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Iminoheterocycles as γ-secretase modulators

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ABSTRACT

The synthesis of a novel series of iminoheterocycles and their structure–activity relationship (SAR) as modulators of γ-secretase activity will be detailed. Encouraging SAR generated from a monocyclic core led to a structurally unique bicyclic core. Selected compounds exhibit good potency as γ-secretase modulators, excellent rat pharmacokinetics, and lowering of $A\beta_{42}$ levels in various in vivo models.

A widely pursued strategy for the treatment of Alzheimer's disease is inhibiting the production of neurotoxic, insoluble amyloid-β (Aβ) peptides, especially $A\beta_{42}$. The final step in the generation of Aβ from the amyloid precursor protein (APP) involves cleavage by γ-secretase.[1] Furthermore, γ-secretase inhibitors (GSIs) have been shown to reduce the amyloid burden in animal models of Alzheimer's disease. Unfortunately, GSIs also inhibit cleavage of other γ-secretase substrates, including Notch. It is this inhibition of Notch processing which is thought to be responsible for adverse events in clinical trials, particularly gastrointestinal and immunological toxicities.[2]

In contrast to GSIs, compounds known as γ-secretase modulators (GSMs) inhibit $A\beta_{42}$ production without interfering in the processing of Notch and other substrates. While the exact reason for the selectivity of these agents remains unclear, they are thought to intervene at an allosteric site on γ-secretase and shift the APP cleavage site so that shorter, soluble, non-toxic peptides (e.g., $A\beta_{38}$) are produced instead of the highly insoluble and neurotoxic $A\beta_{42}$.[3]

Early accounts of cyclooxygenase (COX) inhibitors showed promise in the prevention of Alzheimer's patients. It was later noted that derivatives of COX inhibitors functioned as modulators of γ-secretase independent of their COX activity.[4] Several groups have since explored this strategy towards the development of novel GSMs.[3] However, Flurizan is a weak GSM that failed in clinical trials testing its ability to treat Alzheimer's disease.[5] Therefore, we directed our efforts to develop GSMs which were not derived from the COX inhibitors.

We focused our study on an imidazyl-phenyl series typified by compounds disclosed by Neurogenetics and Eisai (Fig. 1). We sought to replace the aminothiazolyl (Neurogenetics[6]) and lactam (Eisai[7]) portions with iminoheterocycles which have been shown to be brain penetrant structural motifs.[8] Thereby, we could alter the overall molecular properties while maintaining what were believed to be key pharmacophores for γ-secretase modulation. Moreover, the compound from Eisai is a potent GSM ($A\beta_{42}$ IC_{50} = 64 nM) with a good rat PK profile (AUC_{0-6h} = 60.4 μM h, brain:plasma concentration ratio of 0.4 after oral administration). When dosed orally at 30 mg/kg in a transgenic (CRND8) mouse model[9], this compound lowered $A\beta_{42}$ levels in the cortex and CSF.[10–12]

Modeling studies suggested integration of monocyclic iminoheterocycles maintained good overlap with the pharmacophores of the Neurogenetics and Eisai compounds (Fig. 1) and therefore were reasonable targets as potential GSMs.

Starting from (*S*)-1-(4-fluorophenyl)-ethanamine, iminopyrimidinone derivatives were prepared as shown in Scheme 1. Compound **1** displayed moderate potency and functioned as a modulator of γ-secretase.[13] All attempts to form the exocyclic double bond provided the thermodynamic endocyclic regioisomer.[14] The geminal di-methyl analog, **3**, was prepared to avoid double bond migration; however; predominantly the *Z*-exocyclic double bond formed and resulted in a significant loss of potency ($A\beta_{42}$ IC_{50} >20 μM).

Concurrently, an iminohydantoin scaffold was developed originating from (*S*)-1-(4-fluorophenyl)-ethanamine as shown in Scheme 2. The chemistry was further improved at the oxidative amination stage through a two-step $POCl_3$ conversion, followed by amine displacement. Initial efforts were promising as shown in Table 1. Several compounds with sub-micromolar potency were identified exhibiting a γ-secretase modulator profile. Furthermore, the

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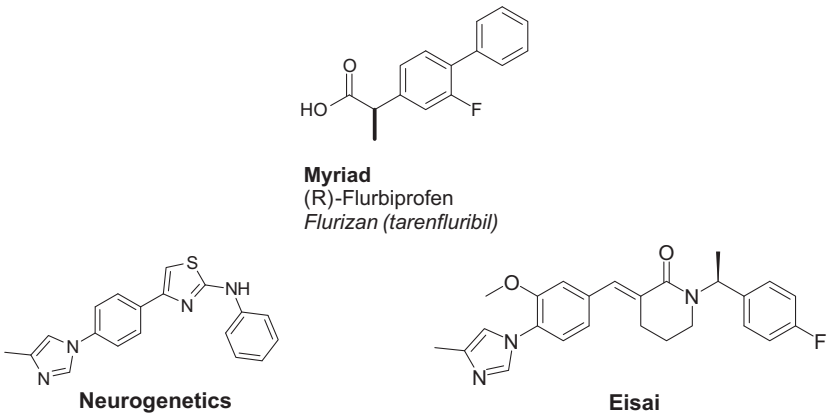
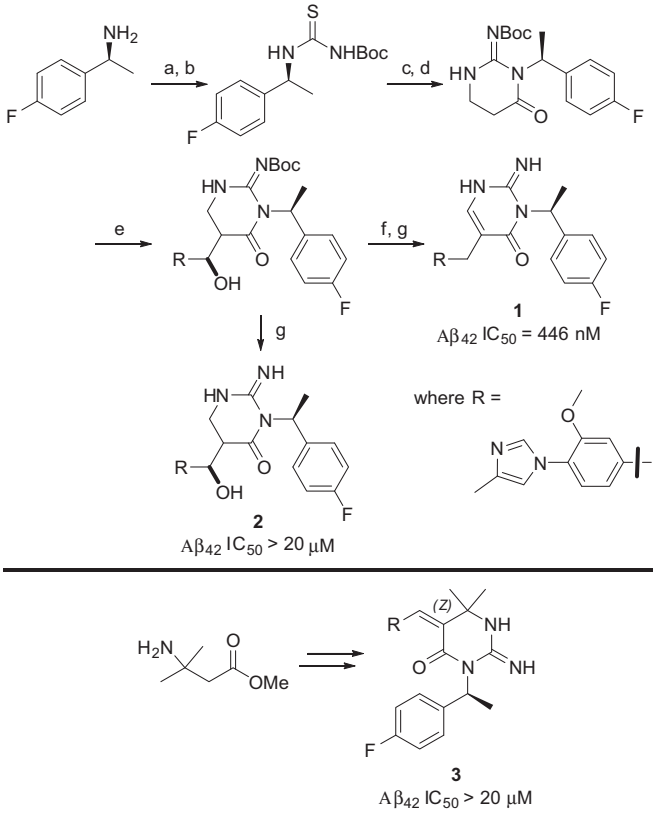


Figure 1. Known γ-secretase modulators.

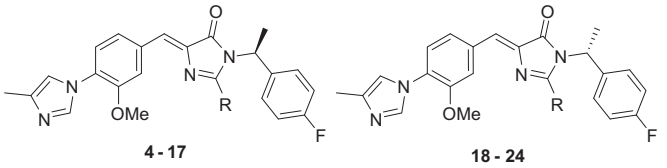


Scheme 1. Reagents and conditions: (a) Cl_2CS, K_2CO_3, DCM/H_2O, rt, 90%; (b) NaH, $BocNH_2$ THF, 0 °C to rt, 76%; (c) EDCI, DIEA, $NH_2(CH_2)_2CO_2CH_3$, 70 °C; (d) NaH, THF, 3% over 2 steps; (e) RCHO, LHMDS, 94%; (f) MsCl, DBU, NaH, CH_2Cl_2, rt, 50%; (g) TFA, CH_2Cl_2, rt, 50% for **1**, 100% for **2**.

R-enantiomer of the α-methyl benzyl was explored. Interestingly, the *R*-enantiomer displayed higher potency when compared to its corresponding *S*-enantiomer. Particularly noteworthy were compounds **19**, **20**, **22**, and **23** which displayed double-digit nanomolar potency while still retaining the γ-secretase modulator profile.

Next, the effect of substitution of the benzylic position was explored as shown in Table 2. Compounds **25**–**31** were prepared using similar intermediates detailed in Scheme 4. In the parent iminohydantoin ($R^1 = NH_2$), the potency appears better with methyl analog, **19**, compared to **25**, **28**, and **30**. Yet, in the ethyl substituted iminohydantoin ($R^1 = NHEt$), the unsubstituted benzylic variant, **26**, shows slightly increased potency compared to the substituted analogs, **20**, **29**, and **31**.

Table 1
Cellular IC_{50} of iminohydantoin analogs[11]

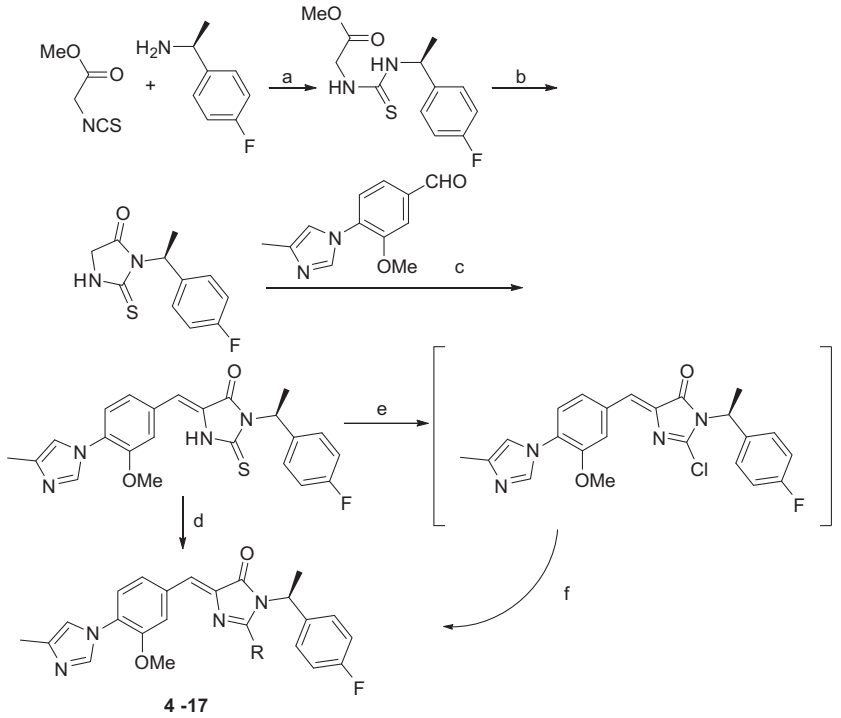


Compound	R	$Aβ_{42}$ IC_{50} (nM)	$Aβ_{total}/Aβ_{42}$
4	OH	163	141
5	OCH_3	864	23
6	NH_2	322	55
7	$NHCH_3$	411	25
8	NHEt	220	97
9	NHcPr	287	136
10	$NHCH_2cPr$	250	71
11	$NH(CH_2)_3CH_3$	258	78
12	Pyrrolidine	671	32
13	Piperidine	955	21
14	$NH(CH_2)_2OH$	218	79
15	$NH(CH_2)_2OCH_3$	301	66
16	$NH(CH_2)_3OH$	163	141
17	$NH(CH_2)_3OCH_3$	287	70
18	OH	3502	nd
19	NH_2	94	65
20	NHEt	88	159
21	$NHCH_2cPr$	158	68
22	$NH(CH_2)_3CH_3$	92	42
23	$NH(CH_2)_2OH$	63	27
24	$NH(CH_2)_3OH$	111	153

An initial venture into preparation of a bicyclic system led to **32** (Scheme 3) with a poor cellular IC_{50}, where $Aβ_{42}$ IC_{50} = 7.1 μM.

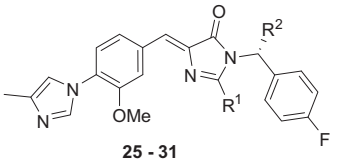
Discouraged by this initial result, we focused our efforts upon a different cyclization strategy that introduced conformational restraint between the benzylic moiety and the iminoheterocyclic core. In this manner, 5-5, 5-6 and 5-7 bicyclic frameworks were investigated as shown in Scheme 4. The key synthetic transformation relied on a latent amine being revealed either by deprotection in the case of the 5-5 system or functional group manipulation in the case of the 5-6 and 5-7 systems. Thus, when subjected to conditions described in the preparation of the monocyclic series, the amine readily displaced the activated thiohydantoin precursor.

The compounds were tested as a racemic mixture unless otherwise specified (Table 3). In general, the ethyl substituent showed better potency than the unsubstituted bicycles in direct comparisons (**35** to **36**, and **37** to **38**). Additionally, the potency appears to drop off slightly with an increase in ring size (**33** vs **37**).



Scheme 2. Reagents and conditions: (a) THF, rt, 100%; (b) NaH, THF, 100%; (c) piperidine, EtOH reflux, then PS-TsNHNH$_2$, rt 95%; (d) Base, *t*-BuO$_2$H, MeOH, rt, 10–20%; (e) POCl$_3$, microwave 170 °C; (f) amine, Et$_3$N, THF, microwave 140 °C, 10–62% over 2 steps (e and f).

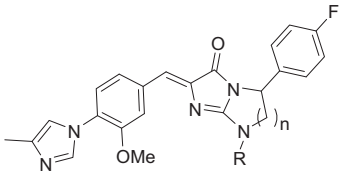
Table 2
Cellular IC_{50} of iminohydantoin analogs[11]



25 - 31

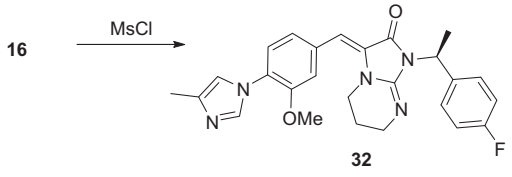
Compound	R^1	R^2	$A\beta_{42}$ IC_{50} (nM)	$A\beta_{total}/A\beta_{42}$
25	NH_2	H	144	139
26	NHEt	H	72	104
27	NHBu	H	85	70
28	NH_2	CH_2OH	139	113
29	NHEt	CH_2OH	91	200
30	NH_2	$(CH_2)_2OH$	229	87
31	NHEt	$(CH_2)_2OH$	171	117

Table 3
Cellular IC_{50} of bicyclic analogs[11]



33 - 39

Compound	*n*	R		$A\beta_{42}$ IC_{50} (nM)	$A\beta_{total}/A\beta_{42}$
33	1	H	*rac*	135	117
34	1	Et	(-)	56	159
35	2	H	(+)	106	188
36	2	Et	(+)	85	71
37	3	H	*rac*	503	40
38	3	Et	*rac*	117	169
39	3	Et	(-)	73	215

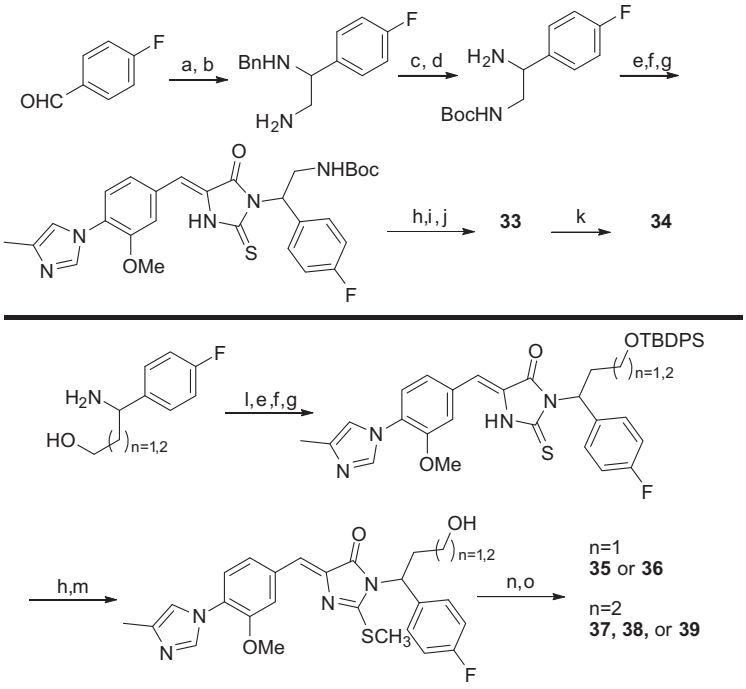


32

Scheme 3. Preparation of a bicyclic analog via N-1 to N-2.

Compounds **20**, **27**, and **39** from both the monocyclic and bicyclic series were selected for further profiling. The selected compounds displayed favorable pharmacokinetic profiles,[15] with high exposure in the plasma and acceptable levels distributed in the brain at 6 h as shown in Table 4. In an in vivo transgenic (CRND8) mouse study[11] dosed at 30 mg/kg orally, compound **20** lowered plasma $A\beta_{42}$ levels 80% while showing minimal (−10%) effect on plasma $A\beta_{total}$ levels. It also had a minimal effect (−12%) on cortical $A\beta_{42}$ levels. Despite significant exposure in both plasma and brain, compound **27** had minimal effect on $A\beta_{42}$ in the CNS in rats, lowering CSF $A\beta_{42}$ levels 10% and 38% at 30 and 100 mg/kg orally, respectively. Echoing these results, compound **39** achieved a very low 8% reduction of CSF $A\beta_{42}$ despite high brain concentrations. The $A\beta_{42}$ samples were taken 3 h after dosing in all of the studies.

Correlation of the PK and efficacy experiments was confirmed by determination of compound exposure in the plasma and the brain in the actual animals used in the efficacy experiments. For compound **27** dosed at 30 mg/kg, plasma and brain levels at 3 h were 7.1 μM and 5.9 μM, respectively. Furthermore, compound **27** was shown not to be a Pgp substrate.



Scheme 4. Reagents and conditions: (a) $BnNH_2$–HCl, NaCN, H_2O MeOH, 94%; (b) LAH, Et_2O, 56%; (c) Boc_2O, CH_2Cl_2, 86%; (d) $Pd(OH)_2$/C, THF, MeOH 94%; (e) methyl 2-isothiocyanatoacetate, THF, 0 °C, 100%; (f) NaH, THF, 89%; (g) 3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)benzaldehyde, piperidine, EtOH reflux, rt 87%; (h) NaH, MeI, DMF, 86%; (i) TFA, CH_2Cl_2; (j) Et_3N, THF, microwave, 68–72%; (k) NaH, EtI, DMF, 43%; (l) TBDMSCl, imid, DMF, 99%; (m) TBAF, HOAc, THF; (n) MsCl, DMAP, Et_3N, DCE; (o) amine, 80 °C, 17–62% over 2 steps.

Table 4
Profiles of selected compounds, dosed at 10 mg/kg orally

Compound	AUC_{0-6h} (μM h)	Plasma C_{max} (μM)	Brain:plasma concentration ratio @6 h	$Brain C_{6h}$ (ng/g)
20	10.1	2.3 @ 2 h	1.3	914
27	10.0	2.3 @ 2 h	0.7	449
39	16.9	4.2 @ 1 h	0.3	275

In summary, the use of the structurally distinct iminoheterocycle core permitted rapid SAR exploration and provided a novel series of small-molecule γ-secretase modulators. Several compounds in this series possess double-digit nanomolar potency in the cell-based assay. Surprisingly, although several compounds exhibited excellent pharmacokinetic profiles with high brain levels, only a modest decrease in $A\beta_{42}$ levels in the cortex and CSF was realized. Additional work in the development of small-molecule γ-secretase modulators will be presented in due course.

Acknowledgments

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10. Unpublished results.
11. *Cell based assay*: Human embryonic kidney (HEK) 293 cells stably transfected with APP carrying both Swedish and London mutations in pcDNA3.1 vector (Invitrogen) were treated with GSM compounds for 5 h. Aβ in conditioned media was measured using MesoScale Discovery (MSD) technology-based sandwich immunoassays. $A\beta_{42}$ was measured using a pair of labeled antibodies TAG-G2-11 and biotin-4G8; $A\beta_{40}$ was measured using an antibody pair of TAG-G2-10 and biotin-4G8; total Aβ was measured using TAG-W02 and biotin-4G8. *CSF and cortex Aβ assays*: Male CD rats (~120 g; Crl:CD(SD); Charles River Laboratories, Kingston, NY) were used for all experiments. Compounds were formulated in 20% hydroxypropyl-β-cyclodextrin and administered orally to male rats at a dosing volume of 5 ml/kg. Three hours after drug administration, cerebrospinal fluid (CSF) was collected from the cisterna magna and the brain was removed immediately following euthanasia with excess CO_2, quickly frozen on dry ice and stored at −70 °C until Aβ analysis. Only visibly clear CSF samples were included. Rat CSF $A\beta_{40}$ and $A\beta_{42}$ was analyzed using AlphaLISA Amyloid Assay kits (Perkin–Elmer) according to the manufacturer's instructions. For brain cortex $A\beta_{42}$ analysis, half of the cortex was homogenized and extracted in 5 M guanidine–HCl/50 mM Tris–HCl, pH 8. The extracts were sonicated and partially purified using a solid phase extraction matrix in 96-well format, the HLB plate (Waters). The samples eluted from the HLB plate were dried and resuspended in freshly prepared PBS/0.5% Tween 20. $A\beta_{40}$ and Aβ were measured using AlphaLISA amyloid assay kits.

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13. The ratio of $A\beta_{total}/A\beta_{42}$ represents the extent of γ-secretase modulation. A ratio of Aβtotal/$A\beta_{42}$ = 1 indicates an inhibitor of γ-secretase, while a ratio >5 indicates a modulator of γ-secretase. Since GSMs shift the cleavage specificity of the enzyme, increasing the production of shorter Aβ peptides while reducing the production of longer species like $A\beta_{42}$, total Aβ production is unchanged while $A\beta_{42}$ production is reduced. Thus, GSMs increase the $A\beta_{total}/A\beta_{42}$ ratio.
14. Conditions: (a) DBU, NaH, MsCl, CH_2Cl_2, rt; (b) DIEA, MsCl, rt then THF, LHMDS −40 °C to 0 °C.
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