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Synthesis and SAR of indole-and 7-azaindole-1,3-dicarboxamide hydroxyethylamine inhibitors of BACE-1

Lawrence R. Marcin *, Mendi A. Higgins, F. Christopher Zusi, Yunhui Zhang, Michael F. Dee, Michael F. Parker, Jodi K. Muckelbauer, Daniel M. Camac, Paul E. Morin, Vidhyashankar Ramamurthy, Andrew J. Tebben, Kimberley A. Lentz, James E. Grace, Jovita A. Marcinkeviciene, Lisa M. Kopcho, Catherine R. Burton, Donna M. Barten, Jeremy H. Toyn, Jere E. Meredith, Charles F. Albright, Joanne J. Bronson, John E. Macor, Lorin A. Thompson

Bristol-Myers Squibb, Research and Development, 5 Research Parkway, Wallingford, CT 06492-7660, USA

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ABSTRACT

Heterocyclic replacement of the isophthalamide phenyl ring in hydroxyethylamine (HEA) BACE-1 inhibitors was explored. A variety of indole-1,3-dicarboxamide HEAs exhibited potent BACE-1 enzyme inhibition, but displayed poor cellular activity. Improvements in cellular activity and aspartic protease selectivity were observed for 7-azaindole-1,3-dicarboxamide HEAs. A methylprolinol-bearing derivative (**10n**) demonstrated robust reductions in rat plasma Aβ levels, but did not lower rat brain Aβ due to poor central exposure. The same analog exhibited a high efflux ratio in a bidirectional Caco-2 assay and was likely a substrate of the efflux transporter P-glycoprotein. X-ray crystal structures are reported for two indole HEAs in complex with BACE-1.

Alzheimer's disease (AD) is an age-related, neurodegenerative disease afflicting an estimated 5.5 million people in the United States and more than 35 million people worldwide.[1,2] The aberrant production and/or clearance of β-amyloid (Aβ) peptides (predominately Aβ1-42) in the brain has been implicated as the underlying cause of the disease.[3] Increased ratios of Aβ1-42/Aβ1-40 have been linked to the formation of neurotoxic Aβ oligomers, Aβ fibrils, and plaque-like deposits of aggregated Aβ.[4] β-Amyloid is produced through the catabolic metabolism of amyloid precursor protein (APP) by the sequential action of two enzymes, β-site APP cleaving enzyme (BACE-1) and γ-secretase. Inhibition of either BACE-1 or γ-secretase decreases the production of Aβ peptides and may provide a disease modifying therapy for the treatment of AD.[5] We have recently detailed our efforts in discovering BMS-708163,[6] a potent γ-secretase inhibitor, and are now disclosing studies toward the optimization of BACE-1 inhibitors.[7,8]

In 2000, crystal structures of BACE-1 in complex with potent peptidic inhibitors, such as OM99-2,[9] provided insight to the rational design of more drug-like ligands. Two years later, isophthalamide hydroxyethylamines (HEAs), such as **1a** and **1b**, emerged as transition state mimetics with reduced peptidic character (Fig. 1).[10] Since their initial discovery, numerous reports have detailed SAR about the isophthalamide aryl ring and disclosed analogs with potent in vitro activity.[11–13] Unfortunately, poor brain exposure, attributed to Pgp-mediated efflux at the blood–brain barrier (BBB), has precluded the central utility of most HEA BACE-1 inhibitors.[7,14–18] Our research program was interested in exploring heterocyclic replacement of the isophthalamide aryl ring, and what effect this change would have on BACE-1 binding, Aβ cellular potency, and brain penetration. Specifically, we had envisioned replacing the aryl ring with a substituted indole.[19] Our motive was twofold: (i) indoles are common to many brain-penetrant pharmaceuticals and (ii) substituted indoles would provide a unique opportunity to fill the enzyme's S2 subpocket and potentially refine BACE-1 potency.

Indole-based HEAs **6** were readily prepared as described in Scheme 1. Indole-3-carboxaldehydes **2** were N-substituted via

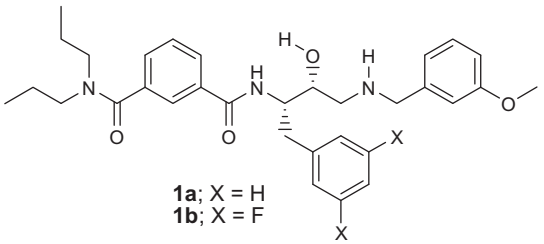

Figure 1. Known isophthalamide HEA BACE-1 inhibitors.

* Corresponding author. Tel.: +1 203 677 6701; fax: +1 203 677 7702.
E-mail address: lawrence.marcin@bms.com (L.R. Marcin).

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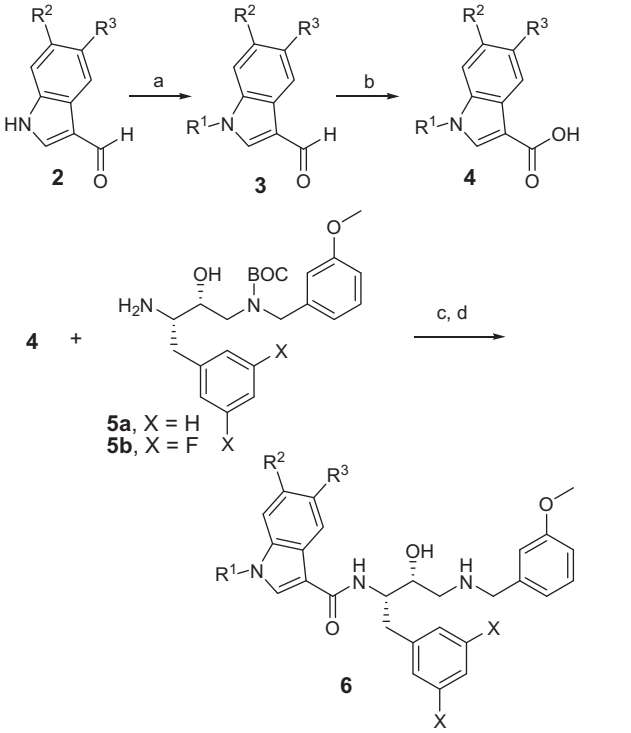


Scheme 1. Reagents and conditions: (a) TEA, CH_3CN, THF, 60 °C, 24 h with carbamoylimidazolium methiodide salts;[21] or NaH, THF, rt, 2–24 h, with carbamoyl chlorides and alkyl iodides; or DMAP, $ClCH_2CH_2Cl$, rt, 18 h with isocyanates; or DIEA, CH_3CN, 24 h, with alkylsulfonyl chlorides; (b) isobutylene, $NaClO_2$, $NaHPO_4$, THF, *t*-BuOH, rt, 24 h; (c) EDC, HOBt, DIEA, DMF, rt, 18 h; (d) TFA, CH_2Cl_2, rt, 2 h. For the definitions of R^1, R^2, R^3, and X refer to Table 1.

standard procedures to afford a variety of *N*-alkyl-, *N*-alkylsulfonyl-, *N*-alkyloxycarbonyl-, and *N*-carboxamidoindoles **3**. Sodium chlorite oxidation of the functionalized carboxaldehydes **3** provided the requisite carboxylic acids **4**. Amide coupling of the carboxylic acids **4** with known 1,3-diaminopropan-2-ols **5a**,**b**[8,20] provided, after BOC deprotection, the desired final products **6**.

Reference compounds **1a**,**b** and novel HEAs **6** were screened for BACE-1 enzyme inhibition[22] and their ability to inhibit secreted Aβ1-40 in cultured HEKsw cells (Table 1).[23] An initial survey of three indole-1,3-dicarboxamides **6a–c** with *N*,*N*-dialkyl substituents of differing length (Et, *n*-Pr, and *n*-Bu) revealed a trend towards greater enzyme inhibition with a longer alkyl chain (BACE-1 IC_{50} = 1500, 240, 94 nM, respectively). While the enzyme inhibition of **6b** and **6c** was similar to that of the analogous isophthalamide **1a**, the cellular activity of the indole carboxamides was significantly inferior. The indole carboxamide and isophthalamide scaffolds both exhibited a shift toward greater enzyme inhibition and cellular activity when the P1 phenyl ring had 3,5-difluoro substitution (Table 1, entries 2 and 6, respectively). However, the cellular potency of the corresponding indole **6d** remained poor (Aβ IC_{50} = 700 nM).

In search of improved cellular potency, we examined a variety of substituted indoles. The *N*-(*n*-butyl)indole **6e** was nearly devoid of BACE-1 inhibitory activity. The *N*-(*n*-butylsulfonyl) analog **6f** and the *n*-butylcarbamate **6g** exhibited modest enzyme potency. The monoalkyl carboxamide **6h** (Aβ IC_{50} = 540 nM) exhibited cellular activity that was comparable to the *N*,*N*-dibutyl analog **6d**. Improved results were observed for the *N*-methyl-*N*-butyl congener **6i** (BACE-1 IC_{50} = 7.4 nM, Aβ IC_{50} = 250 nM). Subsequent SAR ef-

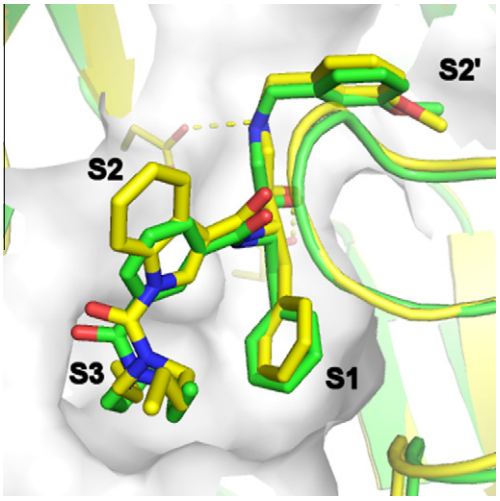


Figure 2. X-ray crystal structures of indole dicarboxamide **6c** (depicted in yellow) and reference isophthalate **1a** (depicted in green) in complex with BACE-1. The BACE-1 flap has been omitted for clarity.

Table 1
BACE-1 binding affinity and cellular Aβ potency for compounds **1** and **6**

Entry	Compd	R^1	R^2	R^3	X	BACE-1 IC_{50}[a] (nM)	HEK-Sw Aβ IC_{50}[a] (nM)
1	**1a**	—	—	—	H	190	120
2	**1b**	—	—	—	F	18	31
3	**6a**	$C(O)NEt_2$	H	H	H	1500	nd
4	**6b**	$C(O)N(n\text{-}Pr)_2$	H	H	H	240	1300
5	**6c**	$C(O)N(n\text{-}Bu)_2$	H	H	H	94	3200
6	**6d**	$C(O)N(n\text{-}Bu)_2$	H	H	F	45	700
9	**6e**	*n*-Bu	H	H	H	38,000	nd
10	**6f**	SO_2n-Bu	H	H	H	3100	nd
11	**6g**	C(O)O*n*-Bu	H	H	F	1100	nd
12	**6h**	C(O)N(H)*n*-Bu	H	H	F	190	540
13	**6i**	C(O)N(Me)*n*-Bu	H	H	F	7.4	250
14	**6j**	C(O)N(Me)*n*-Bu	F	H	F	66	420
15	**6k**	C(O)N(Me)*n*-Bu	CN	H	F	120	220
16	**6l**	C(O)N(Me)*n*-Bu	H	F	F	13	360
17	**6m**	C(O)N(Me)*n*-Bu	H	Cl	F	30	820
18	**6n**	C(O)N(Me)*n*-Bu	H	CN	F	18	310
19	**6o**	C(O)N(Me)*n*-Bu	H	CF_3	F	160	nd
18	**6p**	C(O)N(Me)*n*-Bu	H	CO_2Bn	F	860	nd
19	**6q**	C(O)N(Me)*n*-Bu	H	CO_2H	F	70	3000

[a] Values are means of ⩾2 experiments (nd = not determined).

forts focused on holding the *N*-methyl-*N*-butylcarboxamide moiety constant and examining the effect of substitution at the indole 5- and 6-positions. Small electron withdrawing groups at either position were well tolerated, but a modest preference toward greater enzyme inhibition was observed for 5-substituted analogs. The 5-fluoro, 5-chloro, and 5-cyanoindoles (**6l**, **6m**, and **6n**, respectively) demonstrated potent BACE-1 enzyme activity (IC_{50} = 13–30 nM), but provided no additional improvement in cellular potency (IC_{50} = 310–820 nM).

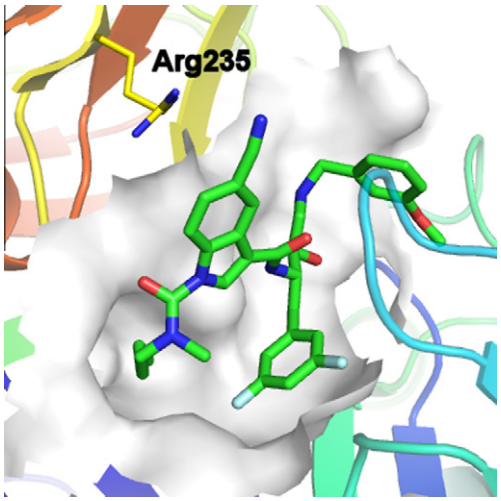


Figure 3. X-ray structure of 5-cyanoindole HEA **6n** in complex with BACE-1. The indole moiety occupies S2 subsite, while the 5-cyano group participates in a π–cation interaction with Arg235.

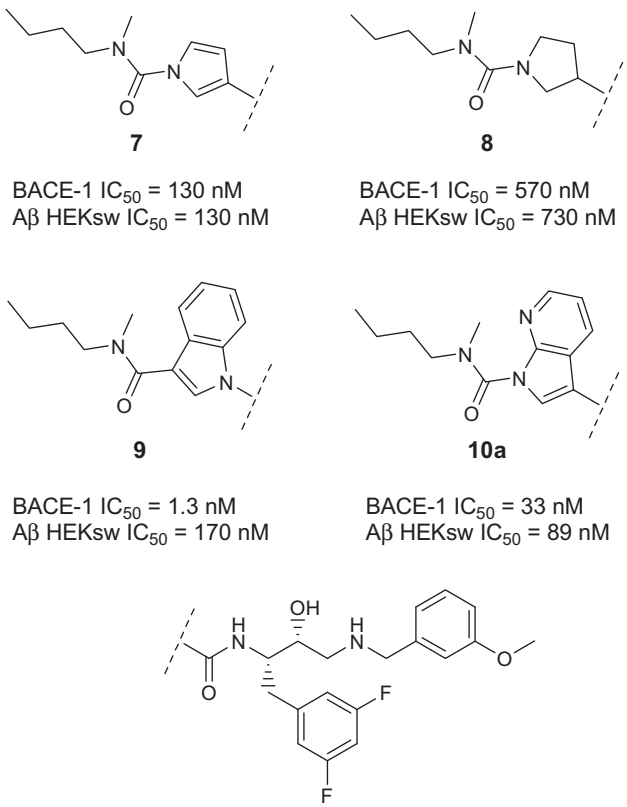
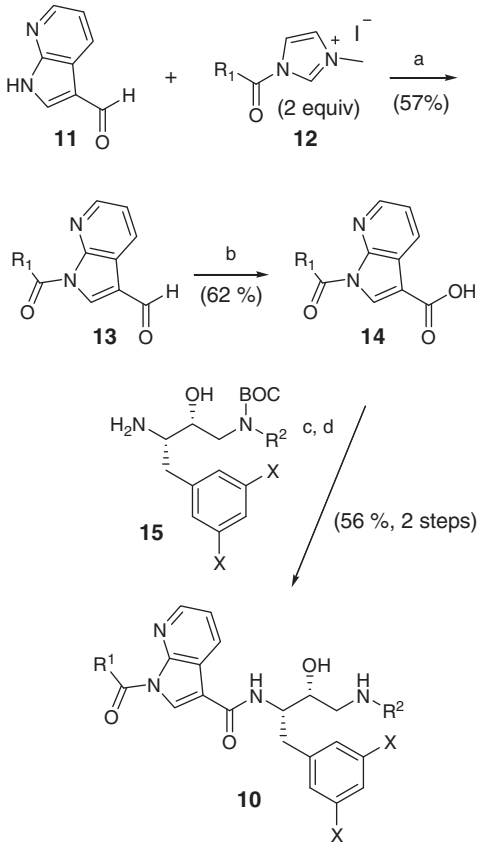


Figure 4. A survey of indole-1,3-dicarboxamide P2 replacements revealed a 7-azaindole-1,3-dicarboxamide **10a** with good cellular activity.

X-ray crystal structures were determined for two indole HEAs in complex with full length BACE-1.[24,25] The structure of indole **6c** and BACE-1 is depicted in Figure 2.[26] The graphic is overlayed with the published crystal structure of isophthalamide **1a**.[27] As anticipated, the indole system extends further into the S2 pocket than the isophthalamide phenyl. Projection of a single carboxamide alkyl chain into the hydrophobic S3 subpocket is similar with both ligands. For both **1a** and **6c**, the second alkyl chain forces the amide carbonyl out-of-plane with respect to the aromatic ring system and enables hydrogen-bonding of the amide carbonyl with Thr232. This same alkyl chain is packed against the ligand's phenylalanyl (Phe) group in the S1 pocket and ultimately creates a continuous hydrophobic surface that bridges the S1–S3 pockets. While this intricate S1–S3 binding assembly appears to be maintained with partially truncated *N*-butyl-*N*-methyl analogs (vide infra), it is likely compromised with the less potent alkyl sulfonamide **6f**, alkyl carbamate **6g**, and secondary carboxamide **6h**. Overlay of the HEA isostere in the catalytic site and the 3-methoxybenzyl group in the S2′ pocket was nearly identical for isophthalamide **1a** and indole **6c**.

An X-ray crystal structure of the 5-cyanoindole HEA **6n** and BACE-1 is illustrated in Figure 3.[28] The 3,5-difluorophenylalanyl group (diF Phe) and *N*-methyl-*N*-butyl carboxamide have combined to fill the hydrophobic S1–S3 continuous pocket in a highly efficient manner. Refined stacking interactions of the diF Phe with Tyr71 of the protein likely contribute to the increased BACE-1 inhibition observed with diF Phe analogs, such as **6i** and **6n**. The 5-cyano group of **6n** is primary solvent exposed, but may participate in a π–cation interaction with Arg235. The cyano group and Arg235 are separated by approximately 3.1 Å. Efforts



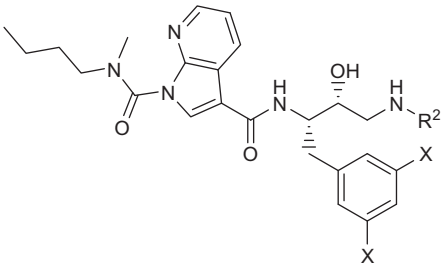
Scheme 2. Reagents and conditions: (a) TEA, CH_3CN, THF, 60 °C, 24 h;[21] (b) isobutylene, $NaClO_2$, $NaHPO_4$, THF, *t*-BuOH, rt, 24 h; (c) EDC, HOBt, DIEA, DMF, rt, 18 h; (d) TFA, CH_2Cl_2, rt, 2 h. Yields in parentheses are representative for example **10n**. For the definitions of R^1, R^2, and X refer to Tables 2 and 3.

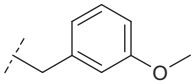
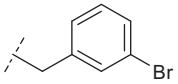
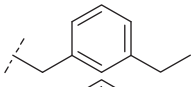
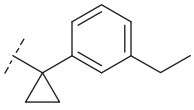
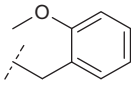
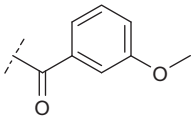
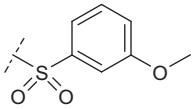
to further optimize the interaction of Arg235 with indole-5-carboxylates, such as **6p** and **6q**, were unsuccessful (Table 1).

Additional heterocyclic replacements of the isophthalamide phenyl group are depicted in Figure 4. The pyrrole **7** and pyrrolidine **8** analogs displayed modest BACE-1 inhibition (BACE-1 IC_{50} = 130–570 nM) with little or no shift in cellular potency. The reversed indole-1,3-dicarboxamide **9** demonstrated excellent enzyme potency (BACE-1 IC_{50} = 1.3 nM), but the functional activity in cells remained weak.[29] The 7-azaindole **10a** provided a significant breakthrough in Aβ cellular potency (Aβ IC_{50} = 89 nM) that warranted further investigation.

Compound **10a** and several related 7-azaindole analogs were prepared from **11**[30] by the general route outlined in Scheme 2 (BACE-1 and HEK-Sw Aβ data appear in Tables 2 and 3). Conservative changes in the P2′ amine that preserved the meta-substituted benzyl motif resulted in potent compounds (**10b–d**), with no clear advantage over the parent **10a**. *Ortho* substitution of the phenyl group was not tolerated as exemplified by **10e**. Amide, sulfonamide, and simple alkylamine analogs were all inactive (**10f**, **10g**, and **10h**, respectively).[31]

Table 2
BACE-1 binding affinity and cellular Aβ potency for 7-azaindole-1,3-dicarboxamides **10a–h**



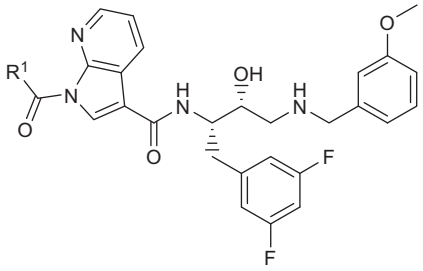
#	R^2	X	BACE-1 IC_{50}[a] (nM)	HEK-Sw Aβ IC_{50}[a] (nM)
10a		F	33	89
10b		H	23	77
10c		F	8.6	170
10d		F	81	360
10e		H	6500	nd
10f		H	>100,000	nd
10g		H	>100,000	nd
10h		H	>100,000	nd

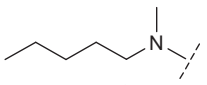
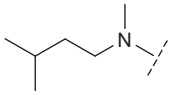
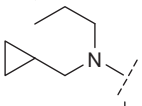
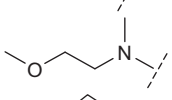
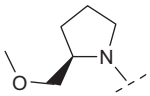
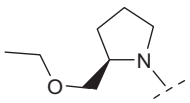
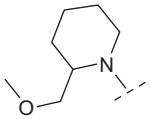
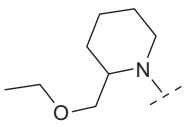
[a] Values are means of ⩾2 experiments.

Continued studies were focused on a more detailed exploration of P3 groups for the 7-azaindoledicarboxamide (Table 3). Other *N*-methyl-*N*-alkyl analogs demonstrated good enzyme activity when one alkyl chain was at least four carbons in length (**10i and 10j**). Good to excellent enzyme potency (BACE-1 IC_{50} = 10–73 nM) was observed for a series of alkyl ether-bearing analogs, **10m–q**. The methylprolinol analog **10n**[32] exhibited the best enzyme activity (BACE-1 IC_{50} = 10 nM) and most robust cellular Aβ activity (Aβ IC_{50} = 26 nM) of all the compounds that were prepared.

The off-target profile[33] and *c* log *P*[34] data for isophthalamide **1b**, indole **6i**, 7-azaindoles **10a** and **10n** are compiled in Table 4. Interestingly, an observation of better cellular potency with decreasing *c* log *P* was evident. This trend may reflect improved cell penetration with more optimal ligand polarity. While all of the BACE-1 inhibitors exhibited some degree of selectivity against the other common aspartic proteases, the methylprolinol azaindole **10n** demonstrated superior results. In our assays, it provided improved selectivity against BACE-2, cathepsin D, cathepsin E, and pepsin versus the reference isophthalamide **1b**.

Table 3
BACE-1 binding affinity and cellular Aβ potency for 7-azaindole-1,3-dicarboxamides **10i–q**



Compd	R^1	BACE-1 IC_{50}[a] (nM)	HEK-Sw Aβ IC_{50}[a] (nM)
10i		32	79
10j		28	94
10k		210	330
10m		73	110
10n		10	26
10o		12	110
10p		30	160
10q		26	170

[a] Values are mean of ⩾2 experiments.

Table 4
Comparison of BACE-1 enzyme inhibition, cellular Aβ activity, and off-target enzyme inhibition for compounds **1b**, **6i**, **10a**, and **10n**

Compound	BACE-1 IC_{50}[a] (nM)	BACE-2 IC_{50}[a] (nM)	HEK-Sw Aβ IC_{50}[a] (nM)	Cathepsin D IC_{50} (nM)	Cathepsin E IC_{50} (nM)	Pepsin IC_{50} (nM)	*c* log *P*
1b	17	230	31	2800	1500	380	4.8
6i	16	43	250	170	130	150	5.9
10a	33	49	89	1900	710	320	5.0
10n	10	800	26	11,000	4000	1000	4.0

[a] Values are means of ⩾2 experiments.

Azaindole HEA **10n** was examined for its ability to decrease Aβ production in wild type rats. Animals were dosed with a single intraperitoneal injection of **10n** at 30 mpk.[35] Plasma and brain samples were harvested and analyzed for drug exposure and Aβ40 levels. Compound **10n** demonstrated robust reductions of 51 ± 28% and 37 ± 16% in *plasma* Aβ at 1.5 and 5 h post injection, respectively. However, no reduction in *brain* Aβ was observed when the animals were euthanized at 5 h. The drug achieved robust peripheral exposure ($[drug]_{plasma}$ = 4200 nM @ t = 1.5 h; 2900 nM @ t = 5 h), but very poor brain exposure ($[drug]_{brain}$ = 57 nM @ t = 5 h). These findings were commensurate with bidirectional Caco-2 permeability assay results (P_{app} A–B <15 nm/s; P_{app} B–A = 184 nm/s), which predicted a B–A/A–B efflux ratio of >12.[36,37] Thus, brain exposure of **10m** was likely limited by P-glycoprotein efflux at the blood–brain barrier.

In conclusion, heterocyclic replacement of the isophthalamide aryl ring in HEA BACE-1 inhibitors was well tolerated. 7-Azaindole-1,3-dicarboxamides with an alkyl ether P3 side chain demonstrated the best combination of BACE-1 enzyme inhibition and Aβ cellular potency. Azaindole HEA **10n** did not achieve sufficient CNS exposure to effect reductions in brain Aβ after a single acute dose. Exposure data and in vitro permeability studies suggested that the brain exposure of **10n** was probably limited by transporter efflux at the BBB. Consequently, it does not appear likely that heterocyclic replacement of the phenyl ring in isophthalamide-derived HEA BACE-1 inhibitors is a viable strategy to improve CNS exposure and achieve significant brain Aβ reductions.[38]

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