP levels in the hippocampus and modestly in the cortex compared to mice receiving vehicles. Western blot analysis proved an increase in NEP protein expression in the cortex region of double-transgenic APP_{swe}/PS_1DE_9 mice treated with compound 7 compared to vehicle treatment. The curcumin analogue treatment drastically reduced $A\beta_{1\text{-}40}$ and $AB_{1\text{-}42}$ levels in the cortex and hippocampus. Other assay results concluded that the treatment of compound 8 **(3b)**, 10 mg/kg (p.o), six times a week for 8 months, decreased Aβ plaque load on the cortex by 28% and the hippocampus by 44% in double-transgenic APP_{swe}/PS_1DE_9 mice (Chen *et al*., 2016).

The chronic histone deacetylase inhibitor (HDACi) treatment to these double-transgenic mice pronounced contextual memory impairments. Injections of class I HDACs (HDAC1, 2, 3, 8) completely restored contextual memory in these mutants AD mice model (Kilgore *et al*., 2010). Curcumin, demethoxy curcumin and *bis*-demethoxy curcumin were reported as potent ligands for HDAC2 with 3 poses *in silico* by employing the retrospective-validated SBVS protocol. The selected docking poses of curcuminoids could be used as insights in further design and discovery novel HDAC2 ligands (Istyastono *et al*., 2016). Thus, the potential of curcuminoids as inhibitor for HDAC2 enable them to be anti-AD agents owing to the overexpression of HDAC2 in AD (Kilgore *et al*., 2010). In line with these, curcuminoids as HDAC inhibitors may change phosphorylation (Zhang *et al*., 2014) and/or decrease the stability of tau protein (Selenica *et al*.,

2014; Cook *et al*., 2014), resulting in reduced clinical features of tauopathies, a class of neurodegenerative diseases associated with the pathological aggregation of tau protein in the human brain.

Transgenic JNPL3 human Tau P301 mice

Okuda *et al*. (2016) examined a curcumin analogue PE859 **(5)** in transgenic JNPL3 human Tau P301 mice. These mice had a mutation in the microtubule-associated protein Tau (Mapt) gene that encodes the Tau protein, proving that Tau dysfunction can directly result in neurodegeneration (Chakrabarty *et al*., 2015). Transgenic JNPL3 human Tau P301 mice showed neurofibrillary tangle (NFT) development in almost all parts of the brain. Consequently, mice were experiencing motor and behavioral deficits. The symptoms were noted to begin at 4.5–6.5 months of age (Lewis *et al*., 2000). PE859 20 mg/kg per day for 4 weeks significantly attenuated insoluble Tau sarkosyl protein levels in transgenic JNPL3 human Tau P301 mice brain. Meanwhile, methylene blue and curcumin did not show this effect (Okuda *et al*., 2016).

Transgenic P301S mice

Song *et al*. (2020) reported the efficacy of analogue C1 **(12)** as an anti-Alzheimer's candidate in three types of transgenic mice (transgenic P301S, 5xFAD, and 3xTg mice). Similar to JNPL3 human Tau P301 mice, P301S mice also had mutations in the Mapt gene. They overexpressed the abnormal Tau protein. These transgenic mice usually experience an imbalance at 4 months of age. Also, excessive accumulation of abnormal Tau protein will lead to neuronal loss (Yoshiyama *et al*., 2007). Mice were treated with curcumin analogue C1 (5 and 10 mg/kg) daily for 3 months. The observations show that the transgenic JNPL3 human Tau P301 mice treated with C1 can last longer on increased speed rotarod (4–40 rpm) than the vehicle group. Furthermore, analogue C1 (5 and 10 mg/kg) also significantly decreased Tau protein level (AT8 and PHF1), conformation-specific Tau (MC1), and total Tau protein in the sarkosyl-soluble fraction of P301S mice brain. Based on immunofluorescence staining, analogue C1 dramatically decreased AT8 protein in P301S mice cortex and hippocampus. This finding proves that analogue C1 inhibits abnormal Tau protein aggregation in the P301S mice brain (Song *et al*., 2020).

5xFAD mice

5xFAD transgenic mice were characterized by increased APP levels and Aβ overexpression in their brains and linked to cognitive impairments (Oblak *et al*., 2021). Song *et al*. (2020) reported that transgenic 5xFAD was treated with analogue C1 (10 mg/kg) daily for 3 months intragastric. The treatment has significantly reduced APP and Aβ levels, accompanied by TFEB activation, increased autophagy, and lysosome activity in the cortex and hippocampus tissue. Immunochemistry and ELISA assay show that analogue C1 significantly decreases Aβ load in the cortex and hippocampus of 5xFAD mice, reducing $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ levels in a whole brain. The analogue C1 showed better activity than curcumin in TFEB-mediated autophagy and lysosome biogenesis. Another finding is analogue C1 found in the 5xFAD mice brain (261.7±55.3 ng/g). This finding also explains the effect of analogue C1 in degrading APP fragments and decreasing Aβ via TFEB, mediating autophagy and lysosome biogenesis in 5xFAD mice brains. Besides, high dose analogue C1 treatment was able to enhance memory deficit better than curcumin in 5xFAD mice.

3xTg mice

The 3xTg transgenic mice expressing more APP and Tau protein in the brain resulted in memory deficits and deteriorated locomotor activity. Song *et al*. (2020) treated 3xTG mice with analogue C1 and curcumin (10 mg/kg) daily for 7 months. They use an open-field test and the Morris water maze task to evaluate memory improvement. In the open-field test, 3xTg mice will remain silent longer after being placed in the middle of the arena than wild-type mice. This study concluded that analogue C1 decreases 3xTG mice freezing time in the central apparatus than in the vehicle. Analogue C1 in high dose increased 3xTg mice exploration around the open-field arena, almost close to wild-type mice. Curcumin did not show any significant effect in restoring 3xTg mice exploration. The 3xTG mice treated with analogue C1 traveled a shorter distance to reach the hidden platform on the Morris water maze task than the vehicle. Besides, 3xTG treated with analogue C1 (10 mg/kg) exhibited better ability in terms of finding quadrant targets.

Efforts to explore novel anti-Alzheimer's candidates have failed in many clinical trials for diverse reasons. Drugs with a single target action cannot provide the desired clinical outcome in AD patients. As a result, anti-Alzheimer's development with a multi-target activity in delaying AD sub-pathologies is a reasonable alternative (Benek *et al*., 2020). As many authors asserted, experimental studies have revealed various curcumin derivatives as potential anti-Alzheimer candidates.

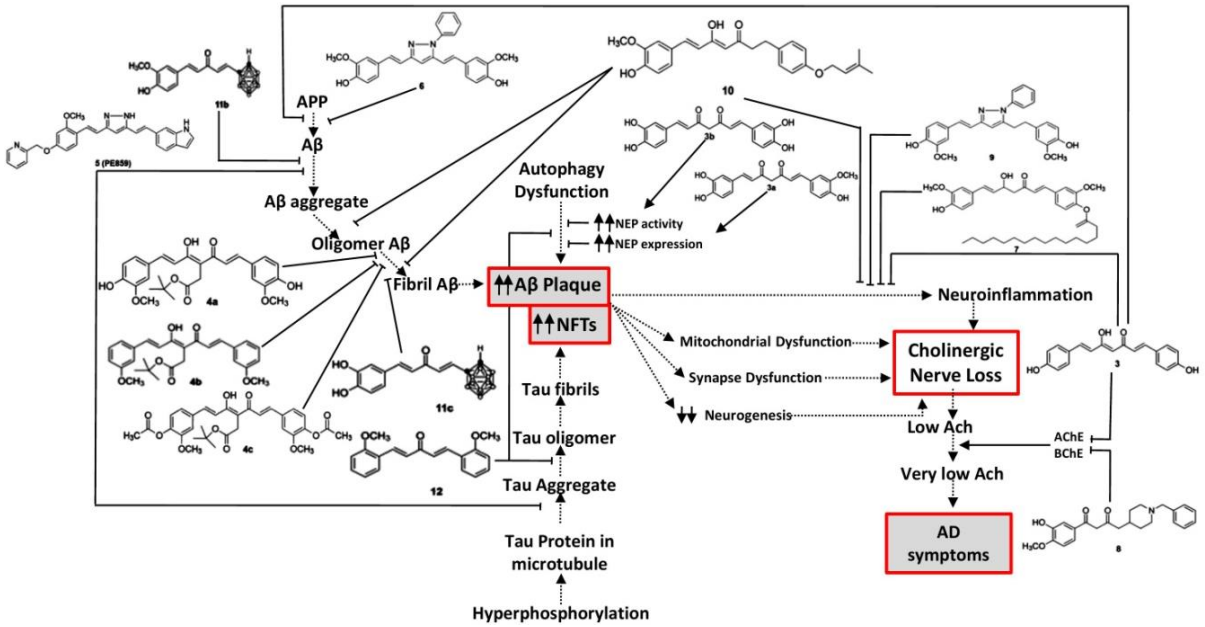

Figure 3. The proposed mechanism of curcumin analogues in alleviating AD symptoms based on *in vitro*, cell-based assay and in vivo studies. ⊣= inhibit; →=trigger; ↑↑=improvement; ↓↓ = reduction

Curcumin analogues are known to have multiple targets for preventing neuronal dysfunction and death as described in Figure 3. In vitro results have showed that bisdemethoxycurcumin **(3)** inhibits AChE and BChE activity (Kalaycıoğlu *et al*., 2017). In addition, this compound also inhibits Aβ biosynthesis and neuroinflammation (Pinkaew *et al*., 2016; Gagliardi *et al*., 2018). C1 **(12)**, which is another curcumin analogue, could improve cognitive deficits in transgenic JNPL3 human Tau P301 mice through its effect on accelerated autophagy and inhibiting Tau protein oligomerization (Song *et al*., 2020). Meanwhile, several other curcumin analogues such as K2T curcumin analogues (**4a, 4b, 4c**), BMAC (**11b and 11c**), and CU6 **(6)** showed inhibition toward just one sub-pathology or single target (Ferrari *et al*., 2017; Azzy *et al*., 2019; Kotani *et al*., 2019) and require further studies to confirm its effect on another pathway. Based on the AD pathophysiology as shown in Figure 3, further research needs to explore the curcumin analogues' effect on adult hippocampal neurogenesis, restoration of mitochondrial function, and synapsis formation.

Curcumin's main problem is its poor solubility and bioavailability. In our review, analogue C 1 **(12)** is the only verified curcumin analogue found in the brains of P301S transgenic mice (Song *et al*., 2020). That means structural modification should be able to produce curcumin analogues with improved pharmacokinetic properties. In addition, curcumin analogues must also be able to cross the blood-brain barrier (BBB) to reach drug targets in AD patients' brains. Hence, further research should aim to explore the pharmacokinetic profile of curcumin analogues and assess their ability to cross the BBB, which is a critical point in the novel anti-Alzheimer's development.

CONCLUSIONS

Alzheimer's disease is a progressive neurodegenerative disease that appears with ageing. Drugs for AD treatment have not provided significant clinical outcomes to date. Several natural compounds have successfully demonstrated potent efficacy in the preclinical trial. However, not one has provided good clinical outcomes and passed in clinical trials, including curcumin. Furthermore, researchers synthesize curcumin analogs through several strategies to suppress AD pathogenesis, particularly as an anti-amyloid and neuroprotector. Modification of curcumin chemical structure has yielded polyhydroxy curcumin, curcumin ester, KT2 series analogue, mono-carbonyl curcumin, prenylated curcumin, BMAC, curcumin hybrid, and CLC. Curcumin analogues effectively decrease AChE and

BChE activity or prevent Aβ fibrillation and oligomerization *in vitro*. Based on the cell-based assay, curcumin analogues showed a potent anti-amyloid effect by enhancing NEP, BACE-1, mRNA MGAT3, and VDR expression, inhibiting $A\beta_{1-40}$ and $A\beta_{1-42}$ biosynthesis. Curcumin analogues have also been shown to scavenge ROS, inhibit expression of iNOS and NF-κB translocation into the nucleus, decreases ROS and NO generation, lipid peroxidation, inhibits the release of IL-1β and TNF-α, trigger Nrf2 translocation into the cell nucleus, and enhance SOD synthesis. These findings support curcumin analogues as a potent neuroprotector that will inhibit neuronal loss in AD patients. *In vivo* studies also found that curcumin analogues enhance mRNA NEP expression in normal B6Ce mice and double-transgenic APPswe/PS_1DE_9 mice brains. The curcumin analogues treatment reduced $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in the cortex and hippocampus of transgenic APPswe/PS_1DE_9, JNPL3 human Tau P301, and 5xFAD mice brain. Also, curcumin analogues enhance locomotors activity and spatial or working memory in several transgenic mouse models for AD drug discovery.

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