J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 48(1): 289-299, 2024
Doi: 10.33483/jfpau.1314900

REVIEW ARTICLE / DERLEME MAKALE

THE PROMISING ROLE OF iNOS INHIBITORS IN ALZHEIMERS DISEASE

ALZHEIMER HASTALIĞINDA iNOS İNHİBİTÖRLERİNİN UMUT VERİCİ ROLÜ

Shkar MARIWAN AHMED[1]*, Gulcin TUGCU[2], Meric KÖKSAL[1]

[1]Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 34755 Istanbul, Türkiye
[2]Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 34755 Istanbul, Türkiye

ABSTRACT

Objective: *This study aims to explore the role of iNOS inhibitors in Alzheimer's disease (AD), a neurodegenerative disorder affecting millions worldwide. The main symptoms of AD include memory loss, cognitive decline, and behavioral changes. While the exact cause remains uncertain, both genetic and environmental factors are believed to contribute. Recent research has emphasized the significance of nitric oxide (NO) in AD development. Specifically, the upregulation of inducible nitric oxide synthase (iNOS) in AD patients leads to excessive NO production during neuronal inflammation, exacerbating AD and dementia. Therefore, the investigation focuses on the potential of iNOS inhibitors as a novel therapeutic approach for AD treatment.*

Result and Discussion: *In this review, we present the current therapeutic strategies available for Alzheimer's disease (AD) and explore the promising potential of iNOS inhibitors in AD treatment. Specifically, we will focus on their capacity to mitigate NO production and examine their potential neuroprotective effects. Additionally, this review will offer an overview of both natural and synthetic iNOS inhibitors, emphasizing the importance of safety considerations during the development of iNOS inhibitors as therapeutic interventions for AD.*

Keywords: *Alzheimer's disease, iNOS, neuroinflammation, nitric oxide*

ÖZ

Amaç: *Bu çalışma, milyonlarca insanı etkileyen Alzheimer hastalığı (AD) ve iNOS inhibitörlerinin rolünü araştırmayı amaçlamaktadır. AD'nin temel belirtileri arasında hafıza kaybı, bilişsel gerileme ve davranış değişiklikleri bulunmaktadır. Kesin neden belirsiz olsa da, genetik ve çevresel faktörlerin katkıda bulunduğu düşünülmektedir. Son araştırmalar, nitrik oksit (NO)'nin AD gelişimindeki önemini vurgulamıştır. Özellikle, AD hastalarında indüklenebilir nitrik oksit sentaz (iNOS) aktivasyonu, nöronal iltihaplanma sırasında aşırı NO üretimine neden olarak AD ve bunamayı kötüleştirmektedir. Bu nedenle, bu araştırma, iNOS inhibitörlerinin AD tedavisinde yeni bir terapötik yaklaşım olarak potansiyelini incelemektedir.*

Sonuç ve Tartışma: *Bu derleme, Alzheimer hastalığı (AD) için mevcut terapötik stratejileri sunuyor ve AD tedavisinde iNOS inhibitörlerinin umut verici potansiyelini araştırıyoruz. Özellikle, iNOS inhibitörlerinin NO üretimini azaltma kapasitelerine odaklanacak ve potansiyel nörokoruyucu etkilerini inceleyeceğiz. Ayrıca, bu derleme doğal ve sentetik iNOS inhibitörlerinin genel bir bakışını sunacak ve AD için terapötik müdahaleler olarak iNOS inhibitörlerinin geliştirilmesi sürecinde güvenlik değerlendirmelerinin önemini vurgulayacaktır.*

Anahtar Kelimeler: *Alzheimer hastalığı, iNOS, nöroenflamasyon, nitrik oksit*

* **Corresponding Author / Sorumlu Yazar:** Shkar Mariwan Ahmed
e-mail / e-posta: shakerakam@gmail.com, **Phone / Tel.:** +905388841510

Submitted / Gönderilme : 15.06.2023
Accepted / Kabul : 26.09.2023
Published / Yayınlanma : 20.01.2024

INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative disease with a complex etiology. It is also the leading cause of dementia in the elderly, which accounts for 60-80% of all cases. According to the World Health Organization (WHO), approximately 50 million people worldwide are now living with dementia today, and this number is expected to triple by 2050 [1,2]. Symptoms of AD include language impairment, progressive memory loss, and visual-spatial impairment, all of which require caregiving [3,4]. Early signs of AD may vary from patient to patient, but typically memory problems are one of the early signs of AD. However, the very early stages of AD may also be indicated by a deterioration in other cognitive abilities [5,6], including the ability to express oneself clearly, problems with vision or spatial awareness, and impaired reasoning or judgment [7,8].

Although the pathology of AD has been studied for decades, the exact cause remains unknown. Recent developments in the diagnostic strategy of AD have aided to diagnose pathological abnormalities in the early stages of the disease. The current understanding of the pathological features of AD, which are the formation of neurofibrillary tangles (NFTs) inside the neurons [9], senile plaques outside neurons, and neuronal loss, is shown in Figure 1 [10]. However, there are other critical factors that promote the onset and development of AD, such as oxidative stress, neuroinflammation, and impaired glucose metabolism in the brain. In addition, amyloid plaques and tau protein-based neurofibrillary tangles may form years before clinical dementia manifests [11,12].

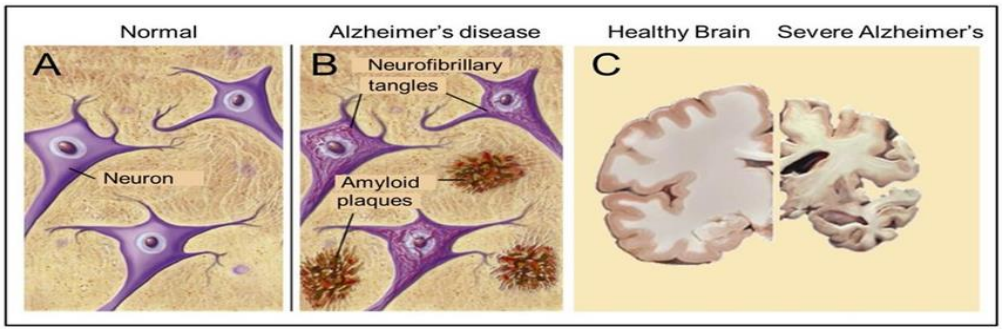


Figure 1. Difference between a healthy brain and the brain of a patient with severe AD. De Loof et al, (2019) OBM Neurobiology journal, from ref [10]

Although scientists have made great in the current decade to find a treatment that stops the progression and development of AD, we are still far from finding an effective treatment strategy [13]. This is because the currently available treatment only treats the symptoms of the disease. In recent years, scientists have focused on the role of neuroinflammation in finding an effective treatment for AD, because neuroinflammation is one of the major causes of the development and exacerbation of AD. Nitric oxide (NO) during neuroinflammation is overproduced by the inducible nitric oxide synthase (iNOS) enzyme in response to inflammatory stimuli, leading to the progression and exacerbation of AD, therefore, finding effective iNOS inhibitors will help in limiting and slowing the progression of AD [14]. This narrative review article will provide a brief overview of AD, and will highlight the promising role of iNOS inhibitors in AD.

Neuroinflammation and Alzheimer's Disease

Recent investigations have demonstrated that neuroinflammation is one of the major causes of the development and exacerbation of AD [15,16]. Interestingly, neuroinflammation could be a beneficial immune response that limits and slows disease progression [17]. For example, in its normal range, neuroinflammation modulates microglial activation and involves in the clearance of Aβ and cellular debris via microglial phagocytosis [18,19]. On the other hand, some researchers believe that neuroinflammation is merely a byproduct of the disease process and may not significantly alter its course. However, evidence from clinical studies and animal experiments has demonstrated that

enhanced neuroinflammatory cascades mediated by primed microglial cells also contribute to AD pathogenesis [20,21].

Neuroinflammation during AD is characterized by microglial activation and astrocyte release of various chemokines and cytokines, which impair blood-brain barrier (BBB) function and stimulate cognitive impairment [22,23]. Furthermore, during neuroinflammation, NO is overproduced by the enzyme iNOS in response to inflammatory stimuli, leading to an exacerbation of AD and dementia [24].

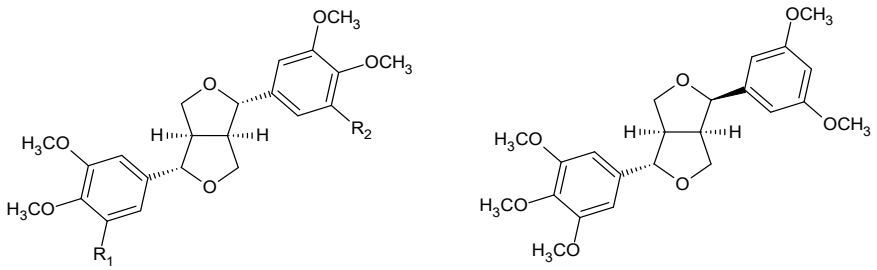
Role of iNOS Inhibitors in Neuroinflammation

NO acts as a mediator in several physiological and pathophysiological processes within the body. It is primarily produced by the inducible isoform of the enzyme, known as the iNOS, in response to inflammatory stimuli [25]. During neuroinflammation, excess NO is produced by iNOS enzymes in response to neuroinflammation. Furthermore, the overproduction of NO will lead to an exacerbation of AD and dementia [26].

To this end, many investigations and studies have been conducted by scientists to develop iNOS inhibitors, which will be an important treatment for the neuroinflammation of AD. However, many iNOS inhibitors showed neuroprotective effects, such as aminoguanidine, and two other amino acid amidines known as GW274150 and GW273629, but most iNOS inhibitors need further studies and investigations to ensure their affectivity and potency. In recent years, computational studies have been used by scientists to screen the inhibitory value of iNOS inhibitors in AD [14].

iNOS Inhibitors from Natural Sources

In recent decades, plants have been used as medicines to treat various diseases, and many plants have been used as anti-inflammatory agents that act by inhibiting the production and expression of NO. Furthermore, compounds isolated from these plants provided a medical chemist with a crucial lead to find effective and safe iNOS inhibitors for the treatment of AD [27]. The anti-neuroinflammatory effect of four ligands, (+)-eudesmin (1), (+)-magnolin (2), (+)-yangambin (3), and epimagnolin B (4) isolated from *Magnolia fargesii* flower buds were tested for their iNOS inhibitory activity against neurodegenerative disorders such as AD (Fig. 2). These four compounds showed IC_{50} values ranging from 10.9-30.0 μM. Among the group, compound 4 showed the most potent inhibition of LPS-induced NO production with an IC_{50} value of 10.9 ± 1.6 μM compared to the positive control drug NG-monomethyl-L-arginine (L-NMMA) IC_{50}=19.2 ± 1.8 μM [28].



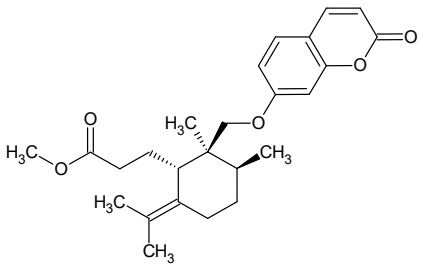
1 : R_1, R_2 = -H
2 : R_1= -OCH_3, R_2 = -H
3 : R_1, R_2 = -OCH_3

4

Figure 2. Absolute configuration of natural iNOS inhibitors isolated from *Magnolia fargesii*

Kohno et al. investigated the potential inhibitory effects of various terpenoid coumarins such as (methyl galbanate, galbanic acid, and farnesiferol A) that have been extracted from *Ferula szowitsiana* DC. Specifically, the study aims to investigate whether these compounds can inhibit the production of NO in RAW264.7 mouse macrophage cells that have been stimulated with lipopolysaccharide (LPS) and interferon-c (IFN-c). Among the terpenoid coumarins that studied, it was observed that in the

presence of methyl galbanate 5 (Figure 3), LPS/IFN-c-induced iNOS mRNA expression was significantly reduced to 52% of the level found with LPS/IFN-c stimulation alone [29].

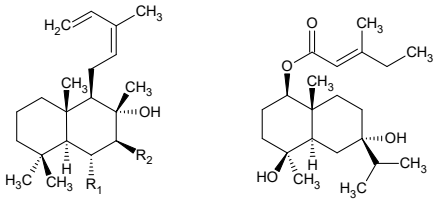


5

Figure 3. Chemical structure of methyl galbanate

In recent years, two other studies for the development of iNOS inhibitors from natural sources were performed by Liu et al. In both studies, the anti-neuroinflammatory effect of compounds isolated from plant sources was evaluated by inhibiting LPS-induced NO release in murine microglial BV-2 cells. In addition, 2-methyl-2- thiopseudourea, sulfate (SMT) was used as a positive control in the studies. In the first study, the anti-neuroinflammatory effects of twelve daphnane diterpenoids isolated from the twigs of *Trigonostemon thyrsoideus* were investigated. The result of this study demonstrated that all the compounds had an inhibitory activity on LPS-induced NO production with an IC_{50} value range of 3.19-24.9 μM [30]. In the second study of the group, isolated sesquiterpenes and terpenes from the flowers of *I. japonica* were evaluated as anti-neuroinflammatory agents for AD through NO inhibition. All of the compounds showed inhibitory activity against NO production, however, five of them possessed a more inhibitory effect on LPS-induced NO release with lower IC_{50} values than 10 μM [27].

Ma et al. investigated the potential inhibitory effects of one new labdane diterpenoid and three new guaiane sesquiterpenoids, alongside ten known compounds from *Blumea balsamifera*. The study focused on assessing the anti-neuroinflammatory effects by impeding the release of NO in murine microglial BV-2 cells induced by LPS. Among these derivatives, three compounds 6-8 showed IC_{50} values ranging from 15.4-22.7 μM, respectively, indicating notable inhibition against LPS-induced NO production in BV-2 cells, with their IC_{50} values falling below 30 μM (Figure 4) [31].



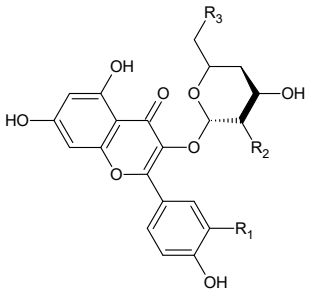
6 : R_1 = -OH, R_2 = -H
7 : R_1 = -H, R_2 = -OH

8

Figure 4. iNOS inhibitors as anti-neuroinflammatory agents for AD isolated from *Blumea balsamifera*

In 2021, two crucial studies have been done to test the anti-neuroinflammatory activity of numerous glycosides isolated from the leaves and stems of *Neoshirakia japonica*, and 7-O-1,2,3-triazole hesperetin derivatives. In the first study, the anti-neuroinflammatory effects of all the isolates were evaluated by inhibiting NO production against LPS-induced BV-2 microglial cells. Notably, three compounds 9-11 exhibited IC_{50} values ranging from 2.7 to 5.5 μM, respectively. These values indicated a higher degree of inhibitory activity compared to the positive control, minocycline, which had an IC_{50}

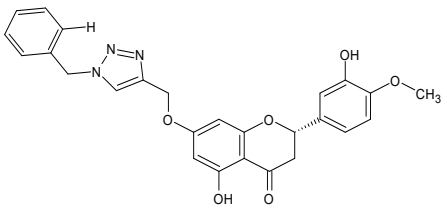
value of 15.6 μM. (Figure 5) [32].



9 : R_1 = -H, R_2 = -OH, R_3 = $-S_4$
10 : R_1 = -H, R_2 = -OH, R_3 = $-S_3$
11 : R_1, R_2 = -OH, R_3 = $-S_2$

Figure 5. iNOS inhibitors as anti-neuroinflammatory agents for AD isolated from *Neoshirakia japonica*

In the second study, the anti-neuroinflammatory of 7-O-1,2,3-triazole hesperetin derivatives was evaluated by Wang et al. Most of the hesperetin derivatives showed better NO inhibitory activity compared to hesperetin (IC_{50}=49.56 ± 2.39 μM). Furthermore, resveratrol (Res) was used as a positive control, and compound 12 (IC_{50}=1.04 ± 0.31 μM) had an eight fold higher NO inhibitory capacity compared to the positive control Res (IC_{50}=7.86 ± 1.49 μM) (Figure 6) [14].



12

Figure 6. Hesperetin derivative potent iNOS inhibitor

iNOS Inhibitors from Synthetic Sources

Synthetic iNOS inhibitors have been extensively studied for their potential therapeutic applications in a variety of disease, including neurodegenerative disorders. These inhibitors are designed to target the iNOS enzyme, which is responsible for the overproduction of NO in response to neuroinflammation. Overexpression of NO can lead to tissue damage and may play a role in the etiology of a number of diseases such as AD. A number of approaches, including structure-based drug design and high-throughput screening, have been used to develop synthetic iNOS inhibitors. These inhibitors have shown promising results in preclinical research, currently being evaluated in clinical trials to determine the efficacy and safety of iNOS inhibitors [26]. Synthetic iNOS inhibitors are classified based on their chemical structures into amidinic and guanidine-based compounds, quinone-based compound, oxadiazole-based iNOS inhibitors, steroidal compounds, and others.

Amidine and Guanidine-based Compounds

Amidine-based iNOS inhibitors, as the name suggests, have an amidine group (-C(=NH)-NH_2) in their structure, which can participate in hydrogen bonding and electrostatic interactions with the active site of iNOS. It has been discovered that these compounds are being investigated as potential treatments for Alzheimer's disease [26].

An example of an amidine-based iNOS inhibitor is GW274150 13 and 1400W 14, which are selective inhibitors of iNOS that have shown promising results in preclinical studies related to AD (Figure 7). GW274150 13 specifically inhibit the activity of iNOS by interfering with its enzymatic function, reducing the production of NO. Also, it has demonstrated anti-inflammatory effects and has been found to attenuate neuroinflammation and neurodegeneration in animal models of AD. Additionally, GW274150 13 has shown neuroprotective properties by reducing oxidative stress and preserving neuronal function. Similarly, 1400W 14 which is another amidine-based iNOS inhibitor is a potent and selective inhibitor of iNOS. It effectively suppresses iNOS activity and the subsequent production of NO. Preclinical studies utilizing 1400W have shown promising results in attenuating neuroinflammation, reducing amyloid-beta (Aβ) deposition, and improving cognitive function in animal models of AD. The inhibition of iNOS by 1400W has been associated with decreased oxidative stress and reduced neurodegeneration [14,26].

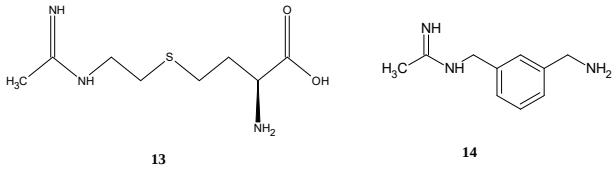


Figure 7. Amidine-based iNOS inhibitors

Guanidine-based iNOS inhibitors have a guanidine group (-C(=NH)-NH-C(=NH)-NH_2) in their structure, which can also interact with the active site of iNOS. Examples of guanidine-based iNOS inhibitors include aminoguanidine 15, and L-NG -nitro arginine methyl ester (L-NAME) 16 (Figure 8).

Two pivotal studies were conducted on guanidine-based compounds in mouse microglial for investigating their iNOS inhibitory activity in AD. The first study was conducted in 2003, during which scientists tested the inhibitory activity of two different guanidine-based iNOS inhibitors for inhibition of AGE-induced NO production in mouse microglial. Compound 15 exhibited the highest rate of iNOS inhibition, reaching 90%. On the other hand, the other guanidine-based iNOS inhibitors, compounds 16 demonstrated inhibition values of 80% for NO production in AD [33].

In addition, Esposito et al. investigated the stimulation of PC12 cells with Aβ (1-42) (1 g/ml), which resulted in a significant increase in NO formation by iNOS enzymes compared to unstimulated cells. Both selective and nonselective iNOS inhibitors significantly reduced the effect of Aβ (1-42). For example, S-methyl-isothiourea (SMT) 17 (Figure 8), a selective iNOS inhibitor, demonstrated the highest level of iNOS inhibition activity in AD, achieving an impressive 80% inhibition rate. Conversely, L-NAME 16, a non-selective iNOS inhibitor, exhibited a lower inhibition rate of 45.6% for iNOS [34].

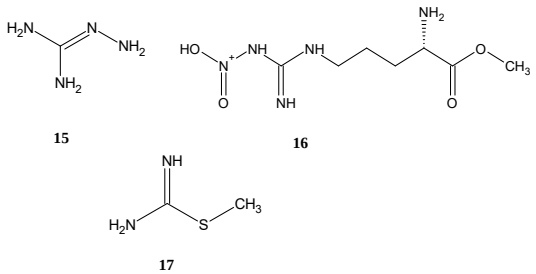
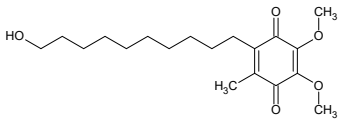


Figure 8. S-methyl-isothiourea and guanidine-based iNOS inhibitors

Quinone-based iNOS Inhibitors

Quinone-based compounds are a class of organic compounds characterized by the presence of a quinone structure in their chemical composition. Quinones are aromatic compounds that consist of a

benzene ring conjugated with two carbonyl groups (C=O) in different positions. Quinone-based derivatives have been investigated as iNOS inhibitors for AD due to their additional antioxidant properties. These compounds are designed to scavenge reactive oxygen species (ROS) and prevent oxidative damage, which is thought to contribute to the pathogenesis of AD. An example of a quinone-based iNOS inhibitor is idebenone 18 shown in (Figure 9), which has been evaluated in preclinical and clinical studies for its potential as a therapeutic agent for AD. Yan et al. conducted a study to investigate the iNOS inhibitory effects of idebenone 18 on LPS-activated BV2 cells for the treatment of neurodegenerative diseases such as AD. The researchers examined the cytotoxicity of idebenone by analyzing its effects at different concentrations (1, 2.5, and 5 μM) on LPS-activated BV2 cells. The findings revealed that idebenone exhibited a dose-dependent reduction in the LPS-stimulated NO production, and decreased mRNA expression of TNF-α, IL-1β, iNOS, and IL-6 in LPS-stimulated BV2 cells. In addition, the highest inhibition of iNOS activity was observed at the concentration of 5 μM [35].



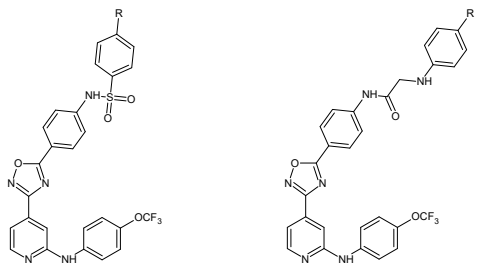
18

Figure 9. Quinone-based iNOS inhibitors

Oxadiazole-based iNOS Inhibitors

Oxadiazole-based iNOS inhibitors are a class of compounds being developed as potential therapeutics for various inflammatory diseases such as neuroinflammation. These compounds have the potential to inhibit iNOS activity and reduce NO production, which may have therapeutic implications for Alzheimer's disease and other inflammatory conditions [36,37].

Two important studies were conducted on 1,2,4-oxadiazole-containing compounds to investigate their NO inhibitory activity in AD. In the first study, a series of novel 3-(4-pyridyl)-5-(4- sulfamido-phenyl)-1,2,4-oxadiazole derivatives were synthesized, and the anti-neuroinflammatory activity of the compounds was assessed in LPS-induced BV2 microglial cells. All compounds showed better activity against NO compared to the positive drug, Res (IC_{50}=10.16 ± 0.12 μM). Compounds 19 and 20 showed the best inhibition rate against NO production, with an IC_{50} value range of 0.47 to 0.72 μM, which was about ten times higher than that of the positive drug Res [36]. In the second study, researchers investigated the potential anti-neuroinflammatory effect of the novel (4-(1,2,4-oxadiazol-5-yl)phenyl)-2-aminoacetamide derivatives in LPS-induced BV2 microglial cells. Among the derivatives tested, compounds 21 and 22 exhibited particularly noteworthy results, displaying more than 20-fold greater iNOS inhibitory activity compared to Res. Notably, compounds 21 and 22 demonstrated IC_{50} values ranging from 0.42 to 0.67 μM, highlighting their potent inhibitory effects on iNOS expression and NO production in AD (Figure 10) [37].



19 : R = -$COCH_3$
20 : R = -$C(CH_3)_2$
21 : R = -H
22 : R = -F

Figure 10. Oxadiazole-based iNOS inhibitors

Steroidal Compounds

Steroid compounds are a class of organic compounds that have a characteristic structure consisting of a sterane ring. These compounds have numerous biological activities, including anti-neuroinflammatory activity in AD [26].

Yang et al. synthesized novel steroidal derivatives, from readily available hyodeoxycholic acid ($C_{24}H_{40}O_4$). In the study, the anti-neuroinflammatory activity of 5α-cholestan-6-one derivatives was evaluated in LPS-stimulated BV-2 microglial cells. As a result of the study, five compounds (23-27) strongly inhibited LPS-induced NO production with a percentage inhibition range of 73.6 – 60%, without causing cell toxicity while dehydroepiandrosterone, which was used as a reference drug in the study, has 55.3% percentage inhibition (Figure 11).

Microglial activation is known to increase the expression of iNOS and COX-2, which are responsible for the production of PGE-2, and NO, and activated microglial cells also increase the production of proinflammatory cytokines such as IL-1b and TNF-a. As a further study, the effects of compound 23 on the mRNA expression of these cytokines were investigated. The result of the investigation clearly indicated that compound 23 which strongly inhibited LPS-induced expression of iNOS, IL-1b, TNF-a, and COX-2 in a dose-dependent manner [38].

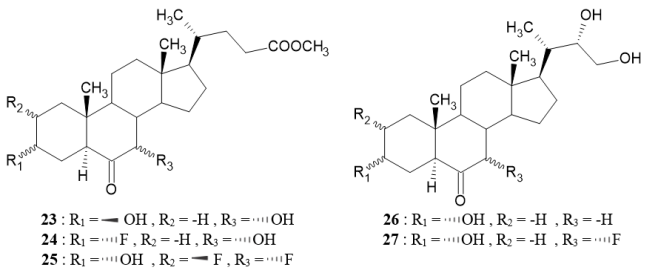


Figure 11. Steroidal compounds as inhibitors of iNOS

Others

Other studies have been conducted to test the iNOS inhibitory ability of various synthetic compounds, such as the study by Watterson et al. that investigated the role of the anti-inflammatory drug K252a 28 (Figure 12), which is a CaMKII inhibitor, that exhibits dose-dependent inhibition of lipopolysaccharide-induced increases in iNOS production and NO accumulation by the BV-2 microglial cell line. Furthermore, the study showed that the anti-inflammatory K252a inhibits the accumulation of NO and IL-1, and other cytokines during neuroinflammation in AD [39].

Zhou et al. explored the effects of rolipram 29 which is a Phosphodiesterase-4 (PDE4) inhibitor, in AD by using bilateral Aβ25– 35 injections into the hippocampus of rats (Figure 12). Different doses of rolipram were administered daily for 25 days after Aβ 25-35 injections. The results of the study demonstrated that rolipram significantly inhibited NO and iNOS pathways in the hippocampus. Furthermore, rolipram improved memory and learning abilities in the Aβ25-35-induced AD rat model [40].

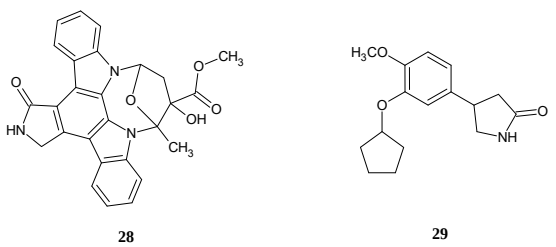


Figure 12. Other synthetic potent iNOS inhibitors

RESULT AND DISCUSSION

In conclusion, iNOS inhibitors have shown potential as therapeutic agents for AD in various studies. Studies evaluating iNOS inhibitors, including amidine, guanidine, quinone-based derivatives, and oxadiazole-based compounds, have demonstrated promising results in various *in vivo* and *in vitro* models of AD. These inhibitors have shown the ability to reduce iNOS activity, inhibit NO production, attenuate neuroinflammatory responses, and improve cognitive function. Some of these compounds have also shown effects on amyloid beta plaque accumulation and oxidative stress.

While the preclinical findings are encouraging, it's important to note that translating iNOS inhibitors into effective clinical treatments for AD has been challenging. Several factors, including the complexity of AD pathology, the multifaceted nature of iNOS signaling, and the need for targeted drug delivery to the brain, contribute to the difficulty in developing successful therapeutics.

Further research and clinical trials are needed to determine the safety, efficacy, and optimal dosing regimens of iNOS inhibitors in the treatment of AD. Combining iNOS inhibitors with other therapeutic approaches, such as anti-amyloid or anti-tau strategies, may also hold promise for improving outcomes in AD patients. Overall, iNOS inhibitors represent a promising avenue for future studies and therapeutic intervention in this major neurodegenerative disease.

ACKNOWLEDGMENTS

The authors are thankful to Spartan’20 for providing the academic license.

AUTHOR CONTRIBUTIONS

Concept: S.M.A., G.T., M.K.; Design: S.M.A., G.T., M.K.; Control: S.M.A., G.T., M.K.; Sources: S.M.A., G.T., M.K.; Materials: S.M.A., G.T., M.K.; Data Collection and/or Processing: S.M.A., G.T., M.K.; Analysis and/or Interpretation: S.M.A., G.T., M.K.; Literature Review: S.M.A., G.T., M.K.; Manuscript Writing: S.M.A., M.K.; Critical Review: S.M.A., G.T., M.K.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

REFERENCES

1. Lane, C.A., Hardy, J., Schott, J.M. (2017). Alzheimer’s disease. European Journal of Neurology, 25(1), 59-70. [CrossRef]
2. Marhánková, J.H. (2023). The role of dementia and Alzheimer’s disease in older adults’ representations of aging and anxieties regarding one’s own future. Journal of Aging Studies, 65, 101127. [CrossRef]
3. Sramek, J.J., Cutler, N.R. (1999). Recent developments in the drug treatment of Alzheimer’s disease. Drugs & Aging, 14(5), 359-373. [CrossRef]
4. Rich, M.B., Blout Zawatsky, C.L., Botta, J.J., Christensen, K.D. (2023). Public perspective on medications to delay Alzheimer’s disease symptoms. Journal of Genetic Counseling, 32(5), 1009-1017. [CrossRef]
5. Lanctôt, K.L., Amatniek, J., Ancoli-Israel, S., Arnold, S.E., Ballard, C., Cohen-Mansfield, J., Ismail, Z., Lyketsos, C., Miller, D.S., Musiek, E., Osorio, R.S., Rosenberg, P.B., Satlin, A., Steffens, D., Tariot, P., Bain, L.J., Carrillo, M.C., Hendrix, J.A., Jurgens, H., Boot, B. (2017). Neuropsychiatric signs and symptoms of Alzheimer’s disease: New treatment paradigms. Alzheimer’s & Dementia: Translational Research & Clinical Interventions, 3(3), 440-449. [CrossRef]
6. Lyketsos, C.G., Carrillo, M.C., Ryan, J.M., Khachaturian, A.S., Trzepacz, P., Amatniek, J., Cedarbaum, J., Brashear, R., Miller, D.S. (2011). Neuropsychiatric symptoms in Alzheimer’s disease. Alzheimer’s Dementia, 7(5), 532-539. [CrossRef]
7. Li, L., He, R., Yan, H., Leng, Z., Zhu, S., Gu, Z. (2022). Nanotechnology for the diagnosis and treatment of Alzheimer's disease: A bibliometric analysis. Nano Today, 47, 101654. [CrossRef]
8. De-Paula, V.J., Radanovic, M., Diniz, B.S., Forlenza, O.V. (2012). Alzheimer’s disease. Protein Aggregation and Fibrillogenesis in Cerebral and Systemic Amyloid Disease, 329-352. [CrossRef]
9. Iseki, E., Tsunoda, S., Suzuki, K., Takayama, N., Akatsu, H., Yamamoto, T., Kosaka, K. (2002). Regional quantitative analysis of NFT in brains of non-demented elderly persons: Comparisons with findings in

brains of late-onset Alzheimer's disease and limbic NFT dementia. Neuropathology, 22(1), 34-39. [CrossRef]
10. De Loof, A., Schoofs, L. (2019). Alzheimer's disease: Is a dysfunctional mevalonate biosynthetic pathway the master-inducer of deleterious changes in cell physiology? OBM Neurobiology, 3(4), 26. [CrossRef]
11. Rojas-Fernandez, C.H., Chen, M., Fernandez, H.L. (2002). Implications of amyloid precursor protein and subsequent β-amyloid production to the pharmacotherapy of Alzheimer's disease. Pharmacotherapy, 22(12), 1547-1563. [CrossRef]
12. Kumar, V., Saha, A., Roy, K. (2020). *In silico* modeling for dual inhibition of acetylcholinesterase (ache) and butyrylcholinesterase (buche) enzymes in Alzheimer's disease. Computational Biology and Chemistry, 88, 107355. [CrossRef]
13. Yiannopoulou, K.G., Anastasiou, A.I., Zachariou, V., Pelidou, S.H. (2019). Reasons for failed trials of disease-modifying treatments for Alzheimer disease and their contribution in recent research. Biomedicines, 7(4), 97. [CrossRef]
14. Wang, M., Fang, L., Liu, T., Chen, X., Zheng, Y., Zhang, Y., Chen, S., Li, Z. (2021). Discovery of 7-O-1, 2, 3-triazole hesperetin derivatives as multi-target-directed ligands against Azheimer's disease. Chemico-Biological Interaction, 342, 109489. [CrossRef]
15. Heneka, M.T., Kummer, M.P., Stutz, A., Delekate, A., Schwartz, S., Vieira-Saecker, A., Griep, A., Axt, D., Remus, A., Tzeng, T.C., Gelpi, E., Halle, A., Korte, M., Latz, E., Golenbock, D.T. (2012). NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature, 493(7434), 674-678. [CrossRef]
16. Welikovitch, L.A., Do Carmo, S., Maglóczky, Z., Malcolm, J.C., Lőke, J., Klein, W.L., Freund, T., Cuello, A.C. (2020). Early intraneuronal amyloid triggers neuron-derived inflammatory signaling in app transgenic rats and human brain. Proceedings of the National Academy of Sciences, 117(12), 6844-6854. [CrossRef]
17. Newcombe, E.A., Camats-Perna, J., Silva, M.L., Valmas, N., Huat, T.J., Medeiros, R. (2018). Inflammation: The link between comorbidities, genetics, and Alzheimer's disease. Journal of Neuroinflammation, 15(1), 276. [CrossRef]
18. Xue, F., Du, H. (2021). Trem2 mediates microglial anti-inflammatory activations in Alzheimer's disease: Lessons learned from transcriptomics. Cells, 10(2), 321. [CrossRef]
19. Yang, Y., Zhang, Z. (2020). Microglia and wnt pathways: Prospects for inflammation in Alzheimer's disease. Frontiers in Aging Neuroscience, 12, 110 [CrossRef]
20. Hampel, H., Caraci, F., Cuello, A.C., Caruso, G., Nisticò, R., Corbo, M., Baldacci, F., Toschi, N., Garaci, F., Chiesa, P.A., Verdooner, S.R., Akman-Anderson, L., Hernández, F., Ávila, J., Emanuele, E., Valenzuela, P.L., Lucía, A., Watling, M., Imbimbo, B.P., Lista, S. (2020). A path toward precision medicine for neuroinflammatory mechanisms in Alzheimer's disease. Frontiers in Immunology, 11, 456. [CrossRef]
21. Ndoja, A., Reja, R., Lee, S.H., Webster, J.D., Ngu, H., Rose, C.M., Kirkpatrick, D.S., Modrusan, Z., Chen, Y.J.J., Dugger, D.L., Gandham, V., Xie, L., Newton, K., Dixit, V.M. (2020). Ubiquitin ligase COP1 suppresses neuroinflammation by degrading C/EBPB in Microglia. Cell, 182(5), 1156-1169. [CrossRef]
22. Blanchard, J.W., Bula, M., Davila-Velderrain, J., Akay, L.A., Zhu, L., Frank, A., Victor, M.B., Bonner, J. M., Mathys, H., Lin, Y.T., Ko, T., Bennett, D.A., Cam, H.P., Kellis, M., Tsai, L.H. (2020). Reconstruction of the human blood-brain barrier *in vitro* reveals a pathogenic mechanism of APOE4 in pericytes. Nature Medicine, 26(6), 952-963. [CrossRef]
23. Sweeney, M.D., Sagare, A.P., Zlokovic, B.V. (2018). Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nature Reviews Neurology, 14(3), 133-150. [CrossRef]
24. Whitson, H.E., Colton, C., El Khoury, J., Gate, D., Goate, A., Heneka, M.T., Kaddurah-Daouk, R., Klein, R.S., Shinohara, M.L., Sisodia, S., Spudich, S.S., Stevens, B., Tanzi, R., Ting, J.P., Garden, G., Aiello, A., Chiba-Falek, O., Heitman, J., Johnson, K.G., Terrando, N. (2022). Infection and inflammation: New perspectives on Alzheimer's disease. Brain, Behavior, & Immunity - Health, 22, 100462. [CrossRef]
25. Cinelli, M.A., Do, H.T., Miley, G.P., Silverman, R.B. (2019). Inducible nitric oxide synthase: Regulation, structure, and inhibition. Medicinal Research Reviews, 40(1), 158-189. [CrossRef]
26. Minhas, R., Bansal, Y., Bansal, G. (2019). Inducible nitric oxide synthase inhibitors: A comprehensive update. Medicinal Research Reviews, 40(3), 823-855. [CrossRef]
27. Liu, F., Dong, B., Yang, X., Yang, Y., Zhang, J., Jin, D.Q., Ohizumi, Y., Lee, D., Xu, J., Guo, Y. (2018). No inhibitors function as potential anti-neuroinflammatory agents for ad from the flowers of *Inula japonica*. Bioorganic Chemistry, 77, 168-175. [CrossRef]
28. Kim, J.Y., Lim, H.J., Lee, D.Y., Kim, J.S., Kim, D.H., Lee, H.J., Kim, H.D., Jeon, R., Ryu, J.H. (2009). *In vitro* anti-inflammatory activity of lignans isolated from *Magnolia fargesii*. Bioorganic Medicinal Chemistry Letters, 19(3), 937-940. [CrossRef]

29. Kohno, S., Murata, T., Sugiura, A., Ito, C., Iranshahi, M., Hikita, K., Kaneda, N. (2011). Methyl galbanate, a novel inhibitor of nitric oxide production in mouse macrophage raw264.7 cells. Journal of Natural Medicines, 65(2), 353-359. [CrossRef]
30. Liu, F., Yang, X., Ma, J., Yang, Y., Xie, C., Tuerhong, M., Jin, D.Q., Xu, J., Lee, D., Ohizumi, Y., Guo, Y. (2017). Nitric oxide inhibitory daphnane diterpenoids as potential anti-neuroinflammatory agents for AD from the twigs of *Trigonostemon thyrsoideus*. Bioorganic Chemistry, 75, 149-156. [CrossRef]
31. Ma, J., Ren, Q., Dong, B., Shi, Z., Zhang, J., Jin, D.Q., Xu, J., Ohizumi, Y., Lee, D., Guo, Y. (2018). No inhibitory constituents as potential anti-neuroinflammatory agents for AD from *Blumea balsamifera*. Bioorganic Chemistry, 76, 449-457. [CrossRef]
32. Zhao, H.Y., Wang, Y.Q., Li, Y.C., Lan, Q., Liao, H.B., Wang, H.S., Liang, D. (2021). Flavonol glycosides and phenylpropanoid glycosides with inhibitory effects on microglial nitric oxide production from *Neoshirakia japonica*. Fitoterapia, 151, 104877. [CrossRef]
33. Dukic-Stefanovic, S., Gasic-Milenkovic, J., Deuther-Conrad, W., Münch, G. (2003). Signal transduction pathways in mouse microglia N-11 cells activated by advanced glycation endproducts (ages). Journal of Neurochemistry, 87(1), 44-55. [CrossRef]
34. Esposito, G., De Filippis, D., Maiuri, M.C., De Stefano, D., Carnuccio, R., Iuvone, T. (2006). Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in β-amyloid stimulated PC12 neurons through P38 MAP kinase and NF-KB involvement. Neuroscience Letters, 399(1-2), 91-95. [CrossRef]
35. Yan, A., Liu, Z., Song, L., Wang, X., Zhang, Y., Wu, N., Lin, J., Liu, Y., Liu, Z. (2019). Idebenone alleviates neuroinflammation and modulates microglial polarization in LPS-stimulated BV2 cells and MPTP-induced parkinson's disease mice. Frontiers in Cellular Neuroscience, 12. [CrossRef]
36. Wang, M., Liu, T., Chen, S., Wu, M., Han, J., Li, Z. (2021). Design and synthesis of 3-(4-pyridyl)-5-(4-sulfamido-phenyl)-1,2,4-oxadiazole derivatives as novel GSK-3B inhibitors and evaluation of their potential as multifunctional anti-alzheimer agents. European Journal of Medicinal Chemistry, 209, 112874. [CrossRef]
37. Liu, T., Chen, S., Du, J., Xing, S., Li, R., Li, Z. (2022). Design, synthesis, and biological evaluation of novel (4-(1,2,4-oxadiazol-5-yl)phenyl)-2-aminoacetamide derivatives as multifunctional agents for the treatment of Alzheimer's disease. European Journal of Medicinal Chemistry, 227, 113973. [CrossRef]
38. Yang, Y.X., Zheng, L.T., Shi, J.J., Gao, B., Chen, Y.K., Yang, H.C., Chen, H.L., Li, Y.C., Zhen, X.C. (2014). Synthesis of 5α-cholestan-6-one derivatives and their inhibitory activities of no production in activated microglia: Discovery of a novel neuroinflammation inhibitor. Bioorganic Medicinal Chemistry Letters, 24(4), 1222-1227. [CrossRef]
39. Watterson, D.M., Mirzoeva, S., Guo, L., Whyte, A., Bourguignon, J.J., Hibert, M., Haiech, J., Van Eldik, L.J. (2001). Ligand modulation of glial activation: Cell permeable, small molecule inhibitors of serine-threonine protein kinases can block induction of interleukin 1β and nitric oxide synthase II. Neurochemistry International, 39(5-6), 459-468. [CrossRef]
40. Zhuo, Y., Guo, H., Cheng, Y., Wang, C., Wang, C., Wu, J., Zou, Z., Gan, D., Li, Y., Xu, J. (2016). Inhibition of phosphodiesterase-4 reverses the cognitive dysfunction and oxidative stress induced by AB25-35 in rats. Metabolic Brain Disease, 31(4), 779-791. [CrossRef]