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Rational design of novel, potent piperazinone and imidazolidinone BACE1 inhibitors

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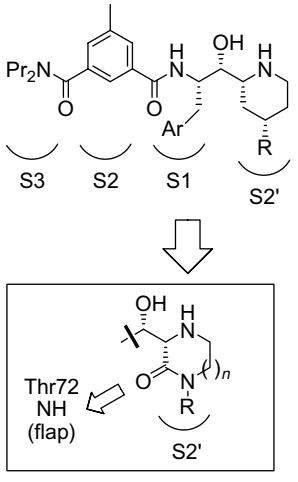
Abstract—Guided by structure-based design, we synthesized two novel series of potent inhibitors of BACE1 and generated extensive SAR around both the prime and non-prime side binding pockets. The key feature of both series is a cyclic amine motif specifically crafted to achieve interactions with both the flap and with the S2′ pocket.

Alzheimer's disease (AD)[1] is a progressive, ultimately fatal neurodegenerative disorder for which there is currently no disease modifying therapy. It affects more than five million people in the United States, fifteen million people worldwide, and has become the seventh-leading cause of death in this country.[1d] A key hallmark of AD is deposition of aggregated β-amyloid peptides ($A\beta_{40,42}$) as plaques in the brain.[2] These peptides are formed through processing of amyloid precursor protein (APP), initiated by β-secretase (BACE1).[3,4] Given that BACE1 knockout mice are viable,[5] and that modest reduction in BACE1 activity produces a sharp decline in AD-like pathology in transgenic mouse models,[6] this enzyme is an attractive therapeutic target to slow or halt the progression of this devastating disease.

As part of our BACE1 inhibitor program, we recently reported a series of novel, potent peptidomimetic piperidines **1**[7a] (Fig. 1) and pyrrolidines.[7] In the former series, substitutions from the 4-position of the piperidine ring improved inhibitor potency either by occupation of the S2′ subsite with large lipophilic groups, for example **1b**, or by formation of a weak hydrogen bond (3.2 Å) to the flap Thr72 NH from a small alkoxy group, for



Cpd	R	BACE1 IC_{50} (nM)	Cell IC_{50} (nM)
1a	H	130	240
1b	Bn	14	522
1c	OEt	7	106

2 (*n* = 1), piperazinones
3 (*n* = 0), imidazolidinones

Figure 1. BACE1 in vitro activity for initial lead piperidines **1** (Ar = 3,5-difluorophenyl)[7a] and design elements of novel piperazinone and imidazolidinone cores **2** and **3**.

Keywords: Alzheimer's disease; β-Secretase; BACE; Aspartyl protease inhibitors; Peptidomimetics.
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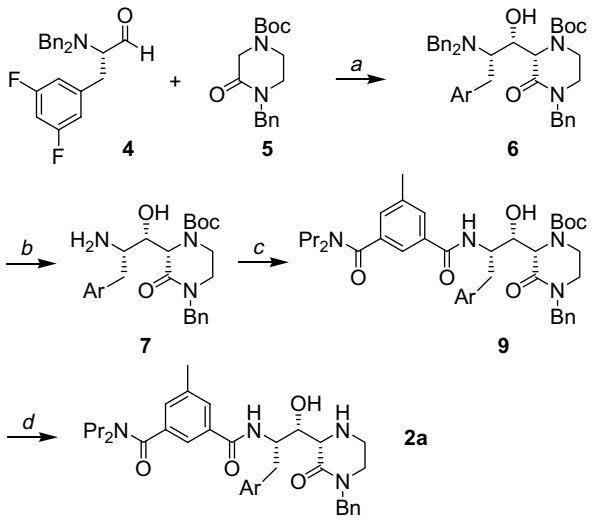


Scheme 1. Synthesis of BACE1 inhibitor **2a** as a representative example for generation of both piperazinone and imidazolidinone analogs **2** and **3** (Ar = 3,5-difluorophenyl): (a) LDA + **5** then **4**, −78 °C, 41%; (b) H_2, $Pd(OH)_2$, HOAc, EtOH, 91%; (c) **8**, EDCI, DIEA 81%; (d) TFA, 79%.

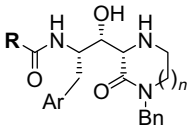
example **1c**. Combining these features to achieve synergistic improvements in potency proved challenging.

Examination of the X-ray crystal structure of piperidine **1b**[7a] in the BACE1 active site strongly suggested that a carbonyl introduced to the 3-position of the ring could achieve a hydrogen bond to Thr72 NH. From these observations, and with the aid of molecular modeling,[8] we designed both piperazinones **2** and imidazolidinones **3**[9] (Fig. 1) as cyclic amine scaffolds that could achieve *both* occupancy of S2′ *and* a hydrogen bond to the flap from the same ring system.

Stereoselective synthesis of these new inhibitors beginning from (*S*)-*N*,*N*-dibenzyl-3,5-difluorophenyl-alaninal **4**[10] is summarized in Scheme 1 for piperazinone analog **2a**. The stereochemistry of the hydroxyl group and cyclic amine ring was set via chirality transfer from the α-amino acid center in a non-chelation controlled Aldol reaction enforced by the *N*,*N*-dibenzyl protecting group.[11] Thus, addition of the conjugate base of *tert*-butyl 4-benzyl-piperazin-3-one-1-carboxylate **5** to aldehyde **4** gave Aldol product **6** with the desired absolute and relative stereochemistry as the major isolable product. Palladium-mediated debenzylation proceeded smoothly to give primary amine **7** that was then coupled to 5-methyl-*N*,*N*-dipropylisophthalic acid **8**[12] in the presence of EDCI/HOBt to give amide **9**. Finally, removal of the cyclic amine Boc group gave BACE1 inhibitor **2a**.

Imidazolidinone analogs **3** were made in similar overall yield following the above route, simply substituting *tert*-butyl 3-benzyl-imidazolidin-4-one-1-carboxylate **10**[13] for piperazinone core **5** in the Aldol step. The modifications of the S2–S3 motifs described in Tables 1 and 2

Table 1. SAR of piperazinone **2** and imidazolidinone **3** BACE1 inhibitors bearing variations of an isophthalamide group in S2/S3 (Ar = 3,5-difluorophenyl)[16]



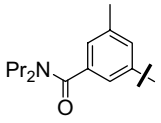
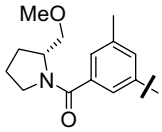
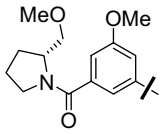
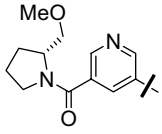
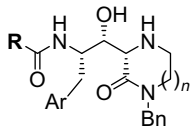
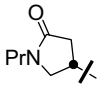
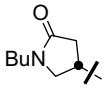
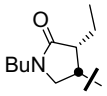
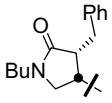
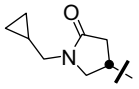
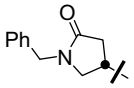
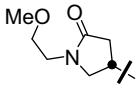
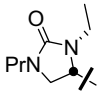
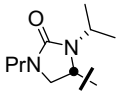
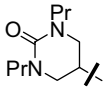
Compound	*n*	R	BACE1 IC_{50} (nM)	Cell IC_{50} (nM)	*C*log *P*[17]
2a	1		3	300	5.4
3a	0		5	1100	4.9
2b	1		1	75	4.2
3b	0		2	700	3.7
2c	1		4	57	3.7
2d	1		2	140	2.9

Table 2. SAR of S2/S3 non-isophthalamide variations of piperazinone **2** imidazolidinone **3** BACE1 inhibitors (Ar = 3,5-difluorophenyl)[16]



Compound	*n*	R	BACE1 IC_{50} (nM)	Cell IC_{50} (nM)
2e	1		9	510
3e	0		905	11,000
2f	1		5	180
3f	0		140	21,500
2g	1		3	240
3g	0		88	12,000
2h	1		16	960
2i	1		10	530
2j	1		22	1400
2k	1		13	1500
2l	1		9	620
2m	1		11	1100
2n	1		22	790

were made employing appropriate replacements[7b,14] of acid **8** in the penultimate coupling step for both cyclic amine series.

We first examined our novel piperazinone and imidazolidinone cores derivatized with isophthalamide-type non-prime side groups. As Table 1 shows, all of these analogs are potent BACE1 inhibitors. Comparison of the activity of piperazinone **2a** (BACE1 IC_{50} = 3 nM) with the original lead piperidine **1b** (BACE1 IC_{50} = 14 nM) shows that additional potency has been gleaned by introduction of the carbonyl group. Overlay of the X-

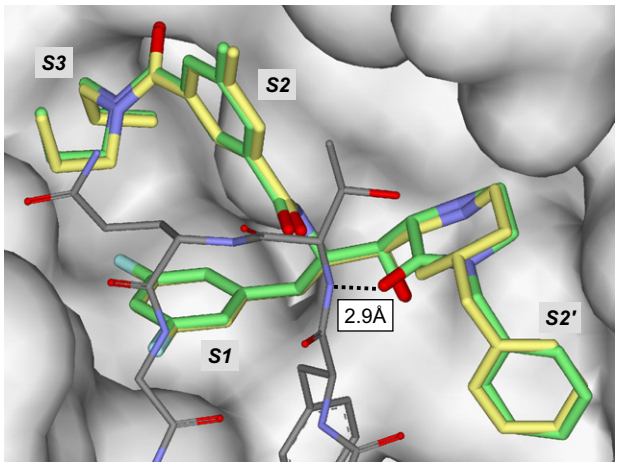


Figure 2. Overlay of BACE1 X-ray structures for piperidine **1b** (yellow) and piperazinone **2a** (green) and showing the 2.9Å hydrogen bond from the carbonyl of the piperazinone ring in **2a** to Thr72 NH of the flap (gray).

ray crystal structures of these two analogs (Fig. 2)[15] confirms that a hydrogen bond (2.9 Å) to the flap Thr72 NH was in fact achieved from the new carbonyl without perturbing the overall binding mode of the analog.

Another observation from Table 1 is that piperazinone **2a** and imidazolidinone **3a** have a larger decrease in cellular activity (200–300-fold) relative to piperidine **1b** (37-fold). This increased shift may be due to the reduced basicity of the piperazinone and imidazol-idinone cores (measured pK_a = 6.0, 3.6, respectively). Given that the BACE1 active site is displayed in an endosomal compartment that is slightly acidic (pH ~5), more basic compounds like piperidines **1**[18] may accumulate inside the compartment to a larger degree than less basic analogs. The cellular shift of the piperazinone analogs could be attenuated by lowering their *C*log *P* via changes to the isophthalamide moiety. In this way we achieved excellent potency both in vitro and in the cell with analogs **2b–d** (cellular IC_{50} = 57–140 nM).

We next turned our attention to pyrrolidinone and cyclic urea non-prime side derivatives (Table 2). Interestingly, the piperazinone core was very tolerant of a variety of these modifications, whereas the imidazolidinone core was not. Looking at the first two entries in Table 2, piperazinone analogs **2e** and **2f** both have BACE1 IC_{50} < 10 nM versus the imidazolidinone analogs **3e** and **3f** with BACE1 IC_{50} > 100 nM. In the third entry, introduction of additional substitution on the pyrrolidinone ring restored some potency to the imidazolidinone analog **3g**, but it was still nearly 30-fold less active than the corresponding piperazinone **2g**.

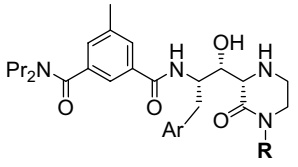
Examination of a wider array of pyrrolidinones as well as cyclic urea derivatives in the piperazinone series produced a set of analogs **2h–n** that generally showed excellent potency against BACE1 (IC_{50} = 9–22 nM). The rightward shift in cellular potency remained high (⩾40-fold) for these analogs. It is noteworthy that the tolerance of the piperazinone series for non-prime side variation was not seen in our piperidine series,[19] and may reflect intrinsically better binding imparted by the combination of S2′ occupation with additional hydrogen bonding to the flap.

In fact, the S2′ binding moiety remains a significant component of BACE1 potency in the piperazinone series, as evidenced by the SAR of modifications to the 4-position of the ring (Table 3).[20] Close analogs of parent **2a** that direct at least some lipophilicity into S2′ (**2s–2w**) showed good to excellent potency (BACE1 IC_{50} = 3–27 nM). Interestingly, a phenyl group directly attached to the lactam nitrogen **2x** cannot attain the necessary trajectory to occupy S2′ and thus has a diminished potency similar to small alkyl groups or no substitution at all (**2o–2q**, BACE1 IC_{50} = 175–345 nM).

Lead piperazinone **2a** and imidazolidinone **3a** were profiled in vivo in 6-week-old, pre-plaque CRND8 mice (Table 4).[2c,21] Both compounds showed modest reduction of $A\beta_{40}$ in the plasma with a single 100 mg/kg sc dose, but did not affect brain levels of the peptide. The PK profiles from these experiments show low brain penetration, which may be due to their being substrates for P-pg as reflected in the Caco-2 efflux ratios. These compounds were further profiled against other human aspartyl proteases (Cathepsins D and E, Pepsin) and generally showed low to modest selectivity (1–15-fold).

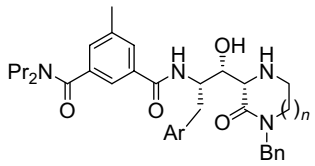
In conclusion, using X-ray crystallography in conjunction with molecular modeling, we have designed a series of novel, potent piperazinone- and imidazolidinone-based peptidomimetic inhibitors of BACE1. These novel cyclic amine aspartyl protease binding motifs combine potency enhancements from simultaneous occupation of S2′ and formation of an additional hydrogen bond to the flap. Piperazinones **2** in particular are tolerant of a wide diversity of modifications to their non-prime side substituents, and we have identified many analogs with BACE1 IC_{50} < 20 nM, a number of which have cellular potency below 200 nM. Two of these new compounds, piperazinone **2a** and imidazolidinone analog **3a**, produced modest inhibition of peripheral $A\beta_{40}$ in a

Table 3. SAR of modification of the substituents in S2′ from the 4-position of the piperazinone ring (Ar = 3,5-difluorophenyl)[16]



Compound	R	BACE1 IC_{50} (nM)	Cell IC_{50} (nM)
2o	H	264	7500
2p	Me	235	7000
2q	Et	175	
2r	Pr	44	4000
2s	CH_2*i*-Pr	13	2000
2t	CH_2*c*-Pr	10	915
2a	CH_2Ph	3	300
2u	CH_2(*o*-MeOPh)	5	550
2v	CH_2(*o*-CNPh)	20	970
2w	CH_2(*o*-ClPh)	27	755
2x	Ph	345	10,000

Table 4. Detailed in vivo, in vitro, and ancillary profiles of piperazinone **2a** and imidazolidinone **3a** (Ar = 3,5-difluorophenyl)



Assay	**2a** (n = 1)	**3a** (n = 0)
CRND8 mouse plasma $A\beta_{40}$[a]	−65 ± 8%	−43 ± 6%
Plasma concentration (ng/mL)	4020	4660
Brain concentration (ng/g)	200	120
B/P ratio	0.07	0.03
Caco-2 efflux ratio (A-B/B-A)	17	3
BACE1 IC_{50} (nM)	3	5
BACE2 IC_{50} (nM)	22	51
Cell IC_{50} (nM)	300	1100
Cathepsin D K_i (nM)	47	32
Cathepsin E K_i (nM)	30	2
Pepsin K_i (nM)	11	4

[a] Four animals per compound, 100 mg/kg sc dose, $A\beta_{40}$ measured at 3 h post-dose. Levels of $A\beta_{40}$ are expressed as mean ± standard error as a percent of vehicle. For additional general experimental details, see Ref. 21.

transgenic mouse model with a single dose. SAR around other, related cyclic amine scaffolds that similarly capture an additional hydrogen bond to the flap will be reported in due course.

Acknowledgments

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7. (a) Iserloh, U.; Wu, Y.; Cumming, J. N.; Pan, J.; Wang, L. Y.; Stamford, A. W.; Kennedy, M. E.; Kuvelkar, R.; Chen, X.; Parker, E. M.; Strickland, C.; Voigt, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 414; (b) Iserloh, U.; Pan, J.; Stamford, A. W.; Kennedy, M. E.; Zhang, Q.; Zhang, L.; Parker, E. M.; McHugh, N. A.; Favreau, L.; Strickland, C.; Voigt, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 418.
8. We employed both the de novo design method Allegrow (v. 020802, Boston De Novo, Boston, MA) as well as minimization using the Polak-Ribiere conjugated gradient algorithm until convergence of 0.001, CFF91 force field, Insight 2000/CDISCOVER (Accelrys Inc., San Diego, CA).
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12. The 5-methyl-*N*,*N*-dipropylisophthalamide group has been used extensively in the patent and journal literature: Maillaird, M.; Hom, R.; Gailunas, A.; Jagodzinska, B.; Fang, L. Y.; John, V.; Freskos, J. N.; Pulley, S. R.; Beck, J. P.; Tenbrink, R. E. PCT Int. Appl. WO 2002002512, 2002.

13. Made by treatment of 3-benzyl-imidazolidin-4-one (Pfeiffer, U.; Riccaboni, M. T.; Erba, R.; Pinza, M. *Liebigs Ann. Chem.* **1988**, 993) with Boc_2O/Et_3N, 83%.
14. (a) The (2*R*)-methoxymethyl)pyrrolidine-5-methyl isophthalamide moiety in analogs **2b** and **3b** (Table 1) was first reported by Elan Pharmaceuticals and Pfizer: John, V.; Maillard, M.; Jagodzinska, B.; Beck, J. P.; Gailunas, A.; Fang, L.; Sealy, J.; Tenbrink, R.; Freskos, J.; Mickelson, J.; Samala, L.; Hom, R. PCT Int. Appl. WO 2003040096, 2003.; (b) The requisite acids for the 5-methoxy isophthalamide analog **2c** and the pyridine dicarboxamide analog **2d** (Table 1) were made by treatment of the corresponding diacids with ((2*R*)-methoxymethyl)pyrroldine, EDCI, HOBT (10–45%); (c) The requisite acids for the cyclic urea derivatives **2l** and **2m** were made from (4*S*)-3-benzyloxycarbonyl-2-oxoimidazolidine-4-carboxylate (Hayashi, K.; Nunami, K.; Kato, J.; Yoneda, N.; Kubo, M.; Ochiai, T.; Ishida, R. *J. Med. Chem.* **1989**, *32*, 289): (i) NaH, alkyl halide; (ii) H_2, $Pd(OH)_2$; (iii) NaH, alkyl halide.
15. Coordinates for the X-ray structure of inhibitors **1b**, **1c**, **2a** and **3a** complexed with BACE1 with have been deposited in the Protein Data Bank (www.rcsb.org), and can be accessed under PDB ID 3CIB, 2QP8, 3CIC and 3CID.
16. Inhibition of BACE1 in vitro was determined using an APP-derived peptide containing the Swedish mutant: (a) Kennedy, M. E.; Wang, W.; Song, L.; Lee, J.; Zhang, L.; Wong, G.; Wang, L.; Parker, E. *Anal. Biochem.* **2003**, *319*, 49; Cellular IC_{50} values for inhibition of $A\beta_{40}$ production were determined by incubating HEK293 cells, stably transfected with the human APP cDNA containing both Swedish and London FAD mutations, with increasing concentrations of BACE inhibitors. $A\beta_{40}$ levels were measured in the cell culture media using an $A\beta_{1-40}$ specific ELISA assay: (b) Zhang, L.; Song, L.; Terracina, G.; Liu, Y.; Pramanik, B.; Parker, E. *Biochemistry* **2001**, *40*, 5049.
17. Calculated using CLOGP version 4.3 (BioByte Corporation, Claremont, CA).
18. Predicted pK_a values for simple 4-benzylpiperidine and 4-ethoxypiperidine are ~10–11 (SciFinder 2007).
19. For example, the corresponding analog of **2f** with the 4-benzyl-piperazin-3-one core replaced by 4-ethoxy-piperidine group, BACE1 IC_{50} = 250 nM (unpublished results).
20. To make the desired variations of the lactam nitrogen substituents, the requisite piperazinone cores were made from commercially-available piperazin-2-one: (i) Boc_2O; (ii) NaH, alkyl halide. The resulting 4-substituted piperazinones were then substituted for core **5** in Scheme 1.
21. CRND8-APP mice are models for early onset (familial) AD that express human APP containing both Swedish and London mutations that enhance the rate of APP cleavage by BACE1 and favor production of $A\beta_{42}$ over $A\beta_{40}$ in the γ-secretase cleavage step: Hyde, L. A.; Kazdoba, T. M.; Grilli, M.; Lozza, G.; Brussa, R.; Zhang, Q.; Wong, G. T.; McCool, M. F.; Zhang, L.; Parker, E. M.; Higgins, G. A. *Behav. Brain Res.* **2005**, *160*, 344.