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Research paper

Design, synthesis, and biological evaluation of compounds with a new scaffold as anti-neuroinflammatory agents for the treatment of Alzheimer's disease

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ABSTRACT

Twenty-eight compounds with a new scaffold were designed and synthesized by assembling fragments derived from known agents such as stilbenes and piperazinyl pyrimidines. Many strategies have been explored to improve the druggability of these series of compounds, such as increasing the distance between two benzene rings in the scaffold and introducing functional groups at designated positions.

These compounds were validated for their anti-neuroinflammatory activity in BV2 cells. Experimental results reveal that the most active compound **8b** can inhibit nitric oxide (NO), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) production with IC_{50} values of 1.0, 2.6, and 0.5 μM, respectively. The compound can also significantly modulate the MAPK pathways through inhibiting the phosphorylation of JNK, ERK1/2, and p38 MAPK without disturbing NF-κB pathway. Parallel artificial membrane permeation assay demonstrated that the most active compound can overcome the blood-brain barrier (BBB). Therefore, this compound can be a promising lead for the treatment of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD), a chronic neurodegenerative disorder, is characterized by memory loss and progressive cognitive impairment [1]. Studies on AD pathogenesis have been conducted for decades. It is hypothesized that acetylcholine, Amyloid β, tau protein and calcium homeostasis play major roles in the disease progress [2]. Increasing evidence indicates that inflammation may have a causal role in AD [3]. Microglia-dominated neuroinflammation is a characteristic feature of AD [4]. Chronic and sustained microglia activation can result in the increased inflammatory mediators including nitric oxide (NO), tumor necrosis factor α (TNF-α), and interleukin 1β (IL-1β) [5,6]. These mediators may directly induce neuronal apoptosis or amplify the local inflammatory response [7]. The inflammatory environment might promote the formation of senile plaques and neurofibrillary tangles, leading to neuronal damage [8]. While neuronal damage can induce microglial activation, which facilitates the propagation of a detrimental cycle of neuroinflammation [9–11]. Therapeutic agents modifying AD through the intervention of neuroinflammation is a feasible strategy [12–15].

Recently, the piperazinyl pyrimidine derivatives were reported as anti-neuroinflammatory agents (Fig. 1) [16–19]. Compound GIBH-130 (Fig. 1) can selectively suppress the IL-1β production with the IC_{50} value of 3.4 nM in LPS-activated microglia. GIBH-130 has been approved by China Food and Drug Administration for clinical trials against AD [17].

Meanwhile, resveratrol has been reported with a wide range of biological effects related to AD including anti-inflammation [20], anti-oxidation [21], and anti-amyloid β (Aβ) aggregation [22]. However, resveratrol's bioavailability is low. Therefore, we designed and synthesized twenty-eight stilbene mimics combining fragments derived from known inhibitors and resveratrol (Fig. 2). The preliminary structure and activity relationship (SAR) against neuroinflammatory has been established, and the mechanism of action was further investigated.

2. Chemistry

As shown in Scheme 1, compounds of series **4**, **5**, **6**, and **8** were synthesized. The intermediate acids were synthesized through a

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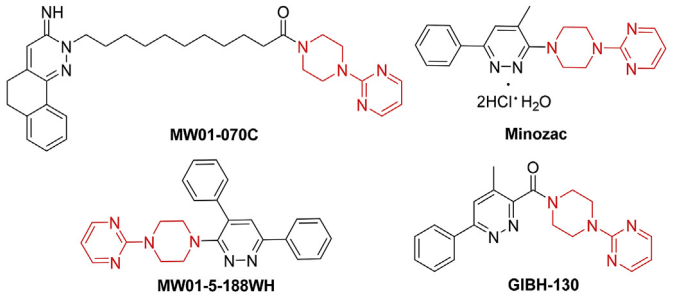


Fig. 1. Structures of reported anti-inflammatory agents.

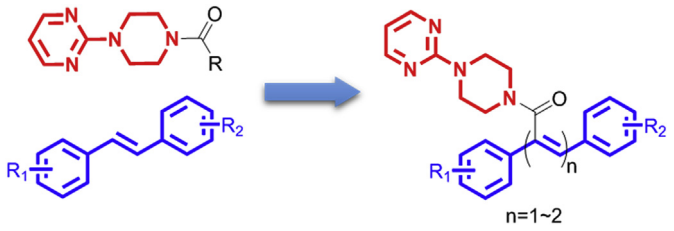


Fig. 2. Design strategy for the stilbene mimics.

Perkin reaction [23] with commercially available phenylacetic acids and benzaldehydes or cinnamaldehydes. The target compounds were prepared via the condensation of the secondary aliphatic amine 2-(piperazin-1-yl) pyrimidine and the intermediate acids using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) and triethylamine mixture as solvent. Twenty-six diphenylethene derivatives and two diphenylbutene derivatives were synthesized. The yields ranged from 45% to 81%.

3. Results and discussion

3.1. Inhibition of NO, IL-1β, and TNF-α production in LPS-induced BV2 cell lines and SAR study

The twenty-eight target compounds were evaluated for their anti-neuroinflammatory activity. Their effects on NO production was tested in LPS-induced BV2 microglia cell lines. Resveratrol (Res) was used as the positive control. The IC_{50} values for each tested compound were calculated and listed in Table 1. The preliminary structure and activity relationship (SAR) was analyzed. Compounds **4b**, **4c**, **4f**, **5h**, **6a**, **6c**, **6e**, **6f**, **6h**, **8a**, and **8b** showed higher inhibition of NO generation in BV2 cells (Table 1). When A-ring and B-ring were not substituted (Scheme 1), such as compound **4a,** the inhibition of NO production was decreased ($IC_{50} = 17.8$ μM) comparing with Res ($IC_{50} = 11.1$ μM). Based on this SAR observation, we synthesized more A-ring and B-ring substituted compounds. Compounds **4b** and **4c**, B-ring substituted with OCH_3 at C-4 or C-2, exhibited improved NO inhibitory activities ($IC_{50} = 9.5$ μM and 7.9 μM). Compounds **4e** and **4f**, B-ring substituted with Cl at C-2 or C-3, also exhibited improved NO inhibitory activities ($IC_{50} = 12.5$ μM and 10.1 μM). However, when B-ring was substituted with Cl at C-4 (**4d**), the inhibitory activity significantly reduced ($IC_{50} > 50$ μM). If B-ring at C-4 was substituted with CF_3 (**4g**), the activity remained unchanged ($IC_{50} = 14.9$ μM) comparing with **4a**. If both C-2 and C-5 were substituted at B-ring (**4h**), the activity would be significantly reduced ($IC_{50} = 48.1$ μM).

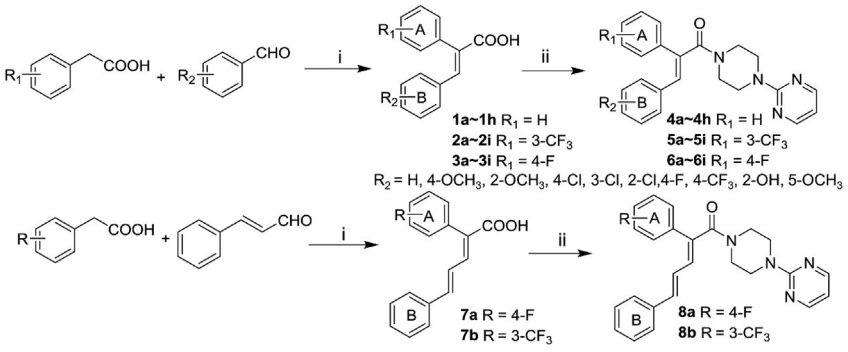
Compounds **5a~5i** are featured a 3-CF_3 group at A-ring, and the substituents at B-ring vary. Compounds **5g** and **5h**, B-ring substituted with F or CF_3 at C-4, showed improved NO inhibitory activities ($IC_{50} = 15.6$ μM and 6.5 μM) comparing with **4a**. Compound **5h** significantly increased the inhibition of NO release. This can be due to the stronger electron-withdrawing inductive effects of the 4-CF_3 group at B-ring. Compounds **5a~5f** and **5i** demonstrated reduced NO inhibitory activities comparing with **4a**.

Compound **6h**, with 4-F at A-ring and 4-CF_3 at B-ring, also demonstrated significantly increased inhibitory activity ($IC_{50} = 3.0$ μM) comparing with **4a**. While compounds **6b** and **6d**, B-ring substituted with 4-OCH_3 or 4-Cl, showed reduced activities ($IC_{50} = 23.1$ μM and 25.1 μM) comparing with **4a**. This indicates that a stronger electron-withdrawing group can improve the inhibitory activity. Compounds **6c** and **6f**, OCH_3 or Cl switched from C-4 to C-2 in B-ring, both showed increased activities ($IC_{50} = 9.8$ μM and 7.3 μM). Compound **6a**, exhibits significantly higher activity than compound **6g**, which has 4-F at B-ring ($IC_{50} > 50$ μM).

In summary, the compounds with 2,5-substitutes at B-ring (2-OH, 5-OCH_3) were unable to demonstrate inhibitory activity ($IC_{50} > 40$ μM).

In order to identify more active compounds, based on the scaffold of GIBH-130 (Fig. 1), we designed and synthesized new compounds (**8a** and **8b**) by increasing the distance between A-ring and B-ring. Compounds **8a** and **8b**, A-ring substituted with 4-F or 3-CF_3, did show significantly increased activities ($IC_{50} = 7.9$ μM and 1.0 μM), which are better than resveratrol.

These compounds were further validated for their anti-neuroinflammatory efficacy with TNF-α and IL-1β assay experiments [19,20]. The results are listed in Table 1. Compound **8b** was the most potent compound against NO, TNF-α, and IL-1β production with the IC_{50} values of 1.0 μM, 2.6 μM, and 0.5 μM, respectively, and was further investigated for anti-neuroinflammatory mechanism of action.



Scheme 1. Synthesis of stilbene mimics **4a**-**4h**, **5a**-**5i**, **6a**-**6i**, and **8a**-**8b**. Regents and conditions: (i) Et_3N, Ac_2O, 90 °C, overnight; (ii) 2-(piperazin-1-yl)pyrimidine, Et_3N, EDC·HCl, CH_2Cl_2, r.t, overnight.