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Discovery of pyrazole as C-terminus of selective BACE1 inhibitors

Yiquan Zou [a,1], Lei Xu [b,1], Wuyan Chen [a], Yiping Zhu [a], Tiantian Chen [a], Yan Fu [a], Li Li [a], Lanping Ma [a], Bing Xiong [a], Xin Wang [a], Jian Li [c], Jianhua He [c], Haiyan Zhang [a,*], Yechun Xu [a,*], Jia Li [b,*], Jingkang Shen [a,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, PR China
[b] The National Center for Drug Screening, 189 Guoshoujing Road, Shanghai 201203, PR China
[c] Shanghai Institute of Applied Physics, Chinese Academy of Sciences, 239 Zhang Heng Road, Pudong New District, Shanghai 201203, PR China

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ABSTRACT

We recently discovered and reported dual inhibitor **5** of AChE and BACE1 with *N*-benzylpiperidine ethyl as C-terminus. Compound **5** showed potent inhibitory activities for BACE1, and could reduce endogenous $A\beta_{1-40}$ production in APP transgenic mice. In present work, we rapidly identified substituted triazole as the C-terminus of compound **5** by replacing the benzylpiperidine ethyl group with click chemistry and tested these synthesized compounds by *in situ* screening assay. As revealed by the crystal structures of BACE1 in complex with our triazole compound **12**, we found that Pro70 and Thr72 located in the flap region were the critical components for binding with these inhibitors. With the aid of the crystal structure, a new series of five-membered heterocyclic compounds was prepared in order to explore the structure–activity relationship (SAR) of this class of molecules. From these efforts, pyrazole was discovered as a novel C-terminus of BACE1 inhibitors. After further modification of pyrazole with variable substituents, compound **37** exhibited good potency in enzyme inhibition assay (IC_{50} = 0.025 μM) and compound **33** showed moderate inhibition effects on Aβ production of APP transfected HEK293 cells. Moreover, these pyrazole derivatives demonstrated good selectivity versus cathepsin D. Our results indicated that the vicinity of Pro70 and Thr72 might be utilized as a subsite, and the discovered pyrazole derivatives might provide useful hints for developing novel BACE1 inhibitors as anti-AD drugs.

1. Introduction

Alzheimer's disease (AD), a form of senile dementia, is characterized by a progressive loss of memory and cognitive ability, now affecting more than 35 million elderly people worldwide with skyrocketing healthcare costs far exceeding $100 billion annually [1]. The pathology of this neurodegenerative disorder usually manifests itself with the presence of extraneuronal aggregation of plaques composed of β-amyloid peptides (Aβ) [2]. Aβ is derived from sequentially proteolytic cleavage of the β-amyloid precursor protein (β-APP) by two aspartic acid proteases, referred as β- and γ-secretase, respectively [3]. Since γ-secretase also cleaves other transmembrane proteins, such as Notch, which is involved in cell differentiation [4] thus chronic high doses of γ-secretase inhibitors may disrupt Notch-mediated processes in the gastrointestinal tract, spleen, and thymus, leading to potential mechanism-based toxicity. In contrast, Notch inhibition is not expected for β-secretase (β-site amyloid precursor protein cleaving enzyme 1; BACE1) inhibitors. Moreover, BACE1 has been identified as the rate-limiting enzyme for Aβ production [3] and BACE1 knockout homozygote mice show complete absence of Aβ production and have no reported side effects [5–7]. Therefore, BACE1 is considered as a promising target for developing drugs to treat and/or prevent AD [8].

However, development of agents capable of inhibiting the BACE1 activity has been proved to be difficult due to the large binding groove of the enzyme. According to the geometry of the long binding groove of BACE1 and the original peptide inhibitors which mimic the sequence pattern of substrate APP [9], BACE1 inhibitors were in general subdivided into three regions: an N-terminal portion, a central core and a C-terminus. In the last decade, these sections were individually subjected to modification

Abbreviations: EDCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HOBt, *N*-hydroxybenzotriazole; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; EtOAc, ethyl acetate; DCM, dichloromethane; THF, tetrahydrofuran.

* Corresponding authors. Tel.: +86 21 50806600x5407; fax: +86 21 50807088.
E-mail addresses: hzhang@mail.shcnc.ac.cn (H. Zhang), ycxu@mail.shcnc.ac.cn (Y. Xu), jli@mail.shcnc.ac.cn (J. Li), jkshen@mail.shcnc.ac.cn (J. Shen).

[1] These two authors contributed equally to this work.

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for reducing the peptidic characters. Many efforts have devoted to find better inhibitors [10], such as via high throughput screening, fragment-based drug discovery and virtual screening. Among them, the peptide mimic inhibitors were extensively studies. Although the N-terminus and central core parts which bound to the hydrophobic S1, S3 and polar S2, S4 subpockets had been optimized extensively (Fig. 1) [11–15], the C-terminus, occupied polar S1′, S2′, S3′, S4′ subpocket, which could hold various sizes and where different polar groups were rarely explored.

Recently, we have developed a novel series of dual inhibitors of AChE and BACE1 through introducing *N*-benzylpiperidine moiety of donepezil at C-terminus. These inhibitors exhibited good BACE1 inhibitory potency in enzymatic assay, and also showed good inhibitory effects on Aβ production of APP transfected HEK293 cells and mild protective effect against hydrogen peroxide (H_2O_2)-induced PC12 cell injury [15]. This previous study demonstrated that the C-terminus was also a critical component in BACE1 inhibitors and spurred us to explore more novel functional groups acting as C-terminus of BACE1 inhibitor. To easily track the improvement from C-terminus of inhibitors, we kept the N-terminus of compound **5** and a facile central core (Fig. 2). By utilizing click reaction and *in situ* screening assay, we could quickly assemble the effective fragments and screen the combined compounds (**10**–**17**) efficiently. Through this technology, we obtained the novel 1,2,3-triazole inhibitors which displayed potent inhibitory effects toward BACE1 and good selectivity over cathepsin D (Table 1). As revealed by the crystal structures of BACE1 in complex with compound **12**, Pro70 and Thr72 in the flap region were important for the binding interactions of these inhibitors. To elaborate the

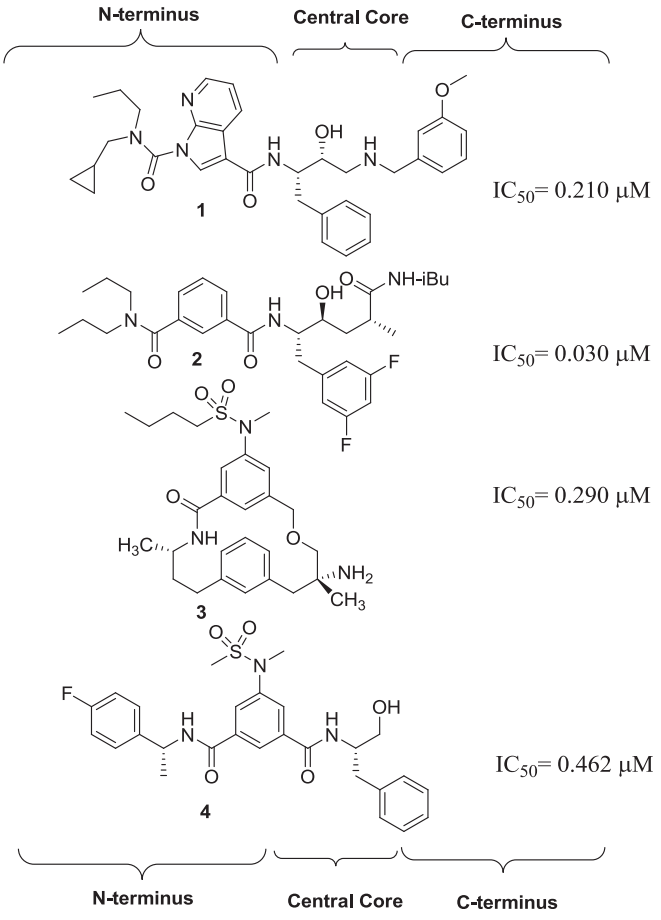

Fig. 1. Representative optimized N-terminus of BACE1 inhibitors (**1**–**4**).

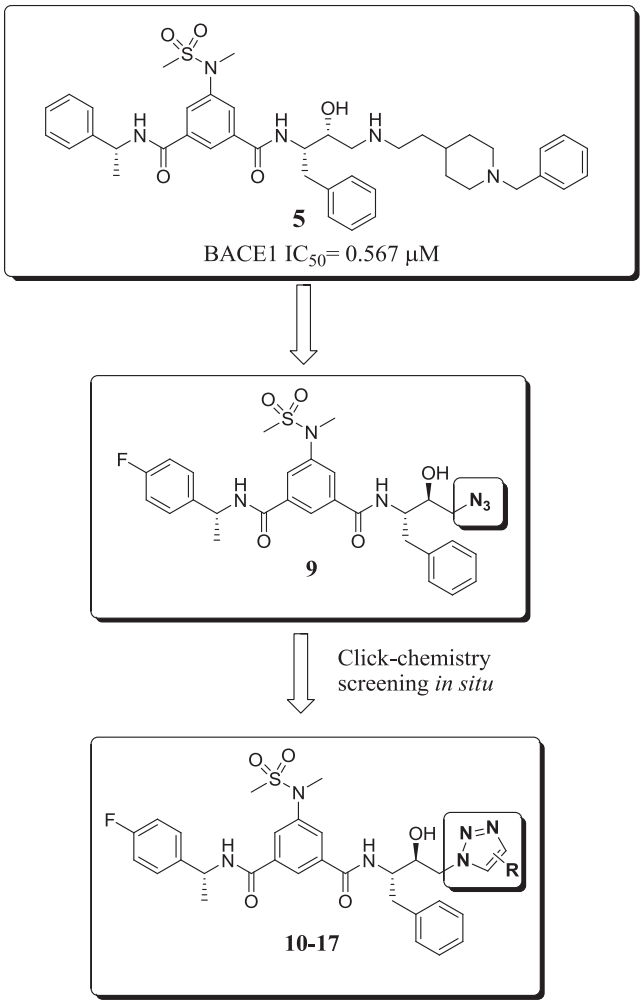

Fig. 2. Click chemistry combined with *in situ* screening approach for discovering β-secretase inhibitors.

structure–activity relationship (SAR) of C-terminus and improve the cellular activity, we replaced triazole with other five-membered heterocycles (compound **20**–**24**). Verified by the crystal structure of BACE1 in complex with compound **20** and **28** and SAR, we found pyrazole derivatives showed potent activities in BACE1 enzymatic assay, good inhibitory effects on Aβ production of APP transfected HEK293 cells and good selectivity over cathepsin D. Herein, we displayed the discovery of pyrazole as novel C-terminus of BACE1 inhibitors which might be beneficial for developing novel selective BACE1 inhibitors as anti-AD drugs.

2. Results and discussion

2.1. Chemistry

The initial compounds (**10**–**17**) were synthesized by click reaction [16]. The Huisgen Cu(I)-catalyzed alkyne-azide cycloaddition (CuAAC, a paradigm of click chemistry) had become a widely used strategy for chemical space exploration in drug design [17]. The synthetic appeal of click reaction relied upon their high yields, simple reaction conditions, tolerance of oxygen and water, and simple product isolation [18]. The Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction between an organic azide and a terminal alkyne would create 1,2,3-triazole derivatives. The synthesis of intermediate azide compound **9** was shown in Scheme 1, and the