

Research Article

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The pharmacological research of Tek-1 relevance to anti-neuroinflammation, a candidate compound based on Telmisartan

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Abstract: In this paper, BV-2 mouse small glial cell inflammation model induced by LPS is established. The experiment used 0.1-10 μM of telmisartan and Tek-1 to incubate with small glial cell and used telmisartan and Tek-1 to incubate with PPAR gamma special heterosexual antagonistic anti-agent GW9662. The article used ELISA method to detect TNF- α effect on small glial cell for telmisartan and Tek-1. The article used real-time quantitative PCR method to detect mRNA level expression effect of CD11b, CD16 and iNOS on small glial cell for telmisartan and Tek-1 and used Western Blot method to detect MAPKs signal pathway and NF- κB signal turned guide pathway effect on small glial cell for telmisartan and Tek-1. Results show that Tek-1 had high affinity with AT1 receptor and inhibited intracellular calcium ion activation which can be for the AT1 receptor antagonists. Meanwhile, Tek-1 can partially activate PPAR gamma compared with full agonists of rosiglitazone.

Keywords: Cartilage rhinoplasty, Goldman technique, Broad nasal tip, Dome-splitting

1 Introduction

Microglial cells are widely distributed in the brain and spinal cord where they comprise the majority of innate immune cells. Their morphological change depends on the microenvironment change in the brain [1]. Under normal circumstances, microglial cells are in a resting state to focus on nutrition and support. When subjected

to external stimuli, microglial cells are activated and transformed into “Amoeba.” Moderately activated microglial cells can not only quickly clear aged or injured brain cells, but also can secrete neural nutrition factor. Excessively activated microglial cells, however, can cause neural damage [2]. For example, lack of blood and LPS stimulus can cause release of large inflammatory factors such as tumor necrosis factor (TNF- α), interleukin-6 (IL-6) and inducible nitric oxide synthase (iNOS) [3,4].

A number of studies have confirmed that one of the mediators of neurodegenerative disease is an inflammatory response induced by aberrant activation of glial cells and subsequent release of neurotoxic factors by invading immune cells. Therefore, suppressing excessive activation of microglial cells and decreasing inflammatory cytokines arising from their excessive activation could provide new treatment strategies for neurodegenerative diseases [5,6].

Research evidence suggests that the PPAR gamma agonist and AT1 receptor antagonists can effectively suppress brain inflammation in AD, cerebral ischemia, multiple sclerosis (MS) and other animal models.

Activation of PPAR gamma can reduce the inflammatory response in the brain by inhibiting microglial activation. PPAR gamma agonists such as rosiglitazone and pioglitazone can introduce dopaminergic cell death of MPTP by inhibiting microglial activation and inhibition. Compared with telmisartan, Tek-1 has dual function of AT1 receptor antagonist and PPAR γ activation. We have proved that telmisartan and Tek-1 can improve the induction of learning and memory impairment by A β and reduce the release of inflammatory cytokines in the brain of mice with IL-6 and MCP-1 in AD mice.

Therefore, we further develop BV-2 cells in mice induced by LPS in vitro model and assess whether Tek-1 can improve the LPS-induced BV-2 in mouse microglial inflammatory responses, and further explore its anti-inflammatory effect and mechanism of signal transduction.

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2 Research methods

The experiment subjects are BV-2 small murine microglial cell line.

(1) Solution preparation. 1ml DPEC is added to 1000ml double distilled water, stirred after an overnight stay, sterilized in high pressure and kept at room temperature. 25ml DPEC water is then added to 75ml ethanol (Table 1).

Electrophoresis buffer is prepared with 15.1g Tris base, 94.0g glycine and 5.0g SDS in 1000ml double boiled water. Film buffer is prepared using 3.03g Tris base and 14.4g glycine in 800ml double boiled water plus 200ml methanol.

(2) Cell culture and drug treatment. BV-2 mouse microglial cells are divided into the following groups: solvent control group, processing group of 1ug/ml LPS, processing group of 0.1uM telmisartan and 1ug/ml LPS, processing group of 1uM telmisartan and 1ug/ml LPS, processing group of 10uM telmisartan and 1ug/ml LPS, processing group of 1uM telmisartan and 1ug/ml LPS and 10μM GW9662 Table 2.

(3) Detection of BV-2 mouse microglial cell supernatant levels of TNF-α by ELISA method. We treat 2×10^4 BV-2 small rat small glial cells grown in 96 well plates using drug regimen. After 24 hours the supernatant from each processing group is collected. TNFα content was determined, following instructions in the manual, by measuring OD₄₅₀ value. We then calculate TNF-α content of each sample by comparing with standard curve (Table 3).

(4) Detection of BV-2 mice microglia CD11b and CD16 and iNOS mRNA expression levels by quantitative real-time PCR method. 1) Extracting total RNA in cells. 2) Reverse transcription. TOYOBO's reverse transcription kit was used to prepare reaction system and conduct reverse transcription according to kit instructions. 3) Real time-PCR. We prepare reaction system following operating instructions provided by real time quantitative PCR kit preparation reaction system (Table 4). Each gene (mice) primer design is shown in table 2.

(5) Western Blot Assay for detection of intracellular expression of NF-κB signaling pathway proteins. 1) Extraction of total protein. 2) Nuclear protein extraction. 3) Determination of protein concentration BCA. 4) Protein-Western blot (Table 5).

The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

Table 1: Versene cell dissociation solution.

Category	Content
Na1	137mM
KC1	2.7mM
KH ₂ PO ₄	1.5mM
Na ₂ HPO ₄ .12H ₂ O	10mM
Glucose.H ₂ O	1mM
EDTA-Na ₂ .2H ₂ O	2mM

Table 2: Membrane protein saturated experimental reaction system.

Category	Total combined pipe	Nonspecific binding tubes
Reaction buffers	100	0
[³ H-ANG]	100	100
ANG	0	100
Membrane proteins	100	100

Table 3: Membrane protein competition experimental reaction system.

Category	Competition compound tube	Nonspecific binding tubes
Reaction buffers	0	0
[³ H-ANG]	100	100
ANG	0	100
Membrane proteins	100	100
Compounds	100	0

Table 4: Primers used in real-time quantitative PCR.

Target gene	Primer sequences	
CD16	forward	TTTGACAC
	reverse	GTCTTCCTT
CD11b	forward	CCAAGACG
	reverse	TTCTGGC
iNOS	forward	GGCAGCCTG
	reverse	GCATTGGA
GAPDH	forward	ACTCCACTCA
	reverse	TCTCCATGGTGG

Table 5: Transfection of plasmid expression plasmid and report program.

Expression plasmid	Transfection of plasmid	
	pSG5-PPAR γ	pGL3-PPRE
contrast	0	0.7
1:1	0.35	0.35
1:2.5	0.2	0.5
1:5	0.12	0.58
1:10	0.06	0.64
1:20	0.03	0.67
1:50	0.01	0.69

3 Results

(1) Telmisartan and Tek-1 can induce the release of TNF- α in BV-2 microglia by LPS.

After BV-2 microglial cells were treated with different drugs, changes in TNF- α content in the cell culture supernatant is shown in Figure 1-2.

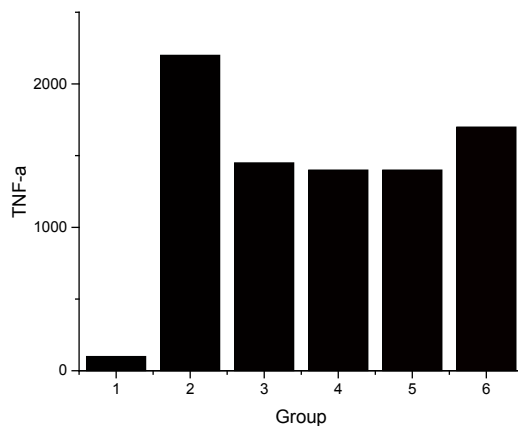
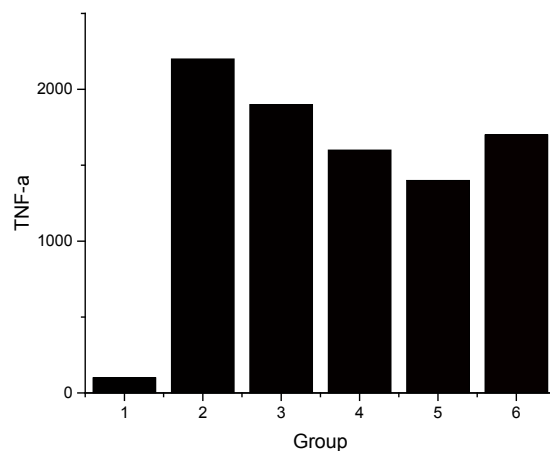
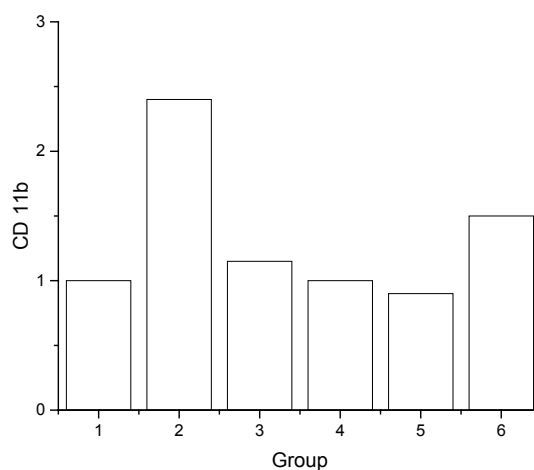
Compared to control group, TNF- α content in the LPS processing group significantly increased ($P < 0.001$). Compared to LPS processing group, 0.1-10 μM telmisartan can significantly inhibit LPS induced BV2 small glial cell production of TNF- α ($P < 0.001$). Compared to telmisartan, 0.1-10 μM Tek-1 has reduced ability to inhibit LPS induced TNF- α production by BV2 microglial cells, with only 10 μM Tek-1 showing significant differences ($P < 0.05$). Meanwhile, telmisartan of 1 μM and Tek-1 can partly be blocked by GW9662.

(2) Telmisartan and Tek-1 can inhibit expression of CD11b, CD16 and iNOS mRNA in mice microglia activated by LPS.

We use real-time quantitative PCR technique for detection of CD11b, CD16 and iNOS mRNA expression levels in BV2 murine microglial cells. The results are shown in Figure 3-5.

Compared to control group, expression level of CD11b, CD16 and iNOS in LPS processing group is significantly increased (p value is respectively for $P < 0.05$, $P < 0.001$ and $P < 0.05$). Compared to LPS processing group, telmisartan can inhibit expression of CD11b, CD16 and iNOS induced by LPS. 10 μM Telmisartan is statistically significant (p value is respectively for $P < 0.001$, $P < 0.05$ and $P < 0.05$).

(3) Telmisartan and Tek-1 can alter expression levels of LPS induced I Kappa b Alpha protein in the cytoplasm and NF Kappa B65 protein in the nucleus of BV2 microglial cells.

**Figure 1:** Telmisartan and Tek-1 prevented TNF- α release in LPS-stimulated BV-2 cells with pretreatment of Telmisartan.**Figure 2:** Telmisartan and Tek-1 prevented TNF- α release in LPS-stimulated BV-2 cells with pretreatment of Tek-1.**Figure 3:** Effects of Telmisartan and Tek-1 on the expression of CD11b in LPS-stimulated BV-2 cells.

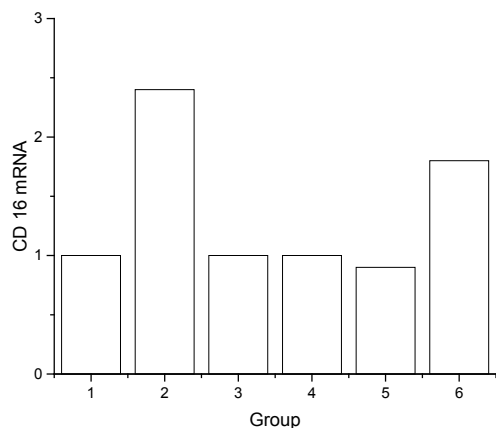


Figure 4: Effects of Telmisartan and Tek-1 on the expression of CD16 in LPS-stimulated BV-2 cells.

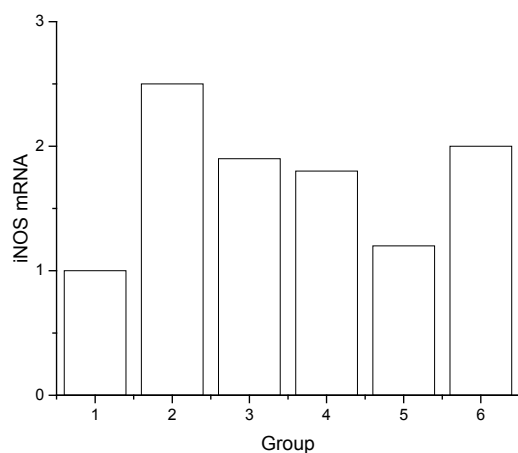


Figure 5: Effects of Telmisartan and Tek-1 on the expression of iNOS in LPS-stimulated BV-2 cells.

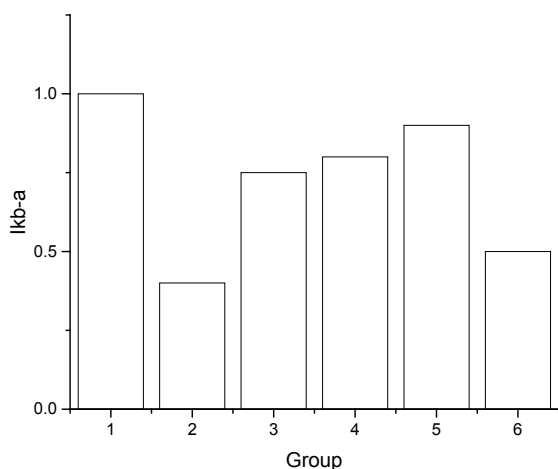


Figure 6: Effects of Telmisartan and Tek-1 on Ikb- α activity in LPS-stimulated BV-2 cells.

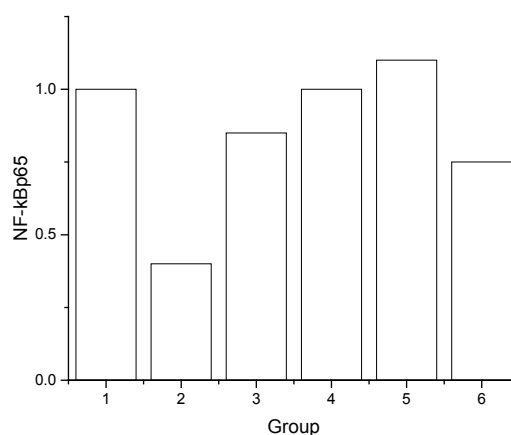


Figure 7: Effects of Telmisartan and Tek-1 on NF- κ Bp65 activity in LPS-stimulated BV-2 cells.

We use Western Blot method for the detection of NF- κ Bp65 and I Kappa b Alpha protein expression in BV 2 murine microglial cells. The results are shown in Figure 6-7.

Compared to control group, Ikb α protein expression in LPS processing group reduced significantly and NF- κ Bp65 nuclear protein expression increased. Compared to LPS processing group, telmisartan and Tek-1 of 0.1-10 μ M can increase significantly Ikb α protein expression. Telmisartan of 0.1-10 μ M can reduce significantly NFkbBp65 levels, but Tek-1 of 0.1-10 μ M has no obvious effects.

(4) Effects of Telmisartan and Tek-1 on LPS-induced p-ERK1/2 and p38 expression in murine microglial cells.

We use Western Blot method for the detection of expression-ERK1/2 and p38 protein expression in BV2 murine microglial cells. The results are shown in Figure 8-9.

Compared with the control group, p-ERK1/2 and p-p38 protein expression were significantly increased in LPS processing group. Compared to LPS processing group, telmisartan of 0.1-10 μ M can reduce p-ERK1/2 and p-p38 protein levels and telmisartan of 10 μ M resulted in a statistically significant difference ($p < 0.05$). Tek-1 of 0.1-10 μ M can reduce p-ERK1/2 and p-p38 protein expression, p-p38 reduction was statistically significant.

4 Discussion

Brain-derived inflammation caused by AD injury is closely related to over activation of the microglia. Over-activation of microglia increases the expression of inflammatory cytokines such as TNF- α , IL-1 and IL-6 so on. In order to further clarify the ability of telmisartan and Tek-1 to inhibit excessive activation of microglial cells and their molecular

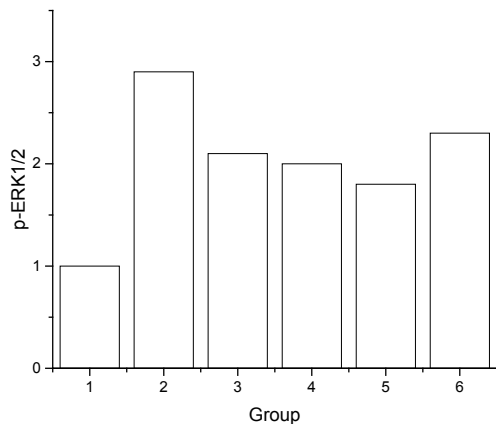


Figure 8: Effects of Telmisartan and Tek-1 on p-ERK1/2 activity in LPS-stimulated BV-2 cells.

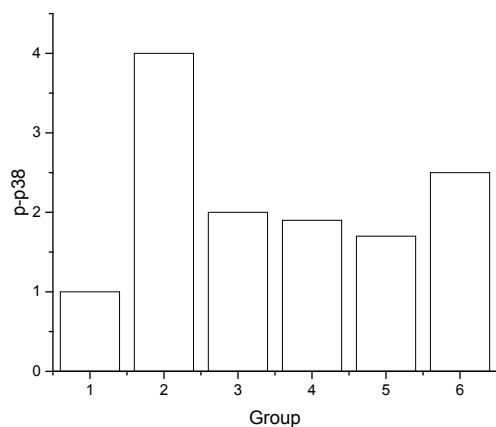


Figure 9: Effects of Telmisartan and Tek-1 on p-p38 activity in LPS-stimulated BV-2 cells

mechanisms, we selected BV-2 mouse microglial cell line in vitro to conduct our study [7]. Microglial cells stimulated by LPS is a commonly used in vitro model to study release of neurotoxic factors and proinflammatory cytokines by microglia [8]. LPS activates microglial cells through Toll TLR-4, inducing the release of proinflammatory cytokines, chemokines and inflammatory mediators which mediate signal transduction mechanisms closely associated with MAPK and NF- κ B signaling pathway activation.

PPARs are a superfamily of nuclear receptors which are mainly involved in lipid metabolism, immune and inflammatory responses through the regulation of gene transcription [9]. Previous results showed that the PPAR gamma agonist 15d-PGJ2 inhibited primary mouse microglial cell activation and star-shaped glial cell release of proinflammatory cytokines including NO, TNF- α , IL-1 β and MCP-1. In addition, Ying WANG have also found that a new type γ/α dual PPAR agonist can reduce hypoxia-

induced expression of TNF- α , COX-2 and p38 by BV-2 mouse microglial cells. Therefore, PPAR gamma plays an important role in inhibiting microglial inflammatory response.

TNF- α is produced by macrophages and binds to the TNF receptors on neurons and enhances cytotoxicity-induced inflammation chain reaction that inhibits production of TNF- α to inhibit inflammation. This study showed that telmisartan and Tek-1 are effective in suppressing LPS-induced microglial cell release of TNF α , however low concentration Tek-1 of 0.1 μ M is not as effective as telmisartan. Furthermore, PPAR gamma antagonists GW9662 can partially reverse the inhibition of TNF- α release by these two compounds, but it is not statistically different. Pablo Garrido-Gil showed that telmisartan can directly activate PPAR gamma and PPAR γ activation indirectly by blocking AT1 receptors where there is an interaction between them. In this experiment, telmisartan and Tek-1 cannot be excluded by blocking AT1 receptors to indirectly activate PPAR gamma. This may be the reason that GW9662 cannot completely block the effects of telmisartan and Tek-1 on TNF- α expression. Therefore, we believe that activation of PPAR gamma by telmisartan and Tek-1 is one of the important mechanisms for decreasing microglial inflammatory response [10].

CD11b is an important marker of microglia and CD16 is the marker for monocyte-macrophage. Their expression shows that microglia are activated and involved in the inflammatory response in the brain. In the case of infection and endotoxin, macrophages and astrocytes are able to produce inducible nitric oxide synthase (iNOS), resulting in NO formation, after which high concentrations of NO primarily effect toxicity by mitochondrial damage, lipid oxidation and DNA damage. We measured, on LPS-induced BV-2 mouse model of microglial inflammatory response, changes in CD11b, CD16 and iNOS mRNA expression induced by telmisartan and Tek-1 [11]. The results showed that telmisartan and Tek-1 can significantly inhibit their expression.. Tek-1 may have a stronger inhibitory effect on iNOS-mediated signaling pathways than that of telmisartan. Meanwhile, PPAR gamma antagonist GW9662 can partly reverse the inhibitory effects of the two compounds on LPS-induced iNOS and CD11b and CD16 expression. Results showed that telmisartan and Tek-1 improve the role of inflammation in the brain by inhibiting excessive activation of microglial cells and reducing release of Inflammatory Cytokines by activated microglia. Telmisartan and Tek-1 effects further prompt that PPAR gamma plays an important role in the aspect of neurological inflammation.

Many signaling molecules are involved in LPS-induced microglial inflammatory response, including ROS, PI3K/

AKT, MAPKs and NF- κ b signaling pathway. When LPS combines with TLR4, it mainly activates MAPKs and NF- κ b signaling pathway mediated by changes of cytokine expression [12].

MAPKs are a class of serine/threonine protein kinases involved in the transduction of signals from membrane to nucleus, where they are involved in the regulation of inflammation-related gene expression. It plays an important role in necrosis including cell cycle regulation, proliferation, differentiation and apoptosis. Activation of microglial cells leads to MAPK signal transduction, resulting in increased expression of iNOS, TNF α and COX2. The results of this study show that ERK, JNK, and p38 phosphorylation levels are elevated when BV2 microglial cells are stimulated by LPS. Telmisartan and Tek-1 can reduce their levels of phosphorylation. This suggested that the MAPK signal transduction pathway is one of the molecular mechanisms of signal transduction regulated by telmisartan and Tek-1 during microglial cells inflammatory response [13].

When NF- κ b combines with inhibitory factor I κ b together, NF κ b enters the nucleus, inhibiting expression of downstream inflammatory factors. When stimulated by foreign factor, I κ b is degraded, leading to activation of NF- κ b and downstream expression of TNF- α , IL-1 β and iNOS. Our research results displayed that LPS stimulation of BV-2 microglial cells reduced I κ b expression and increases NF- κ b expression. Telmisartan and Tek-1 can inhibit LPS induced reduction in I κ b expression and increased NF- κ b expression. Furthermore, PPAR gamma antagonistic agent GW9662 also can reverse the activity of these two compounds to again reduce I κ b protein expression and increase NF- κ b protein. These research results suggest that the reason telmisartan and Tek-1 reduced glial cell inflammatory response is related to activation of PPAR gamma and inhibition of NF- κ b signal pathway. Tek-1 exerts a more obvious effect than telmisartan in inhibiting NF- κ b signal pathway [14].

To sum up, Tek-1 inhibits LPS-induced BV-2 microglial activation and inflammatory responses by activating PPAR gamma and inhibiting MAPKs and activation of NF- κ b signaling pathway.

5 Conclusion

We have been studying the molecule Tek-1, a modified telmisartan molecule with a double role as both an anti-AT1 receptor antagonist and PPAR gamma activator.

Previous experiments showed that Tek-1 has high affinity for AT1 receptor. In AD models, telmisartan is a compound used for its positive neurological benefits. Tek-1 can also significantly improve AD mouse learning memory and learning memory capacity. Tek-1 also inhibits brain inflammation in these same mice through reduced release of IL-6 and MCP-1, meaning that improved learning memory capacity is closely related to reduced inflammatory response. Telmisartan and Tek-1 can obviously inhibit LPS induced release of TNF α from small glial cells and inhibit mRNA expression level. Further molecular mechanism research showed that telmisartan and Tek-1 reduce inflammation through effects on MAPKs and NF- κ b signal pathway. They can reduce phosphate level of ERK, JNK and p38 MAPK. They can also inhibit LPS induced reductions of I κ b protein and increases of NF- κ b proteins. The reason they can inhibit the activation of MAPKs and NF- κ b signaling was associated with partial activation of PPAR gamma receptors. To sum up, Tek-1 showed strong AT1 receptor antagonistic and AT1 receptor affinity. It is similar to telmisartan, which can improve AD-like models of learning and memory function of mice, alongside activating PPAR gamma and inhibiting NF- κ b and MAPK signaling to suppress inflammatory responses in microglial cells.

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Conflict of interest statement: Authors state no conflict of interest

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