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Design, synthesis and evaluation of 2-amino-imidazol-4-one derivatives as potent β-site amyloid precursor protein cleaving enzyme 1 (BACE-1) inhibitors

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ABSTRACT

Inhibition of β-site amyloid precursor protein cleaving enzyme 1 (BACE1) to prevent brain β-amyloid (Aβ) peptide's formation is a potential effective approach to treat Alzheimer's disease. In this report we described a structure-based optimization of a series of BACE1 inhibitors derived from an iminopyrimidinone scaffold **W-41** (IC_{50} = 7.1 μM) by Wyeth, which had good selectivity and brain permeability but low activity. The results showed that occupying the S_3 cavity of BACE1 enzyme could be an effective strategy to increase the biological activity, and five compounds exhibited stronger inhibitory activity and higher liposolubility than **W-41**, with **L-5** was the most potent inhibitor against BACE1 (IC_{50} = 0.12 μM, logP = 2.49).

Alzheimer's disease (AD), a chronic neurodegenerative disorder predominantly occurs among the elderly, is the leading cause of dementia.[1] Current treatments include acetylcholinesterase inhibitors and *N*-methyl-D-aspartate (NMDA) receptor antagonists which just temporarily alleviate the cognitive decline in the initial phase of the disease and do not significantly affect the underlying progress.[2] According to the β-amyloid (Aβ) hypothesis, the abnormal aggregation and deposition of Aβ, generated by the hydrolysis of amyloid precursor protein which could be catalyzed by β-site amyloid precursor protein cleaving enzyme 1 (BACE1), forms senile plaques with neurotoxicity and triggers complex cascades,[3] meanwhile it leads damage to synapses,[4] those are the main causes of AD. Therefore, processes that limit the production and accumulation of Aβ by preventing the formation, inhibiting the aggregation, or enhancing the clearance for Aβ may offer effective treatments for AD. BACE1, as the first rate-limiting enzyme of the amyloidogenic processing pathway, is considered to be a prominent therapeutic target for treating AD by diminishing the formation of Aβ peptide.[5]

Currently, multiple BACE-1 inhibitors have been studied in clinical research. **MK-8931 (Verubecestat)** (Fig. 1), reported by Merck in 2016, could reduce the production of Aβ amyloid peptide by 79% in early clinical trials.[6] Regrettably, **MK-8931**'s clinical trials had been terminated in 2018 because of its ineffective treatment of mild to moderate Alzheimer's disease. **AZD3293** (**Lanabecestat**; **LY3314814**) (Fig. 1), which was reported by Astrazeneca and Eli Lilly in 2017 as an orally active potent BACE1 inhibitor,[7] was also terminated in phase III clinical trial for the same reason like **MK-8931**. **CNP520** (Fig. 1), which was jointly developed by Novartis and Amgen in 2017, had a selectivity, pharmacodynamics and suitable distribution profile for AD prevention studies,[8] is now in clinical phase III trial. **JNJ-54861911** (Fig. 1), which was developed by Janssen in 2016, was a potent brain-penetrant BACE1 inhibitor, achieved high and stable Aβ reductions after single and multiple dosing in healthy participants.[9] It is currently undergoing phase II/III clinical trials. **Elenbecestat (E-2609)**, which was developed by Eisai and Biogen in 2014 and the structure was not public. It was the first substance that had significant effect on Aβ level in clinical studies of mild to moderate Alzheimer's disease.[10] **Elenbecestat** is currently undergoing phase III clinical trials and has passed the 8th safety review of late.

Recently, Wyeth identified the diphenyl iminohydantoin compound **W-41** as a potential BACE1 inhibitor with its IC_{50} was 7.1 μM (Fig. 2).[11–13] Despite **W-41** had relatively weaker BACE1 inhibitory

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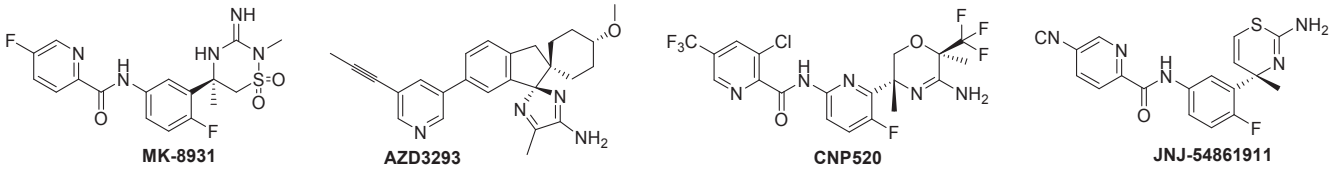


Fig. 1. The chemical structures of BACE1 inhibitors that have entered clinical trials.

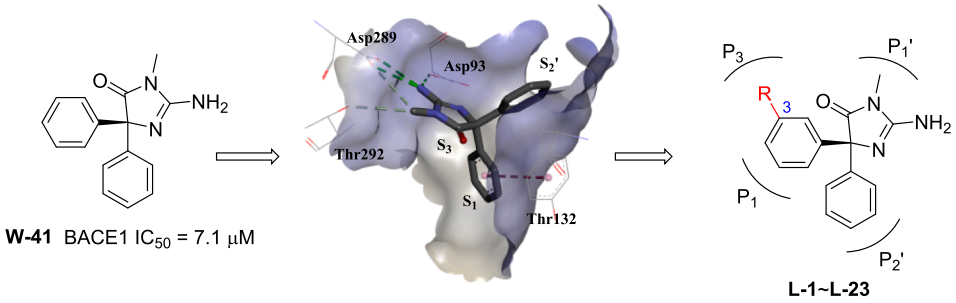


Fig. 2. The design of compounds **L-1**–**L-23** based on the docking study of **W-41** with BACE1 (PDB ID: 4DJU).

activity compared to other compounds, it exhibited substantially improved selectivity against cathepsin D,[14] which was closely related to the side effects of BACE1 inhibitors in animal model,[15] and improved rat pharmacokinetic profile with a brain plasma ratio of 1.8 indicating that **W-41** had good brain permeability, so this inhibitor was appropriate to be a lead compound to improve its inhibitory activity against BACE1.

In this report, we firstly analyzed the X-ray structure of **W-41** binding to the active site of BACE1 using DS 4.0. The results (Fig.2) revealed that H-bonding interactions were formed between the terminal amino group on guanidine moiety and Asp93, Asp289, which were crucial for BACE1 binding.[16] Meanwhile, the two phenyl groups occupied S_1 and S_2' pockets respectively. However, the adjacent S_3 pocket showed no interaction with lead compound **W-41**. We assumed that introducing an additional R group at P_3 section might facilitate the binding ability between **W-41** and BACE1 by occupying the S_3 pocket. Based on that attaching rigid linear fragments at P_3 site was beneficial for improving the BACE1 inhibitory activity,[11] and our docking study indicated that 3-biaryl substitution could occupy the contiguous S_1-S_3 pockets, we tried to link a rigid biaryl substitution at P_3 position of **W-41** to occupy the S_3 pocket. Therefore, a series of aminohydantoin derivatives **L-1**–**L-23** with aryl group substituted at the C-3 position were designed and synthesized to enhance their binding ability with BACE1.

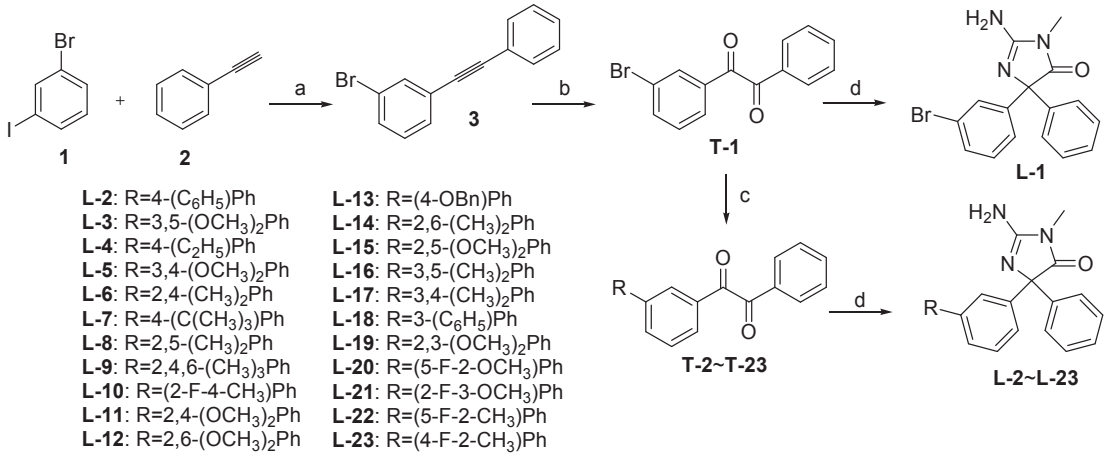
The synthetic routes for aminohydantoins **L-1**–**L-23** were shown in Scheme 1. The Sonogashira coupling of 1-bromo-3-iodobenzene (**1**) with ethynylbenzene (**2**) in the presence of $Pd(PPh_3)_4$, CuI and PPh_3 in trimethylamine (TEA) afforded 1-bromo-3-(phenylethynyl)benzene (**3**) in 96% yield. Then, it was oxidized by heating in DMSO at 140 °C with $Pd(PPh_3)_2Cl_2$ as a catalyst to produce the important intermediate **T-1**, which could be further converted into the disubstituted diketones **T-2**–**T-23** through Suzuki coupling with different arylboronic acids, catalyzed by $Pd(PPh_3)_2Cl_2$ and K_2CO_3, in the mixed solvent of 1,4-dioxane and H_2O (10:1) at 100 °C. At last, the treatment of diketones **T-1**–**T-23** with *N*-methylguanidine hydrochloride in EtOH, using TEA as a base at 80 °C, produced the desired products aminohydantoins **L-1**–**L-23** in excellent yields.

All the compounds were evaluated for their enzymatic inhibition against BACE1 determined by fluorescence resonance energy transfer (FRET) method. As shown in Table 1, the bromine substituted derivative **L-1** showed no inhibitory activity against the enzyme, as the same as multi phenyl substituted derivatives **L-2**, **L-13** and **L-18**, indicating that the right size of the introduced group was significant for occupying the S_3 cavity. Among the single phenyl-substituted derivatives, compounds **L-3**, **L-5**, **L-10**–**L-12**, **L-15**, **L-19**–**L-23** performed certain BACE1 inhibitory activity but the derivatives with only alkyl groups modification on the introduced benzene ring like **L-4**, **L-6**–**L-9**, **L-14**, **L-16** showed opposite results except 2,4-dimethyl substituted derivative **L-17** with its IC_{50} against BACE1 was 0.42 μM. Meanwhile, compounds **L-5**, **L-10**, **L-15**, **L-20** and **L-21**, which contained biphenyl structure with fluorine or methoxy substituted at 2 or 4 position, obviously exhibited much stronger inhibitory activity against BACE1 than the positive control **LY2811376** and the parent analog **W-41**. These results indicated that phenyl group might favor the interaction with the S_3 cavity but require assistance of certain groups like fluorine or methoxy which were substituted at position 2 or 4. Interestingly, when the *meta* and *para* position were both substituted by methoxy group, the obtained compound **L-5** exhibited the most potent inhibitory activity, with its IC_{50} against BACE1 was 0.12 μM.

Subsequently, the logP values of all compounds were measured, considering that they are closely related to the process of drug entering the human body,[17] and the topological polar surface area (tPSA) of all the compounds were also calculated. Generally, central nervous system drugs usually have higher liposolubility, with their logP values are between 2 and 5,17 and small tPSA value which is lower than 90.[18] The results (Table 1) showed that all the compounds had good logP values which were between 2.04 (**L-1**) and 3.18 (**L-2**), and small tPSA which were below 77.15, indicating that they were suitable as central nervous drugs for further research.

To help rationalize the aforementioned structure–activity relationships, a model of BACE1 was constructed and the docking study was carried out. The most potent compound **L-5** was selected to perform the molecular docking study (Fig. 3) with the X-ray structure of BACE1 enzyme (PDB ID: 4DJU), which was obtained from the Protein Data Bank (RCSB PDB).

As expected, in the docking mode with BACE1 (Fig. 3), compound **L-5** deeply extended into the cavity of BACE1, and excellently combined with the hydrophilic and hydrophobic surface. Firstly, the amino group in amidazolidone moiety engaged in hydrogen bonding interactions with Asp93 and Asp289, which were crucial for BACE1 binding.[16] Secondly, the introduced phenyl structure successfully occupied the S_3 hydrophobic cavity, through the hydrogen-bonding formed by Arg189 residue and the methoxy group on it. Thirdly, the docking mode

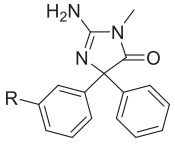


Scheme 1. Reagents and conditions: a) $Pd(PPh_3)_4$ (4 mol %), CuI (2 mol %), PPh_3 (8 mol %), TEA, 50 °C, 16 h, 96%; b) $Pd(PPh_3)_2Cl_2$ (5 mol %), DMSO, 140 °C, 5 h, 60%; c) $RB(OH)_2$ (2 equiv.), $Pd(PPh_3)_2Cl_2$ (10 mol %), K_2CO_3 (3 equiv.), 1,4-dioxane, H_2O, 100 °C, 3 h, 79%–93%; d) *N*-methylguanidine hydrochloride (1 equiv.), TEA (3.5 equiv.), EtOH, 80 °C, 10 h, 64%-74%.

confirmed that the biphenyl motif could occupy S_1 and S_3 pocket respectively.

In summary, using **W-41** as a lead compound, a novel class of aminohydantoins has been designed and synthesized in order to improve the BACE1 inhibitory activities, five analogs **L-5**, **L-10**, **L-15**, **L-20** and **L-21** showed excellent inhibitory activity against BACE1, with **L-5** was the most potent inhibitor (IC_{50} = 0.12 μM). The results proved that occupying the S_3 pocket by incorporating phenyl group at 3-position modified with appropriate groups like fluorine or methoxy was effective to improve the BACE1 inhibitory activity. Furthermore, all the compounds showed good logP values and tPSA. These compounds could be used as potential BACE1 inhibitors for AD treatment and precursors for further modification. Subsequent reports will detail further optimized efforts to obtain inhibitors with improved cell and *in vivo* potency.

Table 1
The inhibitory activity against BACE1 and physical property of compounds **L-1**–**L-23**.



Compound	R	Inhibition rate (%) (at 10 μM)	Activity (IC_{50}, μM)	logP	tPSA[a]
L-1	Br	33.13 ± 0.36	> 10	2.04	58.69
L-2	4-(C_6H_5)Ph	43.90 ± 1.37	> 10	3.18	58.69
L-3	3,5-$(OCH_3)_2$Ph	80.74 ± 0.56	0.8512 ± 0.3445	2.41	77.15
L-4	4-(C_2H_5)Ph	43.53 ± 0.26	> 10	2.56	58.69
L-5	3,4-$(OCH_3)_2$Ph	97.45 ± 0.54	0.1194 ± 0.0007	2.49	77.15
L-6	2,4-$(CH_3)_2$Ph	40.86 ± 0.82	> 10	2.51	58.69
L-7	4-($C(CH_3)_3$)Ph	34.21 ± 2.27	> 10	3.10	58.69
L-8	2,5-$(CH_3)_2$Ph	42.07 ± 5.08	> 10	2.52	58.69
L-9	2,4,6-$(CH_3)_3$Ph	45.73 ± 3.86	> 10	2.55	58.69
L-10	(2-F-4-CH_3)Ph	96.99 ± 0.06	0.1414 ± 0.0046	2.97	58.69
L-11	2,4-$(OCH_3)_2$Ph	75.49 ± 0.86	1.5750 ± 0.0070	2.46	77.15
L-12	2,6-$(OCH_3)_2$Ph	82.11 ± 0.05	0.7205 ± 0.0766	2.02	77.15
L-13	(4-OBn)Ph	37.70 ± 0.93	> 10	2.99	67.92
L-14	2,6-$(CH_3)_2$Ph	34.15 ± 0.62	> 10	2.42	58.69
L-15	2,5-$(OCH_3)_2$Ph	95.05 ± 0.61	0.1953 ± 0.0241	2.54	77.15
L-16	3,5-$(CH_3)_2$Ph	47.16 ± 1.42	> 10	2.56	58.69
L-17	3,4-$(CH_3)_2$Ph	88.31 ± 0.50	0.4249 ± 0.0091	2.49	58.69
L-18	3-(C_6H_5)Ph	20.54 ± 3.72	> 10	3.14	58.69
L-19	2,3-$(OCH_3)_2$Ph	78.63 ± 0.48	1.1090 ± 0.0121	2.15	77.15
L-20	(5-F-2-OCH_3)Ph	95.97 ± 0.31	0.1735 ± 0.0049	2.86	67.92
L-21	(2-F-3-OCH_3)Ph	94.67 ± 2.39	0.2204 ± 0.0005	2.72	67.92
L-22	(5-F-2-CH_3)Ph	92.03 ± 0.49	0.3281 ± 0.0180	2.35	58.69
L-23	(4-F-2-CH_3)Ph	87.70 ± 0.75	0.4562 ± 0.0104	2.34	58.69
W-41	–	62.19 ± 0.22	3.4840 ± 0.0060	1.90	58.69
LY2811376	–	94.22 ± 2.46	0.2693 ± 0.0060	–	–

[a] tPSA were calculated by ChemProp.

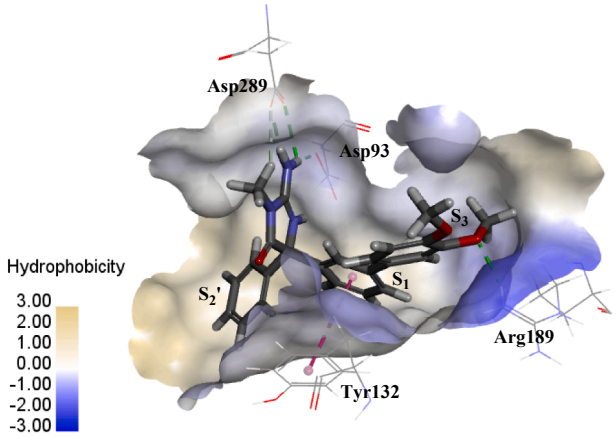


Fig. 3. Interactions between compound **L-5** and BACE1 (PDB ID: 4DJU).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.126772.

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