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Discovery and initial optimization of 5,5′-disubstituted aminohydantoins as potent β-secretase (BACE1) inhibitors

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ABSTRACT

8,8-Diphenyl-2,3,4,8-tetrahydroimidazo[1,5-*a*]pyrimidin-6-amine (**1**) was identified through HTS, as a weak (micromolar) inhibitor of BACE1. X-Ray crystallographic studies indicate the 2-aminoimidazole ring forms key H-bonding interactions with Asp32 and Asp228 in the catalytic site of BACE1. Lead optimization using structure-based focused libraries led to the identification of low nanomolar BACE1 inhibitors such as **20b** with substituents which extend from the S_1 to the S_3 pocket.

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is the leading cause of long-term decline in cognitive function in the elderly. It is estimated that more than 27 million people worldwide have AD and the number of cases is likely to increase in the future.[1] Current AD therapy is based on symptomatic treatment with Aricept™ (Pfizer), Exelon™ (Novartis) and Reminyl™ (Janssen). These acetylcholine esterase inhibitors are effective in patients only for a limited period of time and do not treat the underlying cause of the disease. Thus, there is an extremely urgent need for development of disease-modifying treatment for AD (Fig. 1).

Overwhelming evidence suggests that the formation of β-amyloid plaques is the fundamental cause of AD.[2,3] These plaques are formed by aggregation of amyloid fibrils, which in turn are formed from the amyloid β-peptide (β-amyloid, Aβ), which is generated by sequential proteolytic cleavage of amyloid precursor protein (APP) by β-secretase (BACE1) and γ-secretase.[4] Thus agents that inhibit BACE1 may decrease levels of Aβ, and have therapeutic benefit in AD.[5]

8,8-Diphenyl-2,3,4,8-tetrahydroimidazo[1,5-*a*]pyrimidin-6-amine (**1**)[6] was identified as weak (IC_{50} = 38 μM) inhibitor of BACE1 using a fluorescence energy transfer (FRET)-based HTS screen. An X-ray structure of **1** bound in the BACE1 active site[6] indicates that the guanidine moiety forms key H-bonding interactions with Asp32 and Asp228 in the catalytic site of BACE1. The two phenyl groups occupy the S_1 and S_2' pockets and are conve-

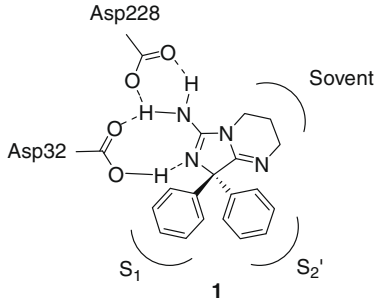

Figure 1. The structure of 8,8-diphenyl-2,3,4,8-tetrahydroimidazo[1,5-*a*]pyrimidin-6-amine (**1**) showing hydrogen bonding interactions between the guanidine moiety and Asp32 and Asp228 of BACE1, and the two phenyl groups projecting into S_1 and S_2' pockets (Model developed based on the X-ray structure PDB ID code: 3IGB[6]).

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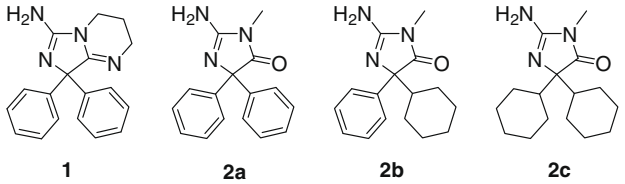
nient handles for SAR optimization, while the six-membered ring of **1** extends toward solvent and does not interact with the enzyme.

On the basis of the X-ray structure it was hypothesized that removal of tetrahydropyrimidine ring of **1**, while lowering the molecular weight, should have little effect on the binding affinity since the resulting compound 2-amino-3-methyl-5,5-diphenylhydantoin (**2a**) or its partially or fully saturated analogs (**2b**, **2c**) should retain all the key interactions with protein.



1 **2a** **2b** **2c**

The aminohydantoins are prepared by reaction of diketones with substituted aminoguanidines via a benzil like rearrangement (Scheme 1).

Under mild base conditions, such as sodium carbonate, the reaction produces almost exclusively the endo-substituted kinetic product **6**, while strong bases, for example, sodium hydroxide, lead preferentially to exo-substituted, thermodynamically favored product **7**.[7,8] Compounds of structure **7** were found to be much less active in the BACE1 FRET assay.

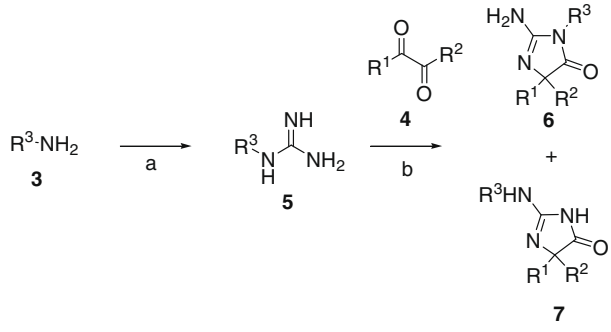
The required substituted aminoguanidines were produced by the reaction of primary amines with 1*H*-pyrazole-1-carboxamidine hydrochloride.[9]

The synthesis of aminohydantoin libraries required access to non-commercial, unsymmetrical, cyclohexyl substituted diketones for example, **12** and **17**, so two routes outlined in Schemes 2 and 3 were devised.[10]

Exhaustive hydrogenation of methyl mandelate **8** affords the hydroxyester **9**, which is oxidized to the corresponding keto ester under Swern oxidation conditions and hydrolyzed to the 2-cyclohexyl-2-oxoacetic acid **10**. The acid is activated as an acid chloride and reacted with a cuprate reagent generated in situ from the appropriate Grignard reagent leading to diketones **12**.

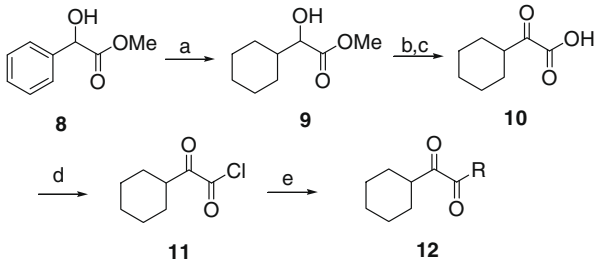
Alternatively, aryl-amido functionalized diketones for example, **16** used for synthesis of aminohydantoin array **20a–h** are prepared by a Sonogashira coupling of Boc-protected bromoaniline **14** with cyclohexylacetylene **13** (Scheme 3). The disubstituted acetylene **15** is oxidized to form the diketone **16**. Removal of the protecting group and amide formation affords **17**.

Analogs **2a–c** were tested in the BACE1 and BACE2 FRET assays to determine potency and selectivity. Cathepsin D, which plays an important role in cellular protein catabolism,[11] was used as an unrelated protease (39% similarity at active site with BACE1) to predict pan-aspartyl protease inhibition.



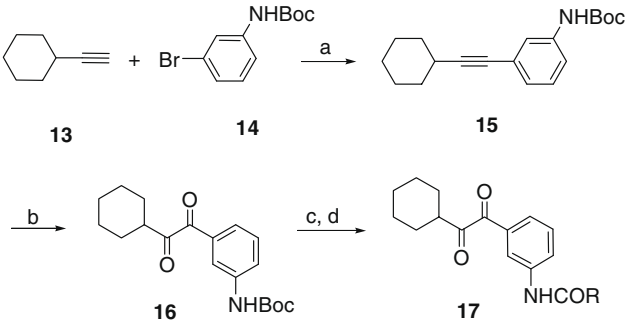
3 **5** **4** **6** **7**

Scheme 1. Reagents and conditions: (a) 1*H*-pyrazole-1-carboxamidine.HCl, DIPEA, DMF, 40 °C, 16 h; (b) Na_2CO_3, EtOH/H_2O (5:1), reflux, 3 h.



8 **9** **10** **11** **12**

Scheme 2. Reagents and conditions: (a) H_2 (50 psi), PtO_2, AcOH, 16 h (99%); (b) $(COCl)_2$, DMSO, −78 °C, 1 h then **9**, 1 h, then Et_3N, 1 h (85%); (c) NaOH, THF–MeOH–H_2O, 5 h (75%); (d) $(COCl)_2$, DMF (cat), DCM, 3 h (100%); (e) RMgBr, CuBr (1 equiv), LiBr (2 equiv), THF, −78 °C, 0.5 h, then **11**, 0.5 h.



13 **14** **15** **16** **17**

Scheme 3. Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI (cat), DIPEA, toluene, 60 °C, 16 h, 99%; (b) $KMnO_4$, acetone, water, 3 h, 65%; (c) DCM–TFA, rt, 1 h, 100%; (d) RCOOH, EDC, DMAP, DCM, rt, 16 h.

BACE2 is the closest related human aspartyl protease to BACE1 (79% identity in the active site).[12] Despite high similarity of BACE2 to BACE1, and the ability of BACE2 to cleave APP within the Aβ domain, its biological function, although not well understood, appears to be independent. BACE2 is likely not responsible for pathogenesis of Alzheimer's disease and it was even postulated that potentiation of this protease in the elderly may protect against AD pathogenesis.[13] Comparative analysis of crystal structures of these two proteases indicates residue differences around S_3, S_2 and S_2' pockets which may allow design of potent inhibitors.[14]

To our surprise, removal of tetrahydropyrimidine ring of **1** had a very pronounced effect, with 10-fold improvement of BACE1 enzyme and cellular activity. This coupled with the reduction in MW and *clogP*, results in aminohydantoin **2a**, with *clogP*, ligand efficiency (LE),[15] LELP[16] and LipE[17] all significantly improved, and in the range of an attractive lead.[16] The improvement in calculated properties also correlates with improved pharmaceutical properties, including permeability (Parallel Artificial Membrane Permeability Assay (PAMPA) and BBB-PAMPA),[18,19] aqueous solubility, brain and plasma exposure and brain/plasma (B/P) ratio (Table 1).

Saturation of one of the phenyl substituents (compound **2b**) gave further improvement of the BACE1 potency while the BACE2 activity was almost unaffected. Full saturation (compound **2c**) did not improve potency; however it had a detrimental effect on brain and plasma concentrations as well as the B/P ratio. We could not find a simple relationship between the B/P ratio and *clogP* or total polar surface area (TPSA). In fact the B/P ratio was counter intuitively highest when *clogP* was lowest. In all cases (**1–2c**) the Cathepsin D activity was well separated from BACE activity.

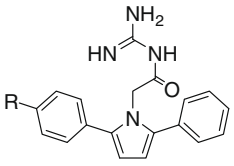
Compound **2b** with its low μM activity in enzyme and cellular assay, good calculated and pharmaceutical properties and brain exposure, was selected for further development. We envisaged that the potency of our series could be improved by introducing substit-

Table 1
BACE1, BACE2, Cathepsin D activities, permeability, brain and plasma concentrations data and calculated properties for compounds **1**–**2c**

Compound	1	2a	2b	2c
BACE1 IC_{50} (μM)	38	3.40	0.99	1.08
Cellular Aβ ELISA EC_{50} (μM)	16	4.17	0.81	1.03
BACE2 IC_{50} (μM)	38	0.77	0.51	0.51
Cathepsin D IC_{50} (or% Inhibition @ 100 μM)	(2)	(34)	24 (38)	(33)
PAMPA (Pe * 10^{-6} cm/s)	1.0	3.0	7.2	2.9
BBB-PAMPA (Pe * 10^{-6} cm/s)	8.6	11.1	12.8	7.7
Brain concentration C_{max} (ng/g)[a]	21	258	99	17
Plasma concentration C_{max}(ng/g)[a]	115	384	208	143
B/P ratio	0.2	0.7	0.5	0.1
Solubility @ pH 7.4 (μg/mL)	55	>100	>100	>100
MW	290	265	271	277
clogP	3.0	1.8	2.8	3.6
TPSA	53	56	56	56
LE[b]	0.22	0.38	0.41	0.41
LELP[c]	16.6	4.7	6.8	8.8
LipE[d]	1.4	3.7	3.2	2.4

[a] Dose 3 mg/kg oral.
[b] LE = −RT(Ln(IC_{50})/HA), HA—non-H atoms.
[c] LELP = clogP/LE.
[d] LipE = −log(IC_{50}) − *clogP*.

uents extending from the S_1 to the S_3 pocket. This strategy was already shown to be successful on the acylguanidine series of BACE1 inhibitors, exemplified by comparing **18** and **19**.[20]



18 R = H IC_{50} = 3.7 μM

19 R = m-Br-Ph-CONH IC_{50} = 0.6 μM

Overlaying compound **2b** with **18** in the BACE1 binding site (Fig. 2), indicated that the *meta*-position of the phenyl ring of **2b** is 'equivalent' to *para*-position of phenyl ring of aminoguanidine **18** and it should allow for introduction of substituents extending into the deeper S_3 pocket.

The possibility of reaching further into the S_1–S_3 pocket was explored with a set of amides **20** (Table 2). 2-Furoic and 3-methyl-2-furoic amides (**20a** and **b**) gave compounds with 10- and 25-fold improved potency over the original aminohydantoin **2a**.

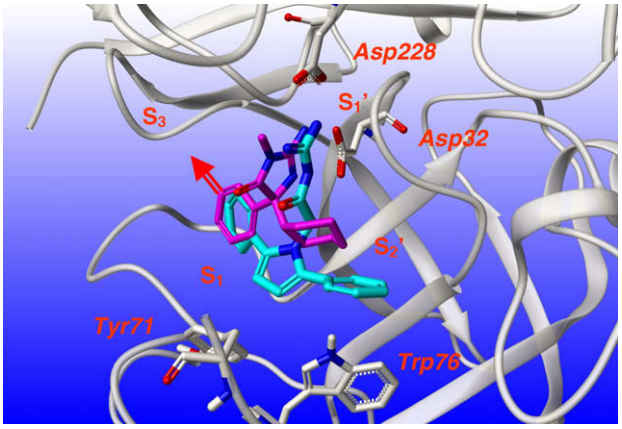
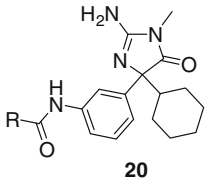
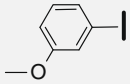
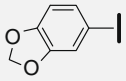


Figure 2. The overlay of compound **2b** (magenta) with **18** (cyan) (PDB ID code: 2QU2) in the BACE1 binding site. The structure alignment is based on protein sequences of both X-ray structures. For clarity, only BACE1 site in complex with compound **2b** is shown in white ribbon.

Table 2
BACE1 and BACE2 activity of compounds **20a**–**l**



20

Compound	R	BACE1 IC_{50} (μM)	BACE2 IC_{50} (μM)
20a		0.10	0.02
20b		0.04	0.03
20c		0.86	0.18
20d		2.85	0.49
20e		0.21	0.04
20f		7.86	1.85
20g		1.36	0.49
20h		0.38	1.15

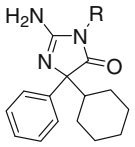
Introduction of the *N*-methyl-2-pyrrole group (**20c**), however was not beneficial. The small *n*-butyric amide (**20d**) led to loss of potency. Introduction of an oxygen atom into the alkyl chain of **20d** lead to fivefold potency improvement (**20e**), while extending the chain by one methylene (**20f**) resulted in significant loss of activity. With the exception of compound **20h**, in the amide series the BACE2 potency was equal or higher than BACE1. Subsequent modeling studies indicated that increased potency of compounds **20a**, **20b** and **20e** may be due to additional hydrogen bonding interactions between the oxygen atom in the furan or ethoxy group and Ser229 through conserved water observed in the X-ray structure of similar lead in this series.[21]

Compound **20b** is a 40 nM BACE1 enzyme inhibitor with an EC_{50} of 180 nM in the Aβ cellular ELISA assay and hence it was more fully characterized (Table 3). While the BACE1 enzymatic activity was improved 25-fold relative to lead **2b**, the improvement of cellular activity was only fivefold, perhaps reflecting the lower per-

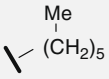
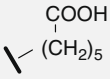
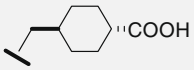
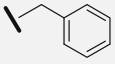
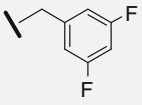
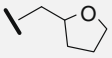
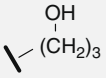
Table 3
BACE1, BACE2, Cathepsin D activities, permeability, brain and plasma concentrations data and calculated properties for compounds **20b**

Property	Value
BACE1 IC_{50} (μM)	0.04
Cellular Aβ ELISA EC_{50} (μM)	0.18
BACE2 IC_{50} (μM)	0.03
Cathepsin D (μM)	17.1
PAMPA (Pe * 10^{-6} cm/s)	1.4
BBB-PAMPA (Pe * 10^{-6} cm/s)	13.7
Solubility @ pH 7.4 (μg/mL)	41
MW	395
clogP	2.7
TPSA	100
LE	0.35
LELP	7.7
LipE	4.7

Table 4
BACE1 activity of compounds **21a–f**



21

Compound	R	BACE1 IC_{50} (μM)	BACE2 IC_{50} (μM)
21a		1.39	0.27
21b		0.95	0.31
21c		0.39	0.12
21d		1.24	0.57
21e		1.08	0.41
21f		1.04	0.17
21g		56% @ 5 μM	1.65

meability of **20b** versus **2b** (1.4 vs 7.2 Pe * 10^{-6} cm/s). The increase in potency resulted from additional functionality, so the MW and TPSA were increased, but the *clogP*, LE, LELP and LipE remained unchanged.

The *N*-methyl group in aminohydantoins of type **2** is oriented towards the hydrophilic S_1' pocket of BACE1 and suggests that extending polar substituents into this pocket may result in additional binding interactions, with for example Arg235. Improved potency by additional interactions in the S_1' region has been achieved in our laboratories on the acylguanidine series[22–24] as well by others.[25,26]

A set of compound **21a–f** bearing polar and hydrophobic groups was selected based on docking experiments. In general, the additional *N*-substituent had minimal impact on BACE1 potency, with the exception of **21c** in which small improvement of potency was realized. The hydroxypropyl group (**21g**), previously shown to be beneficial for the potency of acylguanidines,[22,24] gave surprisingly poor activity. Compounds of type **21** were, however uniformly more active in BACE2 than in BACE1 (Table 4).

In general this hit-to-lead optimization guided by crystallographic and modeling studies led to a 1000-fold potency improvement over the initial hit (compound **1** vs **20b**). The newly designed compounds were selective over Cathepsin D. Future studies will focus on continued optimization of the series, optimizing BACE2 selectivity, and improving the physical and pharmaceutical properties.

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