Methyl-substitution of an iminohydantoin spiropiperidine β-secretase (BACE-1) inhibitor has a profound effect on its potency

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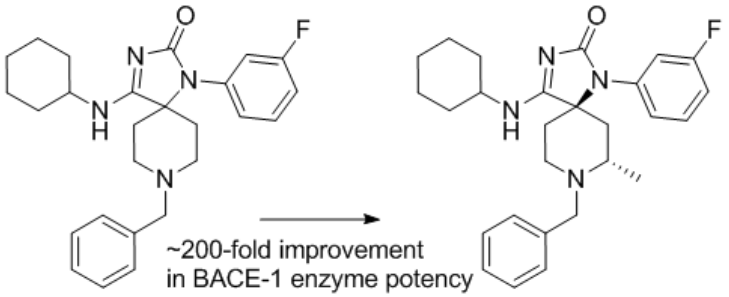

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Methyl-substitution of an iminohydantoin spiropiperidine β-secretase (BACE-1) inhibitor has a profound effect on its potency

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ABSTRACT

The IC_{50} of a beta-secretase (BACE-1) lead compound was improved ~200-fold from 11 μM to 55 nM through the addition of a single methyl group. Computational chemistry, small molecule NMR, and protein crystallography capabilities were used to compare the solution conformation of the ligand under varying pH conditions to its conformation when bound in the active site. Chemical modification then explored available binding pockets adjacent to the ligand. A strategically placed methyl group not only maintained the required pKa of the piperidine nitrogen and filled a small hydrophobic pocket, but more importantly, stabilized the conformation best suited for optimized binding to the receptor.

Beta-secretase (BACE-1) is a transmembrane aspartyl protease that has served as a promising therapeutic target for the treatment of Alzheimer's disease (AD) for many years. [2-5] BACE-1 is an attractive target as its inhibition results in a reduced production of Aβ40-42 thereby decreasing the amount of amyloid accumulation, which appears to be one of several critical steps in the process of neuronal degeneration. [3,6,7] Currently, there are several BACE-1 inhibitors in late-stage clinical trials that will shed further light on whether a reduction of amyloid burden will result in an overall improvement in cognitive function.[8] Since BACE-1 is primarily located in the brain, a small molecule inhibitor would ideally need to possess the appropriate physicochemical properties for CNS penetration.[9,10] This is a daunting task as the BACE-1 active site is large (>1000 Å^3) and quite hydrophilic.[11,12] Previous leads had tended toward high molecular weight (>500 Dalton), high polar surface area (>100 Å^3), and strong P-gp transport susceptibility.[13,14] Therefore the discovery of the BACE-selective spiropiperidine lead **1** (Table 1, BACE-1 IC_{50} 22 μM, Cathepsin D IC_{50} = >1.6 μM, MW= 416, PSA = 47.3) from a high-throughput screen of the Merck sample collection offered a unique opportunity to explore a compound with inherently superior baseline physical characteristics.

In-house optimization of **1**[15] led to compounds **2** and **3** (Table 1, BACE-1 IC_{50} = 11 and 2.8 μM, respectively) that also demonstrated good selectivity over Cathepsin D (Cathepsin D IC_{50} = 220 μM, >505 μM respectively). The first X-ray crystal structure of a spiropiperidine (compound **3**)[16] bound to the

BACE-1 enzyme revealed a hitherto undiscovered binding mode for a BACE-1 inhibitor. In the co-crystal structure, compound **3** was observed to interact with the catalytic aspartic acids via hydrogen bonding to intermediary water molecules, rather than by direct contact. This method of binding induced a conformation change in the flexible "flap" hairpin loop (enzyme residues Val69 to Trp76), significantly expanding the volume of the active site pocket.

The numerous (> 170) publicly available crystal structures of inhibitor/BACE-1 complexes demonstrate that the active site is inherently flexible, accommodating a wide variety of ligands.[17] Most published reports have relied on structure based drug design for optimization toward a static site pose. In contrast, this paper describes a more iterative approach, in which information from X-ray crystallography influenced investigations of ligand conformational preferences in the absence of the enzyme, which in turn guided new analog designs. Interestingly, and presumably as a result of the interplay between enzyme pocket environment and ligand binding conformation, the ligand adopts a chair conformation with an intramolecular steric interaction, in which the fluorophenyl ring is positioned in close proximity to the piperidine ring. MM2 modeling of this conformer showed it is of higher energy than other conformations available to the system (twist boats, other chairs).[18] Additional analogs were sought to explore the series further, with both potency and the capacity to serve as tools for X-ray, modeling and NMR as objectives.

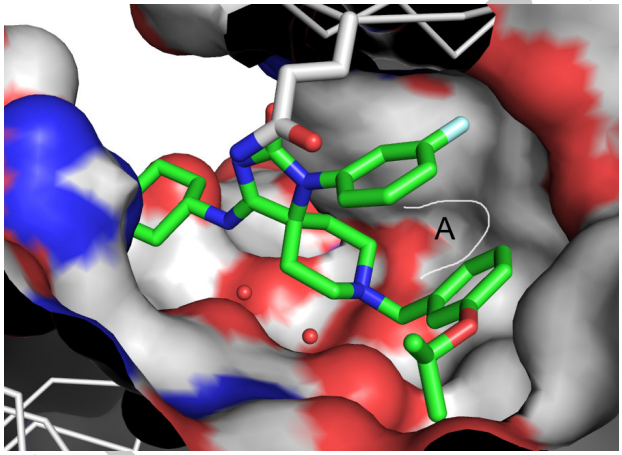
From the X-ray structure of **3** bound to the BACE-1 enzyme it was apparent that the S3 sub-site was not optimally occupied and may provide an area to obtain additional enzyme-binding interactions. This was accomplished through the addition of a meta-biphenyl tolyl group (compound **4,** Table 1), where potency was enhanced 30-fold compared to **3**. Unfortunately addition of the lipophilic aromatic group increased the molecular weight to 524, increased clogP to 5.15, and negatively affected solubility. While an improvement in ligand binding efficiency (LBE) was observed between **3** and **4** it is readily apparent that the potency gains were driven solely through lipophilic interactions as depicted by the drop in lipophilic ligand efficiency (LLE) value from **3** to **4**. This high lipophilicity was reflected in the large shift between the enzymatic and cell-based assay where only moderate cellular activity was observed (sAPPb IC_{50} =5.2 μM) despite the enzymatic activity of 110 nM.

Table 1. Spiropiperidine BACE inhibitors **1, 2, 3** and **4**.

Ideally, additional potency would be achieved with a decrease in molecular weight and lipophilicity (and be reflected in an improvement in LBE and LLE). A survey of small polar groups capable of occupying the S3 sub-site was undertaken (Table 1). Isopropoxy compound **5** possessed improved potency (enzyme and cell assays) and lower P-gp susceptibility as compared to **3**, and lower molecular weight, clogP, and improved permeability (as measured by Papp) and solubility compared to **4**. Although the intrinsic potency of **5** was less than **4**, the cell potency was maintained. The lipophilic ligand efficiency measurement (LLE), in particular, reflected the improvements from **4** to **5**. Brain/plasma PK experiments in mice showed a ratio of ~25% for compounds **4** and **5**, which was also encouraging.

To decrease the overall lipophilicity of the structure a basic amine was added to **5** to give racemic **6**. Compound **6** was more potent than **3** or **5** in both *in vitro* assays, and had significantly improved plasma free fraction, clogP and cell potency (first sub-micro molar compound) compared to **4**. Unfortunately P-gp susceptibility was increased, and is probably underestimated because of the poor apparent permeability (Papp). Other means were sought to improve potency while improving ligand efficiency and physical properties, with an inhibitor of low molecular weight as a key goal. It was proposed that a detailed characterization of the binding and native conformational preferences of the iminohydantoin-spiropiperidine structure would provide new ways to approach the problem.

Figure 1. X-ray co-crystal structure of BACE-1 and spiropiperidine analog **5.** The surface of the "flap" residue Gln73 (gray stick) and water molecules not associated with the catalytic aspartic acids have been removed for clarity.



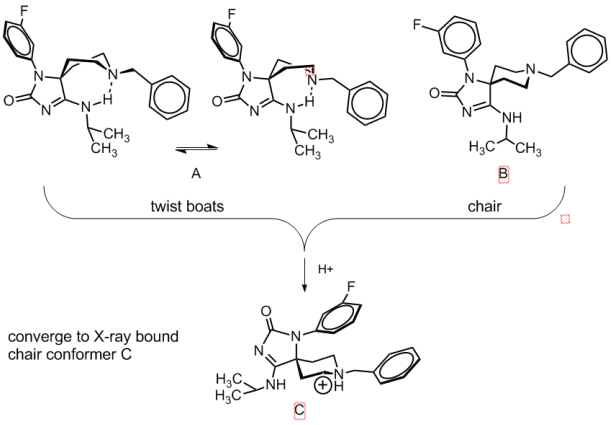
X-ray structures of **5** and **6** co-crystallized with BACE-1 were obtained. As had been observed previously for compound **3**[15], the X-ray co-crystal of compound **5** [16] revealed the piperidine ring of the spiropiperidine in a sterically-congested chair conformation (Figure 1).

Solution state NMR studies[19] were done to determine conformational preference(s) of the spiropiperidines in the absence of enzyme binding. Energetically efficient binding is expected when the solution conformation matches the bound conformation. The free base of **7** was first studied in CD_2Cl_2 solution. [20] Neutral **7** was found by NOE experiments to have a preferred conformation in which the *exo*-cyclic imino hydantoin hydrogen is proximal to the benzylic CH_2 and aromatic ring. These NOEs strongly suggest a hydrogen bond between the labile imino NH and the basic amine of the piperidine ring. Vicinal couplings provide additional clues as to the dominant conformer(s). Due to symmetry, there are four unique vicinal couplings between the piperidine ring protons. All four were found to be in the 6-7 Hz range. There is not a single conformation that satisfies all four coupling restraints. Considering that the major conformer(s) must also satisfy the NH NOE restraints, it is clear that the dominant conformation is actually an equilibrium of the two twist-boat forms of A (Figure 2). One cannot exclude the possibility that the conformer mix also includes small populations of structures B and/or the free base form of conformer C.

Compound **7** was also studied in solution under acidic conditions in order to understand the influence of protonation by the acidic catalytic site of BACE-1. In the presence of acid, **7** exclusively adopts the more sterically demanding chair form C, in which the fluorophenyl group is situated above the piperidine ring. Protonation of the amine prevents hydrogen bonding between the piperidine amine and the hydantoin amino NH. π-stacking of the fluorophenyl and benzyl aromatic rings may also play a role in stabilizing this conformation since a weak NOE

was observed between the protons at the para positions of the benzyl aromatic ring and the fluorophenyl ring.

Figure 2. NMR studies of analog **7,** characterization of the

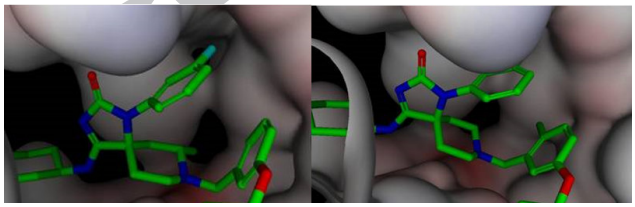


free base in organic solution, followed by treatment with acid.

The enzyme surface depicted in the X-ray structure of compound **5** (Figure 1) was also examined. A large hydrophobic space (deep pocket "A" at the center rear) is created in the S1 pocket by the presence of the inhibitor. Ideally, addition of a small substituent, such as a methyl group, to the spiropiperidine inhibitor could create a favorable lipophilic interaction at that site, and at the same time, also help bias the low energy conformation of the molecule to be that of the bioactive conformation. Optimally, such a substituent would also have the added advantage of maintaining the pKa of the piperidine nitrogen, and thus the key through-water binding interactions with the catalytic aspartic acids.

To estimate the possibility of success for this hypothesis, the attachment of the methyl group to the inhibitor at two sites, one at the 2-piperidine carbon, the other as a *ortho* benzyl substituent, were modeled using the X-ray of compound **6** as a starting point[16]. *A priori*, both positions appeared to possess substituent vectors that could allow access to the pocket, however, modeling clearly predicted that the equatorial 2-methyl piperidine would give the best access to the pocket and thereby desired potency (Figure 3).

Figure 3. Modeling of potential methylated analogs based on the crystallographically determined bound conformation of



compound **6**.

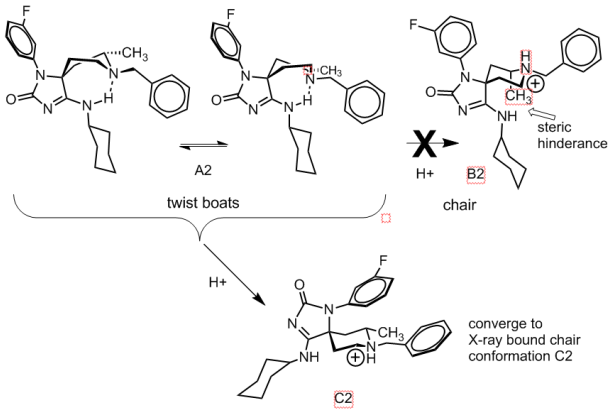
Additional advantages could be predicted from the 2-methyl piperidine substitution, as the energetically preferred conformational options available to the compound would be narrowed. A configuration with an equatorial methyl could be expected to stabilize the chair conformer desired for binding to the enzyme and destabilize the other possible chair conformers due to higher-energy methyl-proton gauche and axial-axial interactions (*vida infra*).

The target compounds were prepared using Ugi reaction conditions (similar to those already described[15 21] starting with the racemic 1-benzyl-2-methyl-4-ketopiperidine to give major and minor diastereomeric reaction products in a ratio of 10:1 and overall yield of 10%. The major isomer was found to be the *cis* analog **8** (where the methyl and aza-fluorophenyl of the iminohydantoin are in a *cis* orientation) and the minor *trans* **9** (Table 2).

The solution conformations of **8** and **9** were studied by NMR. The major *cis* diastereomer **8** was found to exist under neutral conditions as a mixture of conformers. The dominant conformer is a boat where the methyl group is pseudo-equatorial. Under acidic conditions, **8** transitioned to a complex mixture of protonated amine isomers (and presumably conformers thereof).

NMR analysis of the minor *trans*-product **9** indicated it exists as a mixture of twist-boat conformers (A2) under neutral conditions. The bioactive chair form (C2) was cleanly produced under acidic conditions (Figure 4). The alternative, more sterically demanding chair conformation (B2) was not observed. The methyl group achieves an energetically favorable equatorial position in conformation C2 without incurring other interactions that would be detrimental to binding. Minor isomer **9** was therefore predicted to be the more potent diastereomer.

Figure 4. Minor *trans* Ugi product 9 solution NMR conformation study.



The predicted activities of the two Ugi products was confirmed in our BACE-1 enzyme assay (Table 2). The *trans* diastereomer **9** predicted to have the desired configuration of the methyl group was ~ 100-fold more potent in versus BACE-1 (BACE-1 pH 6.5 IC_{50} = 69 nM) compared to the undesired *cis* diastereomer **8** (BACE-1 pH6.5 IC_{50} ~ 7.2 µM). Resolution of **9** into its enantiomers identified **10** as the active enantiomer (BACE-1 IC_{50} = 55 nM). Compound **10** is ~200-fold more potent versus BACE-1 than the des-methyl parent **2**. The significant improvement in potency as a result of the "magic methyl effect" is reflected in the calculated ligand efficiencies LBE, BEI and LLE (Table 2).[22]

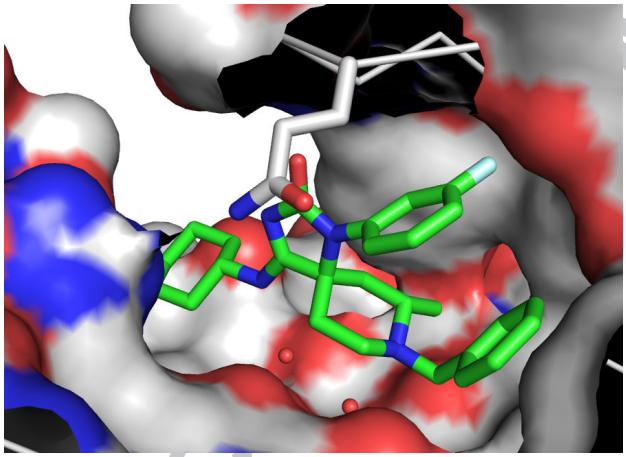
Table 2: Comparison of methylated and un-methylated spiropiperidine analogs.

X-ray crystallography of the active enantiomer **10** bound to BACE-1 (Figure 5) [16] further confirmed the enzyme structure, the position of the methyl group extending into the pocket, and the chair form, as predicted by computational chemistry.

The limitations of gas-phase modeling to capture the intricacies of enzyme/solvent/ligand interactions are well recognized, but can serve as a first approximation for study.

When such an analysis is done for the spiropiperidines an interesting correlation was observed. Computational comparison of the des-methyl spiropiperidine analogs to methylated analogs suggests a lowering of energetic costs required to achieve the bioactive chair conformation upon addition of the methyl group.[23] [24] The des-methyl spiropiperidine template accesses the bioactive conformation within 4 kcal/mole of the ground state, while the 7-methyl bioactive conformation is within 1.9 kcal/mol of the ground state, an improvement of ~ 2.1 kcal. This energy stabilization is approximately equal to the ΔΔG calculated for methyl versus des-methyl analogs based on their observed potency, suggesting that conformational stabilization plays a prominent role in improving potency. Improved potency is also attributed to the lipophilic interaction of the methyl group with the BACE-1 pocket. ITC experiments conducted on compound **2** revealed that binding of the piperidine to BACE-1 (Kd 4.2 μM) was associated with ΔH = -5.536 kcal/mol and ΔS of 6.3 kcal/mol[25], indicating exothermic binding with a favored entropic requirement, presumably due to the de-solvation of waters occupying the active site and surrounding the ligand. Addition of the methyl group could be expected to be beneficial in that regard. A more flexible and higher yielding synthetic route was needed to access analogs to further probe this interaction (*vida infra*).

Figure 5. X ray of active enantiomer **10** bound to BACE-1. The surface of the "flap" residue Gln73 (gray stick) and water molecules not associated with the catalytic aspartic acids have been removed for clarity.

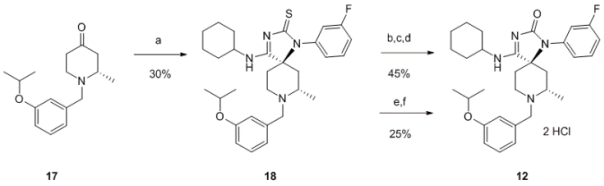


In pursuit of a high potency tool compound, two potency-enhancing groups (equatorial methyl and S3-isopropoxy) were combined in a single analog. Compound **12** (Table 2) was initially prepared in low yield (<0.3%) starting from racemic 1-benzyl-2-methyl-4-ketopiperidine, followed by resolution with chiral chromatography.[15] It was gratifying to see a further 9-fold and 5-fold improvement in *in vitro* and cell BACE-1 potency, respectively, (BACE-1 IC_{50} = 6 nM, sAPPb IC_{50} = 63 nM), compared to **10.** Compound **12** was not selective versus BACE-2 (IC_{50} = 2 nM) but showed good selectivity versus Cathepsin D (IC_{50} = 16.3 μM). Polar surface area and cLogP were maintained at a tolerable values and protein binding still provided a significant free fraction. Permeability was also maintained. The increase in MW offset potency increases so that no improvement in LBE or BEI ligand efficiency was observed compared to **10**, but a good improvement in LLE ligand efficiency was seen.

Unfortunately, the Ugi sequence was low yielding and optimization of the synthetic route was needed. Since potassium isocyanate (KOCN) is poorly soluble in methanol, simply switching to potassium isothiocyanate, (KSCN), which is soluble in MeOH, and using higher reaction temperature (70 ^{o}C) allowed greater conversion of the starting materials and reduced side products (Scheme 1). In addition the ratio of desired to undesired Ugi diastereomers was improved from 1:10 to 1:2. The yield of the desired product **18** was thus improved from 2% to 30%.[26]

The thio-Ugi product **18** could then be converted to **12** by first transforming the thiocarbonyl to the iminothiochloride with thionyl chloride and then to the iminoether with methanol, which was then hydrolyzed to racemic **12** with acetic acid and methanol (one pot, 3 steps ~45% yield). Resolution on reverse phase chiral column gave the single enantiomer **12** in 3% overall yield, a 10-fold improvement over the original route.[26]

Scheme 1. Thio-Ugi route to compound **12**



(a) **17** (racemic)[26], 3-fluoroanaline HCl, cyclohexyl isocyanide, potassium thiocyanate, methanol, 0-70^{o}C. (b) thionyl chloride (c) methanol (d) HOAc/methanol (e) chiral resolution (f) HCl/ether

Further improvement in route flexibility and access to analogs was found by preparation of the resolved N-CBZ protected piperidine ketone **20** and formation of the iminohydantoin **24** through a two-step process consisting of a Strecker reaction followed by cyclization (Scheme 2). The Strecker was performed with either $TiCl_4$/TMSCN/DCM or with $Zn(CN)_2$/HOAc to give a 1:2 or 3:2 ratio of desired (**22**) to undesired (**21**) diastereoisomers. The reaction yield could be further improved by converting the undesired diastereomer **21** to the desired isomer **22** by treatment with TMSCN/MeOH. Ring closure could then be carried out in a two step - one pot procedure consisting of treatment with Cl_3CONCO followed by MeOH/H_2O/TEA to give a mixture of *cis* **23** and *trans* **24**. The desired enantiomerically pure imino hydantoin isomer **24** could be isolated from the starting ketone **20** in 38% overall yield. Treatment of **24** with neat cyclohexylamine proceeded in 65% yield and the CBZ could then be removed by hydrogenation and the piperidine alkylated with different alkyl substituents as chosen. The overall yield of **12** was 3%, similar to the thio-Ugi route, but with a great improvement in the ability to rapidly explore the synthesis of analogs.[26]

Using the Strecker route, racemic analogs of compound **12** replacing the methyl group with larger substituents such as ethyl, propyl, cyclopropyl and allyl were prepared to probe the lipophilic interaction of the methyl group with the enzyme (Table 2). While ethyl substitution was somewhat tolerated (only ~ 5-fold loss in potency), propyl, cyclopropyl and allyl substitution was accompanied by ~50-100-fold losses. Substituents larger than methyl, which should be more likely to stabilize the bioactive chair form, were in practice not as good for potency. The methyl substituent appears to be of optimal steric bulk to occupy the lipophilic pocket without displacing the rest of the molecule from its favored enzyme-bound position. Compound

12 was therefore chosen as a potent lead with good properties with which to explore in vivo efficacy.

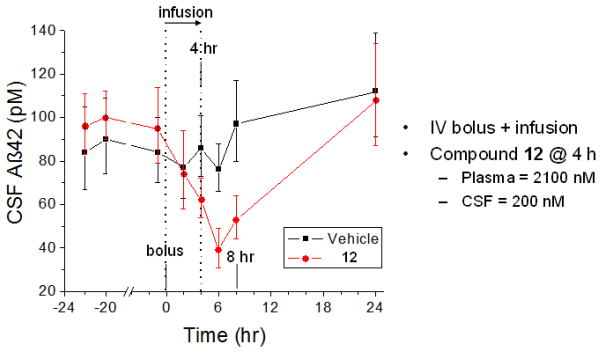
Scheme 2. Strecker route to compound **12**.

With sufficient quantities now synthetically available from the improved synthetic routes, **12** was characterized for its suitability to explore Aβ reduction *in vivo*. The compound was a moderate P-gp substrate in human, as was revealed when the borderline P-gp ratio measured at the routine screening compound concentration (5 μM) was repeated at a more stringent, lower compound concentration (1 μM) (human P-gp B:A/A:B ratio = 2.7 @ 5 μM, 4.4 @ 1 μM). The compound was a strong P-gp substrate in mouse (mouse B:A/A:B ratio = 25 @1 μM). A brain to plasma ratio of 0.25 and reduction of brain Aβ40 and Aβ42 by 30% was observed 3 hours after **12** was dosed IP (5% methylcellulose vehicle) in mice @ 30 mpk.[27] Although encouraging, the absence of mouse protein binding data prevented detailed interpretation of these results. As compounds with high P-gp efflux ratios are not expected to be highly brain penetrant, it is probable that the brain to plasma ratio may have overestimated the free brain exposure.[28] [29] A more detailed study in ported Rhesus was undertaken.

Compound **12** was studied for its ability to reduce CSF Aβ after IV bolus and 4 hour infusion in ported rhesus [30] (Figure 6). The CSF/plasma fraction unbound (fu) ratio of **12** at 4 hours after bolus was 0.79.[31] At 8 hours after bolus the compound reduced CSF Aβ40, Aβ42, and sAPPβ by 54, 62 and 44% compared to baseline.

Thus, **12** was shown to be a good tool to study the reduction of CSF Aβ in monkeys. PK studies showed that the piperidine nitrogen-benzyl methylene linkage was susceptible to cleavage by metabolic oxidation. Further work in this series focused on finding analogs with improved metabolic stability and lower P-gp susceptibility and a future publication outlining this work is planned. In addition, the series has served as inspiration to other research groups, who have explored analogously constrained structures as BACE-1 inhibitors.[32]

Figure 6. Reduction of Aβ by compound **12** after IV infusion in Rhesus



- Baseline adjusted Aβ reduction in CSF vs. vehicle at 8 h after bolus:
  - Aβ1-40: 54% (28% in plasma)
  - Aβ1-42 62%
  - sAPPβ 44% (49% at 24 h)

In summary, a novel mode of binding was discovered by X-ray crystallography of a spiropiperidine BACE-1 complex. Modeling revealed the computed high(er) energy of the spiropiperidine ligand-bound conformation. To better understand the relative energetic costs required for this binding mode, NMR experiments were used to study the solution-state conformations of the spiropiperidines and their dependence on pH. The NOE derived distances demonstrated that pH and configurational substitution play a significant role in determining conformational preferences for the spiropiperidine structure. Stabilization of the conformation best suited for optimized binding to the receptor via a conformational constraint, coupled with a highly-tailored occupation of the BACE-1 binding pocket, improved inhibitor potency by ~200 fold. This was efficiently accomplished by the addition of one appropriately placed methyl group and subsequently corroborated through X-ray crystallography.

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REFERENCES AND NOTES

Experimental conditions for the synthesis of compounds, NMR data and descriptions of molecular modeling calculations are available in Supplemental Materials.

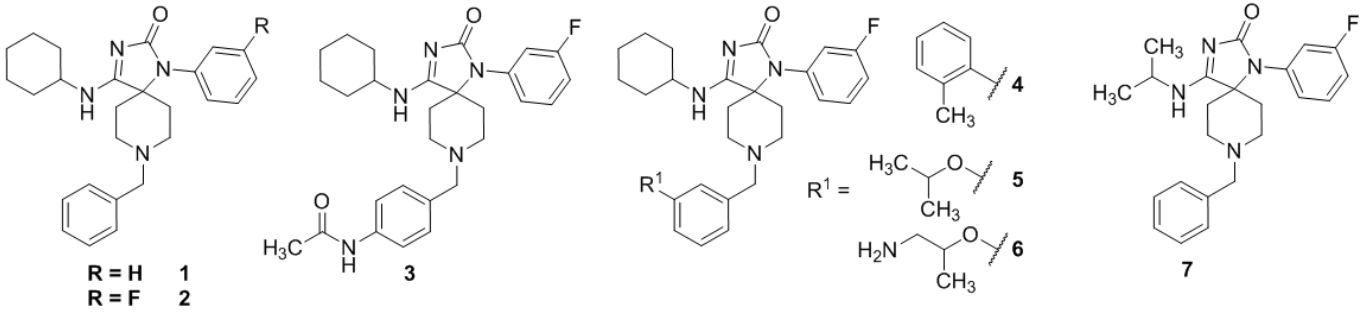
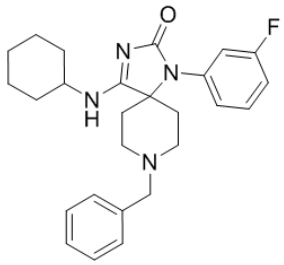


Table 1. Spiropiperidine BACE inhibitors **1, 2, 3** and **4**.

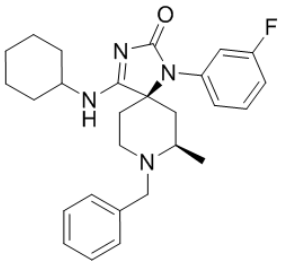
No.	BACE-1 IC_{50} (μM)[a]	sAPPβ (μM)[b]	PSA[c]	clogP[d]	PB(h)[e]	P-gp[f]	Papp[g]	LBE[h]	BEI[i]	LLE[j]	Brain/plasma[k]
1	22 ± 2.5[l]	>30	47.3	2.9				0.05	4.0	-1.2	
2	11 ± 0.98	32[m]	47.9	2.1	92%	3.4	25	0.06	4.5	-1.4	
3	2.8 ± 0.12	8.2 ± 1.7	77.8	2.3		19	<10	0.15	11.3	3.3	
4	0.11 ± 0.04	5.2 ± 2.4	49.3	5.2	insol	1.8	8	0.18	13.2	1.8	0.26
5	0.45 ± 0.05	5.0 ± 0.2	57.2	2.9	99%	1.0	28	0.18	12.9	3.4	0.23
6[m]	0.18 ± 0.08	0.71± 0.3	83.2	1.8	76%	4.2	1.2	0.18	13.3	4.9	
7	13[n]		47.9		57%	2.5	45				

[a] isolated enzyme IC_{50} at pH 6.5[33 34] [b]cell-based activity in presence of 10% fetal bovine serum.[34] [c]Polar surface area. [d]octanol/water partition coefficient, [e]human protein binding extrapolated from 10% serum to 100%. [f]B:A/A:B ratio in MDR1 transfected cells at 5 μM compound[35] [g]Permeability measured in porcine control cells, number times 10^{-6} cms, [h,i,j]Ligand binding efficiencies calculated from BACE-1 potency, see ref.[36] [k]brain to plasma ratio (after dosing @ 30 mpk IP in APP-YAC mice[37], 0.5% methylcellulose vehicle, 2h time point). [l](n=6), [m]racemic. [n](n=2). Blank = not done.

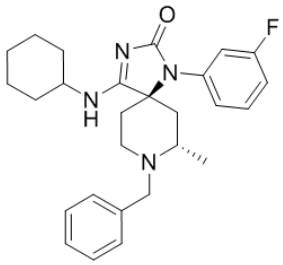
Table 2: Comparison of methylated and un-methylated spiropiperidine analogs



2

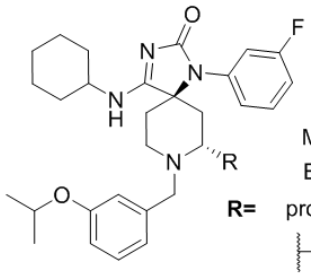


8



9 racemic

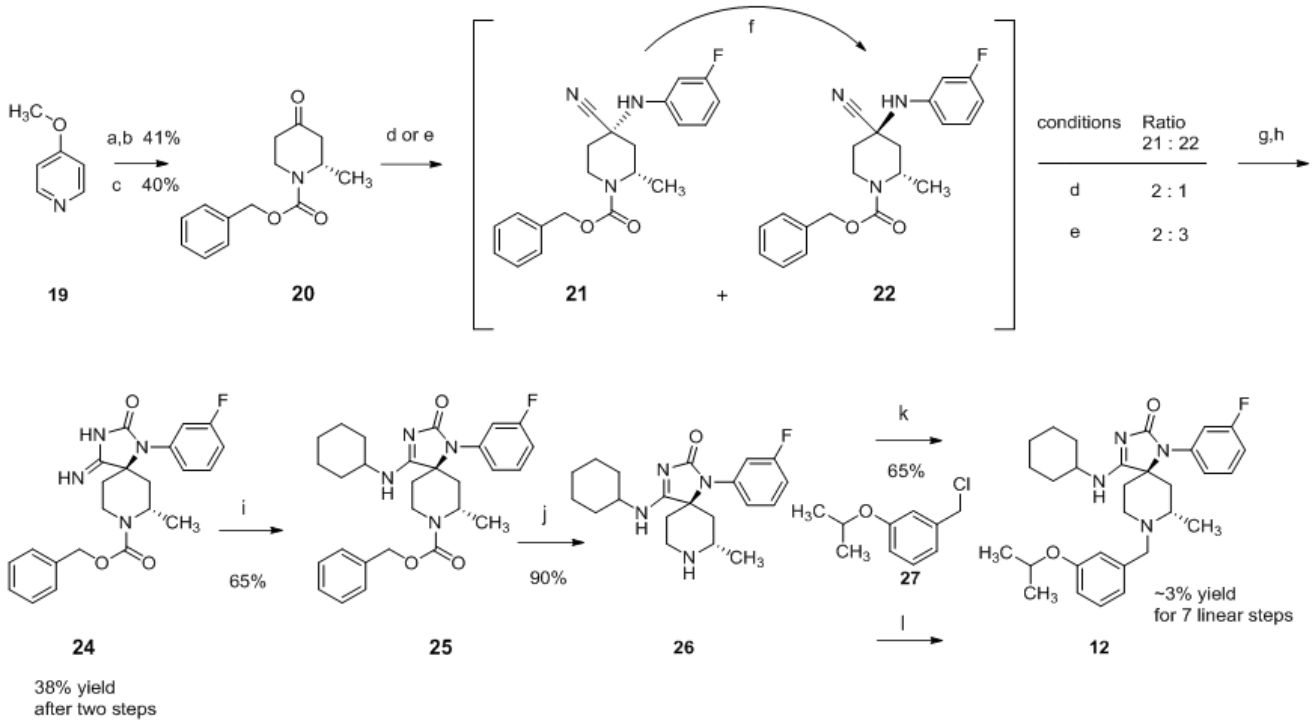
10 single enantiomer



R= Me **12**; Et **13**; propyl **14**;  **15**; allyl **16**

No.	BACE-1 IC_{50} (μM)[a]	sAPPβ (μM)[b]	PSA[c]	clogP[d]	PB(h)[e]	P-gp ratio[f]	Papp[g]	LBE[h]	BEI[i]	LLE[j]
2	11 ± 0.98	32[k]	47.9	2.1	92%	3.4[l]	25	0.06	4.5	-1.4
8	7.2[k]		47.9							
9[m]	0.069 ± 0.013	0.80 ± 0.16		3.4	94	3.9	24			
10[n]	0.055 ± 0.004	0.327 ± 171	47.9	3.2	87	3.4	20	0.22	16.2	4.0
11[o]	66.0[k]									
12[p]	0.006 ± 0.0005	0.063 ± 0.02	57.2	3.7	93	4.4[q]	24	0.22	16.2	4.5
13[m]	0.063	0.408								
14[m]	0.378	1.5								
15[m]	0.660	>10								
16[m]	0.300	5.6								

[a-e,g-j]See Table 1, [f]human P-gp B:A/A:B ratio at 1 μM compound, [l]P-gp ratio at 5 μM compound, [k]n=2, [m]racemic, [n]early eluting enantiomer of **8**, [o]late eluting enantiomer of **8**, [p]single enantiomer, [q]P-gp B:A/A:B ratio observed at 5 μM was 2.7, Blank= not done.



Scheme 2. Strecker route to compound **12**.

a) CBZCl, MeMgBr; b) Zn/HOAc; c) resolution chiral column; d) TiCl4/TMSCN/CH_2Cl_2 50 ^{0}C 30 min.; e) $Zn(CN)_2$/HOAc 50 ^{0}C, 1hr; f) TMSCN/MeOH; g) $Cl_3CC(O)N=C=O$; h) MeOH/H_2O/TEA; i) cyclohexylamine, neat; j) H_2/Pd/C/MeOH; k) **27**(3 steps, see supplemental data), DMF/K_2CO_3/70-80 ^{0}C (+/- NaI) 16h; l) chiral chromatography to remove 5% undesired enantiomer.

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[17] **www.rcsb.org** search "beta secretase".

[18] MM2 minimization in ChemBio3D of un-protonated compound **2** protein-bound conformation C (Figure 2) results in conversion to boat (total energy decrease from 319 to 35.22 kcal/mole). Upon simulated heating, conversion between boat forms A and chair form B is observed, chair form C is not observed.

[19] NMR methods: Samples were generally studied at a concentration of ~1mg/mL in the indicated organic solvent. Data were acquired using a Varian Inova 600 spectrometer operating with a sample temperature of 25°C. In the case of compound **7**, the sample temperature was also lowered to -20°C, but no perturbation attributable to a change in conformers was observed. Standard 1D and 2D pulse sequences were used. Samples were titrated with acid via the introduction of small amounts of TFA headspace vapor into the headspace of the NMR tube followed by mixing.

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