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Pyridinyl aminohydantoins as small molecule BACE1 inhibitors

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ABSTRACT

A novel class of pyridinyl aminohydantoins was designed and prepared as highly potent BACE1 inhibitors. Compound (S)-**4g** showed excellent potency with IC_{50} of 20 nM for BACE1. X-ray crystallography indicated that the interaction between pyridine nitrogen and the tryptophan Trp76 was a key feature in the S2′ region of the enzyme that contributed to increased potency.

Alzheimer's disease (AD) is the most common cause of dementia and is rapidly becoming the most prevalent problem in the aging human population. Although the cause of AD is still unclear, deposition of β-amyloid peptide (Aβ) in the brain is one of the few recognized hallmarks of AD pathogenesis.[1] It has been well established that Aβ is produced from membrane-bound β-amyloid precursor protein (APP) by the sequential proteolytic cleavage of two aspartyl proteases, β- and γ-secretase. β-Secretase (BACE1, also called Asp2 or memapsin2), has been identified as the enzyme responsible for the initial processing of APP.[2,3] Hence, the inhibition of this enzyme represents a strategy for the development of disease-modifying therapeutics for the treatment of AD.[4]

In a program to design and develop novel small molecule BACE1 inhibitors, a weak BACE1 inhibitor (IC_{50} = 38 μM), aminoimidazole **1** was discovered via high-through screening (Scheme 1).[5] Initial SAR around **1** quickly led to aminohydantoin **2** which showed a 10-fold improvement in BACE1 potency (IC_{50} = 3.4 μM).[6] Replacement of one of the phenyl groups in **2** with a pyridine gave pyridinyl aminohydantoin **3** which maintained the ligand's affinity for the BACE1 enzyme (IC_{50} = 2.7 μM).

We have previously reported that introduction of a 3-substituent on the phenyl group (projected in the S1 pocket) of compound **2** resulted in improvement in BACE1 activity through enhanced S3 pocket interactions.[6] We have now investigated whether the same trend would hold true for the pyridine analog **3**, and examined whether additional interactions between the ligand and protein backbone of the S3 pocket, by functionalization of the phenyl ring, could enhance the potency of derivatives of **3**.

Here, we report the design and synthesis of pyridinyl aminohydantoins **4** as small molecule BACE1 inhibitors. Particular focus was on exploration of the S3 pocket by introduction of substituents at the 3-position of the phenyl ring in **3**. In addition, through the use of an X-ray crystal structure, we observed a key interaction between the pyridine nitrogen in derivatives of **4** with Trp76 in the S2′ pocket, and we chose to utilize this in our design strategy.

Two approaches were used to prepare the target molecules **4**: (I) direct aminohydantoin formation from diketone intermediate **5**, and (II) derivatization of pyridinyl aminohydantoin intermediate **6** (Scheme 2).

As shown in Scheme 3, the diketone intermediates **5** used in strategy (I) were obtained via oxidation of 4-(3-arylphenylethynyl)pyridine **9** which was prepared starting with 3-aryl ethynylbenzene **7**. Sonogashira coupling of **7** with 4-bromopyridine **8** gave diaryl substituted ethyne **9** in good to excellent yield.[7] Alternatively, **9** could be prepared by Sonogashira coupling of commercially available 3-bromoiodobenzene **10** and 4-ethynylpyridine **11** followed by Suzuki coupling of the resulting 4-(3-bromophenylethynyl)pyridine **12** with aryl boronic acids or by Stille coupling of **12** with aryl stannanes.[8] Oxidation of **9** with $KMnO_4$ afforded diketone **5** in good to excellent yield.[9] Treatment of **5** with *N*-methyl

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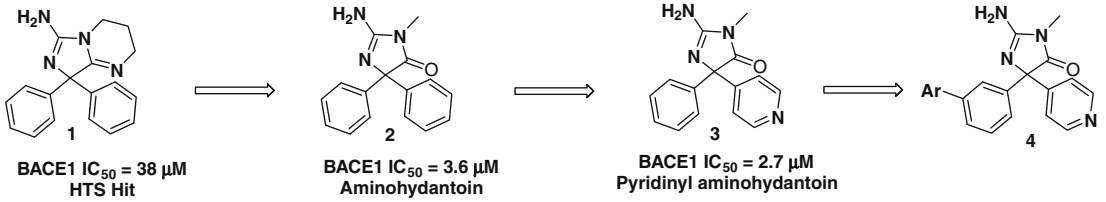
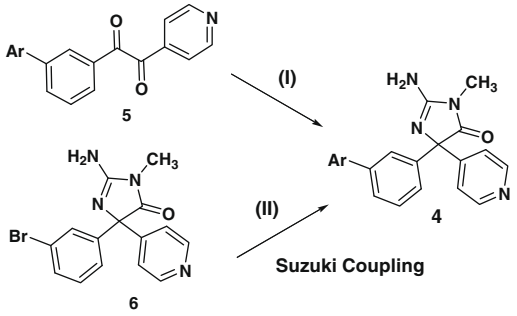
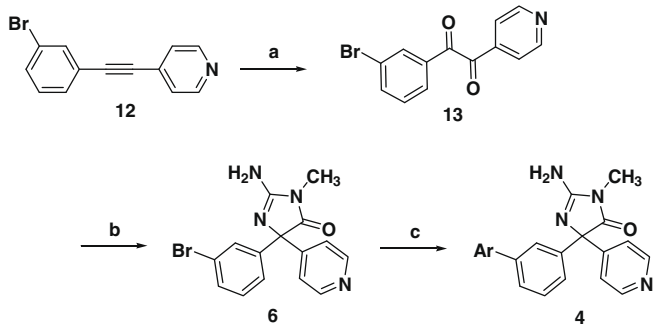


Scheme 1. From HTS hit to pyridinyl amino hydantoin.



Scheme 2. Strategy for the synthesis of pyridinyl aminohydantoins **4**.



Scheme 4. Reagents and conditions: (a) $KMnO_4/NaHCO_3/MgSO_4/H_2O$, rt, 75%; (b) *N*-methylguanidine/Na_2CO_3/EtOH/H_2O, reflux, 61%; (c) $ArB(OH)_2$/tetrakis(triphenylphosphine)palladium/Na_2CO_3/DME, reflux, 51–95%.

guanidine in the presence of Na_2CO_3 produced 3-arylphenyl pyridinyl aminohydantoin **4**.[10]

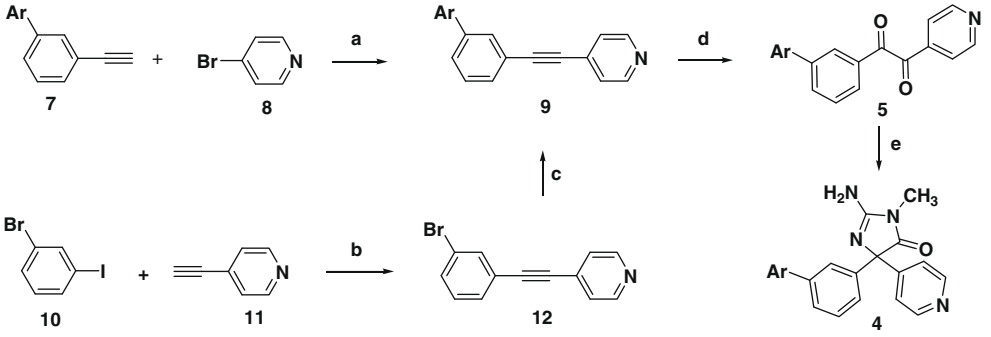
The precursor 3-bromophenyl pyridinyl aminohydantoin **6** used in the strategy (II) for Suzuki coupling, was prepared from 4-(3-bromophenylethynyl)pyridine **12** (Scheme 4). Oxidation of **12** with $KMnO_4$ under similar reaction conditions as **9** afforded bromo diketone **13** in good yield. Treatment of **13** with *N*-methyl guanidine as described above produced 3-bromophenyl pyridinyl aminohydantoin **6**, the precursor for Suzuki coupling. Standard Suzuki coupling of **6** with boronic acids furnished final products **4**.[11]

BACE1 and BACE2 affinities of the target ligands are summarized in Table 1. As an additional measure of selectivity, inhibition of cathepsin D was determined for selected compounds exhibiting potent BACE1 activity. The data show that introduction of a 3-aryl group to the benzene ring of **3** was beneficial for BACE1 affinity. Simple 3-phenyl analog gave 20-fold improvement in potency at BACE1 (**4a** vs **3**), which confirmed our previous findings[6] that extending in the S3 region improves potency. Replacement of phenyl with 3-methoxyphenyl (**4b**), 3-cyanophenyl (**4d**) or 3-fluorophenyl (**4e**) provided analogs with further improved BACE1 potency (54–89-fold), with the exception of the trifluoromethoxyphenyl derivative (**4c**) that resulted in only an 11-fold increase in ligand potency. Difluorophenyl derivatives (**4f**, **4g**, **4h**) also showed improved BACE1 activity up to 67-fold.

Furthermore, 3-heteroayl analogs (**4i**–**m**) provided derivatives with improved potency. For example, the thienyl and furyl derivatives showed a 4–11-fold improvement in BACE1 potency (**4i** and **4j** vs **3**), while the 2-pyrazinyl (**4k**) had 38-fold increase in ligand potency, giving a compound with an IC_{50} of 70 nM at BACE1. The 3-pyridinylphenyl and 2-fluoropyridin-3-ylphenyl groups were previously studied in related aminohydantoins with comparable results.[6] Similar trends were also found when these two groups were incorporated in pyridinyl aminohydantoins **4**. Both compounds **4l** and **4m** showed 45- and 89-fold BACE1 potency improvement compared to the parent derivative **3**.

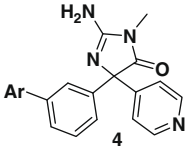
The majority of the prepared analogs were equally potent in both BACE1 and BACE2 activity, with the exception of compound **4m**, which demonstrated 33-fold selectivity versus BACE2. On the other hand, most of the tested compounds demonstrated high selectivity (>100-fold) for BACE1 versus cathepsin D.

Racemic pyridinyl aminohydantoin **4g** was separated into enantiomers (*S*)-**4g** and (*R*)-**4g**, with the (*S*)-enantiomer showing higher affinity than the (*R*)-enantiomer. The (*S*)-configuration of enantiomer **4g** is supported by X-ray studies. As shown in Figure 1, the amino group of hydantoin **4g** interacts with both aspartic acids (Asp32 and Asp238) of the enzyme backbone and the N3 nitrogen



Scheme 3. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$/CuI/TEA/DMF, 65 °C, 59–88%; (b) $Pd(PPh_3)_2Cl_2$/CuI/TEA/DMF, 65 °C, 87%; (c) $ArB(OH)_2$ or $ArSnBu_3$/tetrakis(triphenylphosphine)palladium/Na_2CO_3/DME or toluene, reflux, 40–77%; (d) $KMnO_4/NaHCO_3/MgSO_4/H_2O$, rt, 49–96%; (e) *N*-methylguanidine/Na_2CO_3/EtOH/H_2O, reflux, 37–52%.

Table 1
Biological activities of pyridinyl aminohydantoins[a]



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Compound	Ar	BACE1 IC_{50} (μM)	BACE2 IC_{50} (μM)	Selectivity BACE2/BACE1	Cathepsin D IC_{50} (μM)
3	H	2.68	2.01	0.8	ND
4a	Ph	0.13	0.14	1.1	30.1
4b	3-MeOPh	0.03	0.02	0.7	12.2
4e	3-CF_3OPh	0.25	0.14	0.6	14.1
4d	3-NCPh	0.04	0.04	1.0	24.2
4e	3-FPh	0.05	0.08	1.6	20.8
4f	2,3-diFPh	0.04	0.13	3.3	13.1
4g	2,5-diFPh	0.05	0.12	2.4	9.4
(*S*)-4g	2,5-diFPh	0.02	0.10	5.0	5.5
(*R*)-4g	2,5-diFPh	3.81	0.70	0.2	26.4
4h	3,5-diFPh	0.06	0.06	1.0	34.1
4i	3-Thienyl	0.24	0.19	0.8	31.6
4j	3-Furyl	0.63	0.57	0.9	46.8
4k	2-Pyrazinyl	0.07	1.01	14	ND
4l	3-Pyridinyl	0.06	0.48	8.0	ND
4m	2-Fluoropyrindin-3-yl	0.03	1.00	33	17.4

ND = not determined.

[a] A homogenous, continuous fluorescence resonance energy transfer (FRET) was used to assess compound inhibition for BACE1, BACE2, Cathepsin D, Pepsin, and Renin activities. The BACE1 and BACE2 activities were based on the cleavage of peptide substrate Abz-SEVNLDAEFR-Dpa (Swedish substrate), while peptide substrate MOCAc-GKPILFFRLK (Dnp)-D-R-NH2 was used for Cathepsin D. Kinetic rates were calculated and IC_{50} values were determined by fitting the % inhibition, as a function of compound concentration, to the Hill equation ($y = ((B * Kn) + (100 * xn))/(Kn + xn)$).

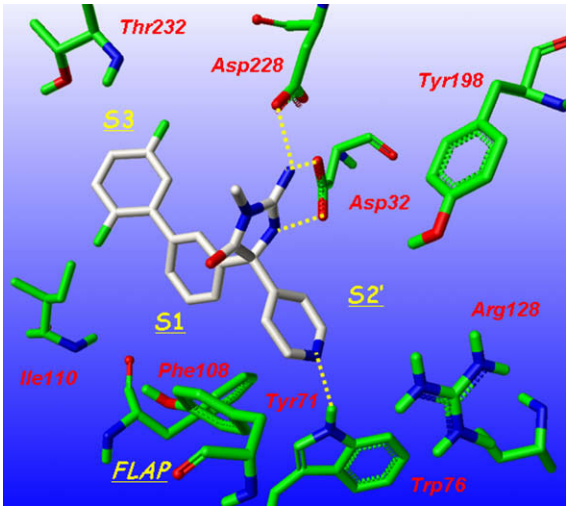

Figure 1. Crystal structure of BACE1 complexed with (*S*)-**4g** (PDB ID code: 3LHG) highlighting the key hydrogen-bonding interactions in yellow dashed lines between the catalytic aspartic acids Asp32 and Asp228 and the aminohydantoin ring, as well as between pyridine nitrogen and Trp76 at S2′.

of the imidazole ring interacts with Asp32 in a coplanar fashion. In addition, the pyridine nitrogen at S2′ interacts with the tryptophan Trp76. The difluoro phenyl moiety orients itself into the S3 pocket and enhances binding affinity for the BACE1 enzyme.

In summary, a novel class of pyridinyl aminohydantoins **4** was explored to develop a better understanding of BACE1 SAR in the S3 pocket pf the enzyme. This work revealed that more potent BACE1 inhibitors could be prepared taking advantage of several key interactions with the enzyme. X-ray crystallography indicated that the interaction between pyridine nitrogen and Trp76 was a key feature in the S2′ region of the enzyme that contributed to increased potency. When enantiomers of the hydantoin were resolved, for example, (*S*)-**4g** with IC_{50} of 20 nM, they proved to be significantly more potent than the *R*-isomer, in line with the X-ray analysis. These potent BACE1 leads can be optimized further and may prove to be useful tools for biological studies.

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