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Discovery of fused 5,6-bicyclic heterocycles as γ-secretase modulators

Jun Qin [a,*], Pawan Dhondi [a], Xianhai Huang [a], Mihirbaran Mandal [a], Zhiqiang Zhao [a], Dmitri Pissarnitski [a], Wei Zhou [a], Robert Aslanian [a], Zhaoning Zhu [a], William Greenlee [a], John Clader [a], Lili Zhang [b], Mary Cohen-Williams [b], Nicholas Jones [b], Lynn Hyde [b], Anandan Palani [a]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA
[b] Department of Neurodegenerative Diseases, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

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ABSTRACT

We herein report the discovery of four series of fused 5,6-bicyclic heterocycles as γ-secretase modulators. Synthesis and SAR of these series are discussed. These compounds represent a new class of γ-secretase modulators that demonstrate moderate to good in vitro potency in inhibiting $A\beta_{42}$ production.

Despite Alzheimer's disease being identified more than 100 years ago, it still remains an urgent medical challenge.[1–3] Alzheimer's disease is a neurodegenerative disorder with clinical symptoms of cognitive impairment and progressive loss of bodily functions and language ability. The disease is characterized pathologically by neuronal loss, neurofibrillary tangle (NFT) formation, and extracellular deposition of amyloid-β (Aβ) peptide plaques. Although there are several hypotheses about the cause of the disease, a predominant one is the amyloid hypothesis.[4,5] According to this hypothesis, reduced clearance and/or increased aggregation of the Aβ peptides, especially $A\beta_{40}$ and $A\beta_{42}$, play a key role in the pathogenesis of Alzheimer's disease.

Aβ peptides are generated by initial cleavage of the amyloid precursor protein (APP) by β-secretase to form a C-terminal fragment (CTF), which is subsequently proteolyzed by γ-secretase to produce Aβ peptides ranging from 37 to 42 amino acids in length. Despite accounting for only 10% of the overall Aβ peptide production, $A\beta_{42}$ is the major component of amyloid plaques. Several classes of γ-secretase inhibitors (GSIs) have been reported to demonstrate efficacy in reducing overall production of Aβ peptides in plasma, cerebrospinal fluid (CSF) and brain.[6,7] However, γ-secretase inhibitors can cause mechanism-based toxicity due to disruption of the processing of other γ-secretase substrates such as Notch and ERB4. Cleavage of Notch is important for cellular gene transcription, and inhibition of Notch cleavage by GSIs results in side effects such as thymus atrophy and intestinal goblet cell hyperplasia.[8,9]

On the other hand, γ-secretase modulators (GSMs) selectively inhibit the production of $A\beta_{42}$ without blocking the overall function of γ-secretase on CTF and other substrates such as Notch. Therefore this class of molecules should not cause Notch-related side effects and could offer a better safety profile than GSIs.[10] It is believed that GSMs interact with γ-secretase at an allosteric site, inducing a conformational change in the protease and causing a shift of cleavage specificity to preferentially produce shorter, more soluble Aβ-peptides such as $A\beta_{38}$.[11–16] However, the exact location of this allosteric site on γ-secretase remains unknown. Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, indomethacin, sulindac sulfide and tarenflurbil were the first class of GSMs reported to selectively reduce the production of $A\beta_{42}$ in favor of shorter peptides such as $A\beta_{38}$.[17–19] In addition, they did not inhibit the cleavage of other transmembrane protein substrates such as Notch by γ-secretase.[20] However, they demonstrated limited potency ($A\beta_{42}$ IC_{50} = 25–200 μM) and did not improve cognition in clinical trials in AD patients.[21,22]

Eisai reported a GSM **1** which contains a cyclic cinnamamide motif (Scheme 1).[23–25] It demonstrated good in vitro potency and selectivity as a GSM in our hands (Cell $A\beta_{42}$ IC_{50} = 64 nM, $A\beta_{total}$ IC_{50}/$A\beta_{42}$ IC_{50} ratio = 226).[26] This compound also showed robust in vivo efficacy to reduce CSF and brain $A\beta_{42}$ (−55% CSF $A\beta_{42}$ and −44% brain $A\beta_{42}$, 3 h after a single oral dose of 30 mg/kg).[27] Inspired by this novel structure, we decided to modify the core of **1** to identify new chemical entities with improved potency and ancillary profile, while removing the cinnamamide double

* Corresponding author. Tel.: +1 908 740 3904; fax: +1 908 740 7664.
E-mail addresses: jun.qin@merck.com, jun2005qin@yahoo.com (J. Qin).

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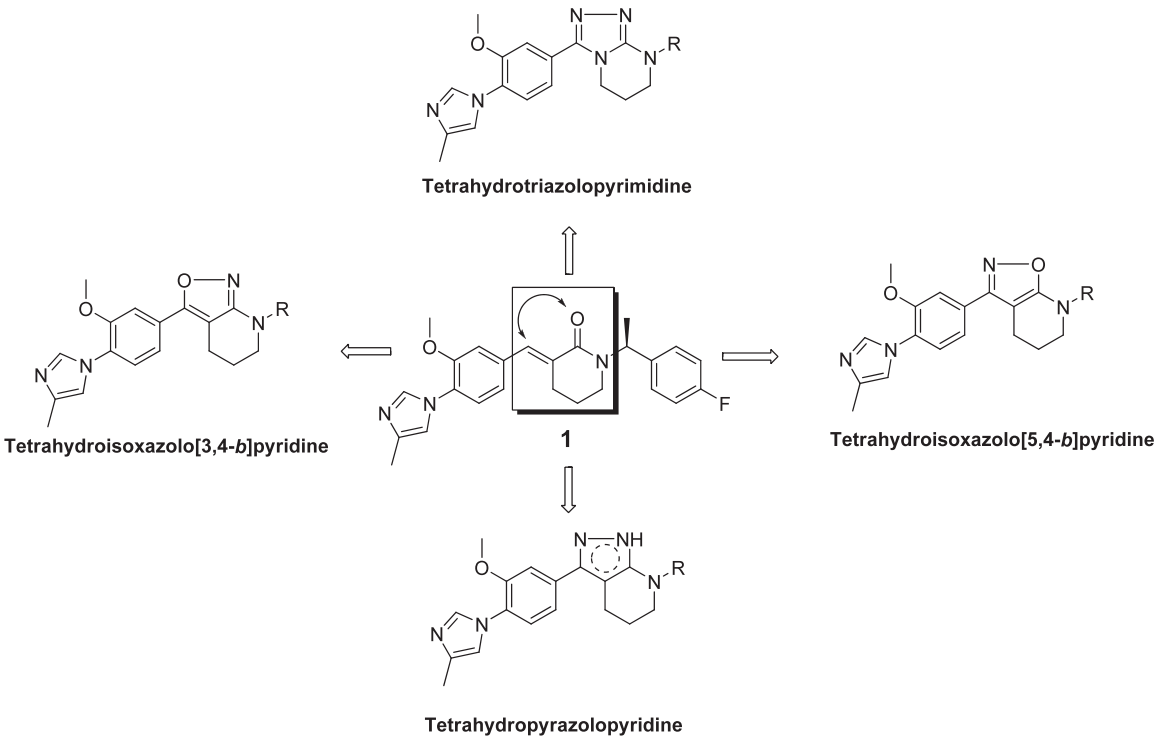
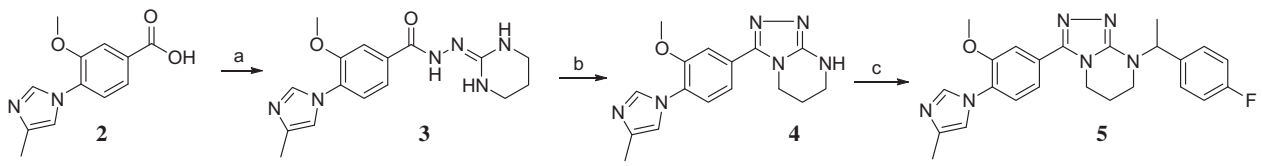


Scheme 1. Design of fused 5,6-bicyclic γ-secretase modulators.

bond, which could be a potential Michael acceptor. We explored core modifications by forming an additional five-membered ring between the cinnamamide double bond and the carbonyl group in the central core. This modification led to four series of compounds containing a fused 5,6-bicyclic structure as the central core as depicted in Scheme 1. We conducted SAR on the R region of each of these series. Herein we discuss the synthesis and SAR data for each of these series.

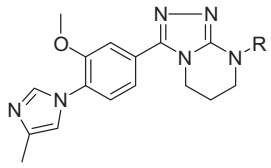
We first looked at the tetrahydrotriazolopyrimidine series, and the synthesis is outlined in Scheme 2. The acid **2** was coupled with hydrazinopyrimidine to afford **3**. With pyridine as solvent at elevated temperature, **3** underwent intramolecular cyclization to form triazolopyrimidine core **4**, which was then alkylated with 1-(1-bromoethyl)-4-fluorobenzene to afford **5**. Analogues **6**–**24** with different *N*-substitution were prepared by a similar route to **5**, and SAR data of these compounds is summarized in Table 1. Compound **5** with a 1-(4-fluorophenyl)ethyl group had moderate activity to inhibit $A\beta_{42}$. Longer alkyl chains attached to the benzylic carbon improved the potency and selectivity (**6** $A\beta_{42}$ IC_{50} = 156 nM, **7** $A\beta_{42}$ IC_{50} = 145 nM). Replacement of the simple alkyl chain at the benzylic carbon with a cyclopropyl group (**8**, **9**) was not tolerated. Compound **10** with hydrogen substitution at the benzylic carbon showed potency similar to that exhibited by **5**. Substitutions on the phenyl ring also affected the activity. Halogen substitutions generally were better than non-halogen substitutions (**13**–**14** vs **15**–**16**). Compound **12** with a 1-(3,4,5-trifluorophenyl)ethyl group and **14** with a 3,4-dichlorophenyl ring were the most potent analogues in this series (**12** $A\beta_{42}$ IC_{50} = 116 nM, **14** $A\beta_{42}$ IC_{50} = 126 nM). Longer *N*-alkyl substitutions as in the case of **17** and **18** did not help improve the potency. Amide derivatives **19**–**22** were generally not favored, although **21** had moderate activity. Urea compound **23** was not active up to 20 μM. *N*-Arylsulfonamide analogue **24** showed weak potency. Changing the R from the 1-(4-fluorophenyl)ethyl of **5** to a hydrogen atom (**4**) completely eliminated the activity. Overall, *N*-benzyl derivatives were favored over urea, amide and *N*-arylsulfonamide compounds. In order to determine the ability of compounds **12** and **14** to reduce the amount of $A\beta_{42}$ in CSF, each compound was dosed orally in rats at 30 mg/kg. Unfortunately, neither compound showed statistical efficacy in this in vivo model, despite good in vitro potency. Examination of the pharmacokinetics of **12** and **14** revealed that each had limited brain exposure (**12** Pl. C_{3h} = 9.00 μM, brain C_{3h} = 0.49 μM; **14** Pl. C_{3h} = 22.8 μM, brain C_{3h} = 0.52 μM).[28]

Given the disappointing in vivo data from the tetrahydrotriazolopyrimidine series, we decided to work on tetrahydroisoxazolo[3,4-*b*]pyridine and tetrahydroisoxazolo[5,4-*b*]pyridine series, which could offer better brain penetration due to lower basicities and higher *c* log *P* values. The preparation of the tetrahydroisoxazolo[3,4-*b*]pyridine series is depicted in Scheme 3. A Sonogashira coupling of **25** with pent-4-yn-1-ol, followed by protection of the alcohol afforded **26**. Cycloaddition of **26** with nitrile oxide, generated in situ from dibromoformaldoxime, gave **27**. Deprotection of



Scheme 2. Reagents and conditions: (a) 2-hydrazino-1,4,5,6-tetrahydropyrimidine, HATU, *i*-Pr_2NEt, DMF, 88%; (b) pyridine, 120 °C, 54%; (c) NaH, 1-(1-bromoethyl)-4-fluorobenzene, DMF/THF, 4%.

Table 1
Cell $A\beta_{42}$ IC_{50} and γ-secretase modulator selectivity of tetrahydrotriazolopyrimidines **4–24**



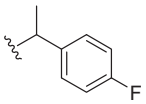
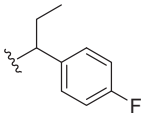
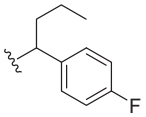
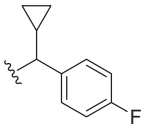
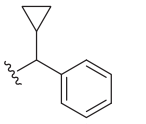
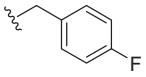
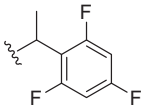
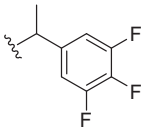
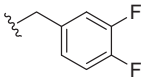
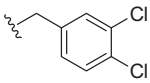
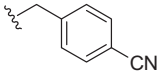
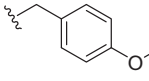
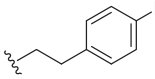
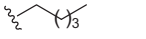
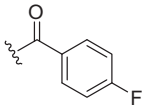
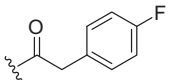
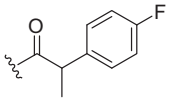
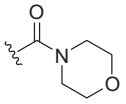
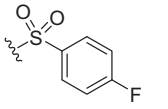
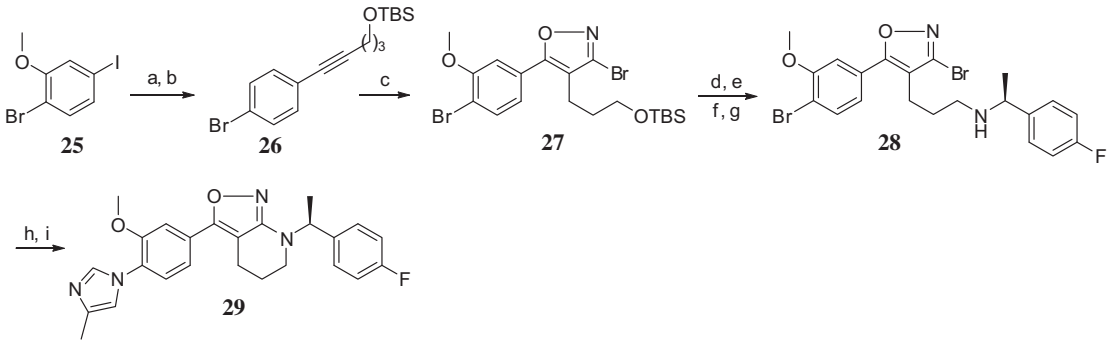
Compound	R	$A\beta_{42}$ IC_{50}[a] (nM)	$A\beta_{total}$ IC_{50}/$A\beta_{42}$ IC_{50} ratio
4	H	20,000	1
5[b]		509	39
6		156	128
7		145	138
8		16,374	1
9		20,000	1
10		649	31
11		342	58
12[c]		116	165
13		552	36
14[d]		126	159
15		2531	3
16		1409	14
17		619	32
18		1406	14

Table 1 (*continued*)

Compound	R	$A\beta_{42}$ IC_{50}[a] (nM)	$A\beta_{total}$ IC_{50}/$A\beta_{42}$ IC_{50} ratio
19		19,316	1
20		1919	10
21		485	41
22		2069	10
23		20,000	1
24		2215	9

[a] IC_{50} was reported as an average of multiple determinations ($n \geqslant 2$).
[b] *c* log *P* = 4.5.
[c] *c* log *P* = 4.7.
[d] *c* log *P* = 5.4.

TBS ether **27**, followed by oxidation of the resulting alcohol to an aldehyde and the reductive amination of this aldehyde with (*S*)-1-(4-fluorophenyl)ethanamine provided **28**. Final compound **29** was delivered after ring closure at elevated temperature and displacement of aromatic bromide with 4-methyl imidazole. Compounds **30–32** were synthesized by a similar method to **29**. The SAR data in Table 2 showed that this tetrahydroisoxazolo[3,4-*b*]pyridine series showed moderate but reduced activity relative to **1**. Varying the R substitution of **29** to that of **30–32** had little effect on potency. Compound **31** was dosed orally at 30 mg/kg in rats, but did not show statistical efficacy. Meanwhile, the tetrahydroisoxazolo[5,4-*b*]pyridine series was synthesized as shown in Scheme 4. *N*-Alkylation of **33** with 1-(1-bromoethyl)-4-fluorobenzene afforded **34**, which was converted to **35** by Aldol condensation with 3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl) benzaldehyde and Swern oxidation of the resulting alcohol intermediate. Finally, oxime ether formation and dehydrative ring closure delivered the product **36**, which was resolved by chiral HPLC to provide its two enantiomers **37** and **38**.[29] The latter was 3-fold more potent than the former. The SAR data for the rest of the analogues **39–41**[29] is presented in Table 2. Changing the R substitution from the 1-(4-fluorophenyl)ethyl of **38** to a 1-(4-fluorophenyl)propyl (**39**) or to a 1-(3,5-difluorophenyl)ethyl (**40**) decreased potency 2-fold, whereas the 1-(3,4,5-trifluorophenyl)ethyl derivative **41** maintained similar activity to that of **38**. Overall, the tetrahydroisoxazolo[3,4-*b*]pyridine and tetrahydroisoxazolo[5,4-*b*]pyridine series displayed moderate in vitro activity and did not offer a better profile than the tetrahydrotriazolopyrimidine series. Although these two series of compounds have relatively lower basicities (higher *c* log *P* value) than tetrahydrotriazolopyrimidine series in general, which suggest a possibility of better brain penetration, compound **31** did not show improved in vivo potency. The lack of in vivo activity could be partially

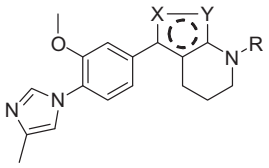


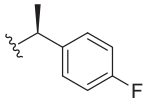
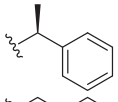
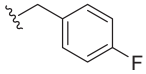
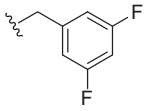
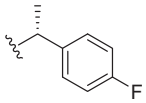
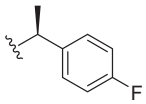
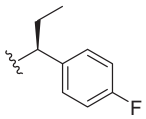
Scheme 3. Reagents and conditions: (a) pent-4-yn-1-ol, $PdCl_2(PPh_3)_2$, CuI, DIEA, THF; (b) TBSCl, imidazole, CH_2Cl_2, 99% for two steps; (c) dibromoformaldoxime, $NaHCO_3$, CH_2Cl_2, 50 °C, 30%; (d) HF, THF, 99%; (e) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; (f) (*S*)-1-(4-fluorophenyl)ethanamine, $MgSO_4$, CH_2Cl_2; (g) $NaBH_4$, MeOH, 80% for three steps; (h) BEMP, CH_3CN, 180 °C, 15%; (i) 4-methyl imidazole, Cu_2O, Cs_2CO_3, 4,7-dimethoxy-1,10-phenanthroline, polyethylene glycol, butyronitrile, 30%.

attributed to its limited in vitro potency (**31**, $A\beta_{42}$ IC_{50} = 356 nM). Therefore, we need to identify compound with not only low basicity but also good in vitro potency for displaying in vivo activity.

Having explored the previous three series, we turned our attention to the tetrahydropyrazolopyridine series. Synthesis of this tetrahydropyrazolopyridine series is outlined in Scheme 4. From intermediate **35**, analogue **42** was obtained through hydrazone formation and cyclization in one pot.[30] Compound **42** showed good in vitro activity ($A\beta_{42}$ IC_{50} = 164 nM) (Table 3). Changing the R substitution from the 1-(4-fluorophenyl)ethyl of **42** to a 1-(3,4,5-trifluorophenyl)propyl (**43**) maintained similar activity. Enantiomer **44**[31] was examined in our rat in vivo model, where it demonstrated moderate, but statistically significant efficacy in reducing rat CSF $A\beta_{42}$ (−26% $A\beta_{42}$, 3 h after 30 mg/kg po dosing). The improved in vivo activity of **44** seems to correlate well with its good in vitro potency and lower basicity (*c* log *P* = 6.6) than the previous

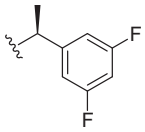
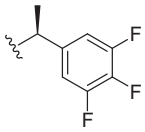
Table 2
Cell $A\beta_{42}$ IC_{50} and γ-secretase modulator selectivity of tetrahydroisoxazolo[3,4-*b*]pyridines **29**–**32** and tetrahydroisoxazolo[5,4-*b*]pyridines **37**–**41**



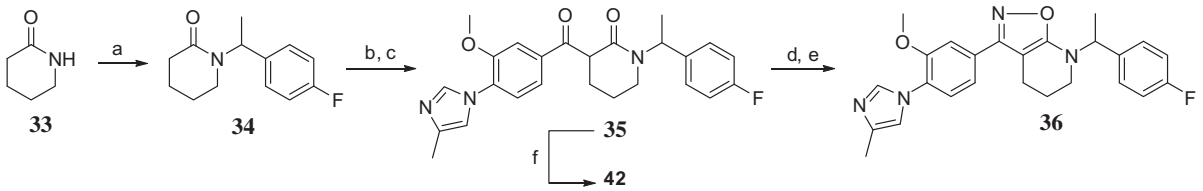
Compound	X	Y	R	$A\beta_{42}$ IC_{50}[a] (nM)	$A\beta_{total}$ IC_{50}/$A\beta_{42}$ IC_{50} ratio
29[b]	O	N		465	38
30	O	N		537	37
31[c]	O	N		356	48
32	O	N		440	46
37	N	O		1392	14
38[d]	N	O		448	29
39	N	O		875	17

(*continued on next page*)

Table 2 (*continued*)

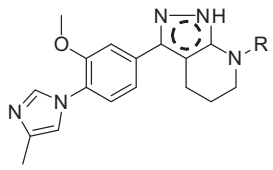
Compound	X	Y	R	$A\beta_{42}$ IC_{50}[a] (nM)	$A\beta_{total}$ IC_{50}/$A\beta_{42}$ IC_{50} ratio
40	N	O		935	21
41	N	O		540	37

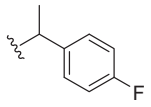
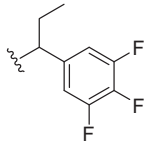
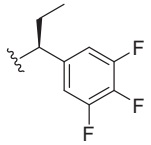
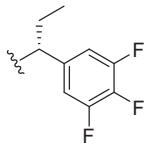
[a] IC_{50} was reported as an average of multiple determinations ($n \geqslant 2$).
[b] *c* log *P* = 5.8.
[c] *c* log *P* = 5.5.
[d] *c* log *P* = 5.8.



Scheme 4. Reagents and conditions: (a) NaH, 1-(1-bromoethyl)-4-fluorobenzene, THF, 57%; (b) *t*-BuLi, 3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)benzaldehyde, −78 °C, 50%, THF; (c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 94%; (d) $NH_2OH{\cdot}HCl$, KOAc, MeOH, 89%; (e) P_2O_5, EtOH, 80 °C, 29%; (f) NH_2NH_2, P_2O_5, EtOH, 75 °C, 21%.

Table 3
Cell $A\beta_{42}$ IC_{50} and γ-secretase modulator selectivity of tetrahydropyrazolopyridins **42–45**



Compound	R	$A\beta_{42}$ IC_{50}[a] (nM)	$A\beta_{total}$ IC_{50}/$A\beta_{42}$ IC_{50} ratio
42[b]		164	46
43		201	99
44[c]		122	164
45		209	96

[a] IC_{50} was reported as an average of multiple determinations ($n \geqslant 2$).
[b] *c* log *P* = 5.9.
[c] *c* log *P* = 6.6.

three series. With this encouraging preliminary data in hand, we plan to explore the potential of this series and the results will be reported in the near future.

In summary, we have explored four series of 5,6-bicyclic heterocycles as γ-secretase modulators. Compounds from all series demonstrated moderate to good in vitro potency. Among these series, we have identified the tetrahydropyrazolopyridine core as a promising new lead series. Specifically, compound **44** demonstrated good in vitro and moderate in vivo efficacy as a γ-secretase modulator.

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26. *Cell based in vitro assay method:* Human embryonic kidney (HEK) 293 cells stably transfected with APPsw-Ion 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 the antibody pair of TAG-G2-10 and biotin-4G8; total Aβ was measured using TAG-W02 and biotin-4G8.
27. *In vivo assay method:* Male CD rats (~100 g; Charles River Laboratories) were orally administered a compound (formulated in 20% hydroxypropyl β cyclodextin; 5 ml/kg) and 3 h later tissues were collected. Immediately following euthanasia with excess CO_2, cerebrospinal fluid (CSF) was collected from the cisterna magna and frozen on dry ice; only clear samples were analyzed. Blood (from the vena cava) and brains were collected from the animals immediately following CSF collection. Blood was briefly kept on ice until it was spun at 6000 rpm for about 10 min and plasma was removed. Brains were immediately frozen on dry ice. All samples were stored at −80 °C until analysis. Rat CSF $A\beta_{40}$ and $A\beta_{42}$ were analyzed using AlphaLISA Amyloid Assay kits (Perkin-Elmer) according to the manufacturer's instructions. For brain cortex $A\beta_{42}$ analysis, a half cortex from one hemisphere 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 $A\beta_{42}$ were measured using AlphaLISA amyloid assay kits.
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30. The exact isomeric structures of pyrazoles were not determined.
31. Absolute configuration was assigned based on chiral HPLC retention time when correlated to closely related series of enantiomers of known absolute configuration. HPLC condition: chiral AD column, hexane/*i*-PrOH/Et_2NH = 100:25:0.1.