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Discovery of 2-methylpyridine-based biaryl amides as γ-secretase modulators for the treatment of Alzheimer's disease

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ABSTRACT

γ-Secretase modulators (GSMs) are potentially disease-modifying treatments for Alzheimer's disease. They selectively lower pathogenic Aβ42 levels by shifting the enzyme cleavage sites without inhibiting γ-secretase activity, possibly avoiding known adverse effects observed with complete inhibition of the enzyme complex. A cell-based HTS effort identified the sulfonamide **1** as a GSM lead. Lead optimization studies identified compound **25** with improved cell potency, PKDM properties, and it lowered Aβ42 levels in the cerebrospinal fluid (CSF) of Sprague-Dawley rats following oral administration. Further optimization of **25** to improve cellular potency is described.

Alzheimer's disease (AD) is the most common dementia affecting more than 5 million patients in the US. According to the amyloid hypothesis,[1–3] aggregation of the amyloid β-protein (Aβ), particularly its 42-residue variant (Aβ42), plays a direct role in the pathogenesis of AD, and therefore agents that could lower Aβ levels in the brain should be a useful treatment for AD. Aβ is generated through sequential cleavage of amyloid precursor protein (APP) by β-secretase (BACE) and γ-secretase (GS).[4] These enzymes have become popular AD drug discovery targets over the last decade and several inhibitors of each enzyme have been advanced into clinical trials.[5–7] However, development of γ-secretase inhibitors (GSIs) are challenging, largely due to mechanism-based toxicity associated with inhibition of other γ-secretase substrates such as Notch.[8,9] An alternative approach, called γ-secretase modulation, involves lowering pathogenic Aβ42 levels by shifting the enzymatic cleavage sites to generate shorter and less aggregation-prone forms of the Aβ peptide such as Aβ37 and Aβ38.[10–19] The processing of other γ-secretase substrates such as Notch does not appear to be affected by the γ-secretase modulators (GSMs), thus avoiding side effects associated with GSIs. Herein, we describe the discovery of an orally efficacious 2-methylpyridine-based amide GSM **25**[20] through lead optimization of a high throughput screen (HTS) lead sulfonamide **1**.

At the start of our program only a few nonsteroidal anti-inflammatory drugs (NSAID)-derived carboxylic acids and imidazole-based heterocycles represented by compounds **2** and **3** were disclosed chemotypes of γ-secretase modulators (GSMs).[11] Both **2** and **3** have the same imidazole phenyl moiety that was subsequently used in a wide variety of GSMs.[11]

We used a high-throughput homogeneous time-resolved fluorescence (HTRF) assay to screen for inhibitors of Aβ42 production in Human Embryonic Kidney 293 cells (HEK293 cells) with stable expression of guinea pig Swedish mutant SFV-APP695sw. A cell

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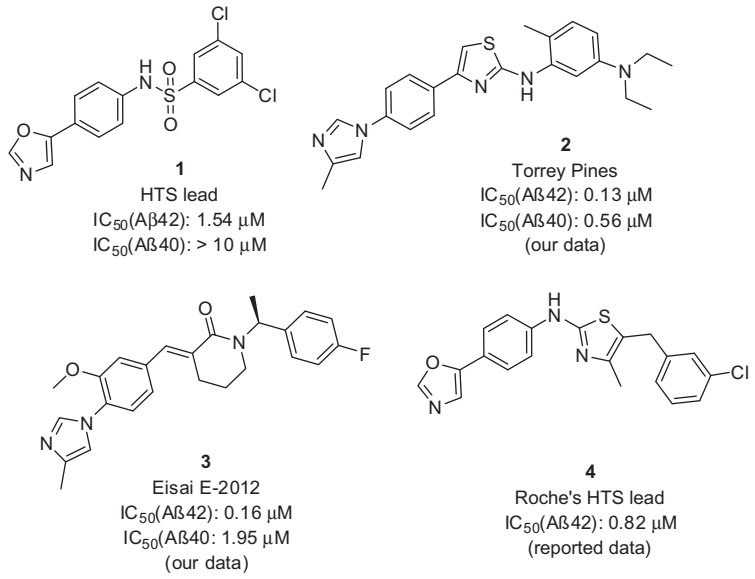
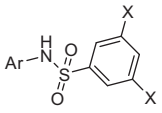


Figure 1. Non-NSAID based GSM and the HTS lead **1**.

viability assay (Cell Titer-Blue from Promega) was used to eliminate cytotoxic compounds. A Notch luciferase reporter gene assay (in which a luciferase construct was inserted under the control of the Notch promoter, in TE-671 cells and Luciferase luminescence was measured following three days of compound treatment) was used to eliminate nonselective GSIs. Compound **1** was identified as a novel GSM which is structurally related to the early generation GSMs **2**–**3** and Roche's recently reported oxazole-based GSM lead, **4** (see Fig. 1).[21]

Lead **1** selectively inhibited the formation of Aβ42 (IC_{50} = 1.54 - μM) over Aβ40 and Notch (both IC_{50} > 10 μM). In our initial optimization, we explored the replacement of the oxazole group by other five and six-membered heteroaryls (Table 1). Replacement of the nitrogen in the oxazole by CH resulted in over

Table 1
Sulfonamide SAR



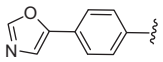
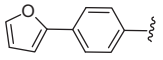
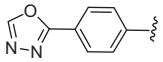
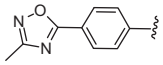
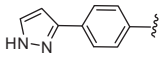
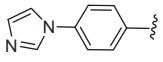
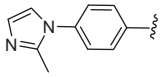
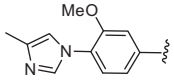
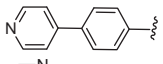
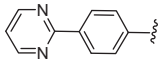
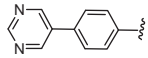
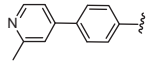
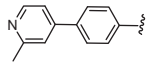
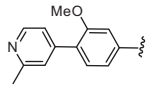
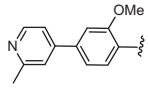
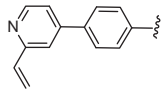
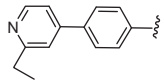
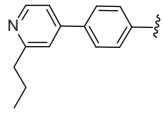
Compd	Ar	X	Aβ42 cell IC_{50}[a] (μM)	RLM[b] (μL/min/mg)	HLM[b] (μL/min/mg)	CYP3A4 IC_{50}[c] (μM)	CYP2D6 IC_{50}[c] (μM)	Mouse B/P[d]
1		Cl	1.54 ± 0.0.36	166	32	1.6	2.9	0.03
5		Cl	>10	>399	>399	13	>27	
6		Cl	2.44	287	94	27	15.1	0.16
7		Cl	>10	>399	>399	>27	>27	
8		Cl	>10	>399	384	15.3	11.8	
9		Cl	2.10 ± 0.23	<14	<14	0.1	0.1	0.07
10		Cl	9.4	113	22	>27	>27	
11		Cl	1.06 ± 0.08	NA	NA	NA	NA	
12		Cl	2.75 ± 0.46	85	35	0.6	0.2	0.09
13		Cl	>10	>399	>399	16.2	>27	

Table 1 (*continued*)

Compd	Ar	X	Aβ42 cell IC_{50}[a] (μM)	RLM[b] (μL/min/mg)	HLM[b] (μL/min/mg)	CYP3A4 IC_{50}[c] (μM)	CYP2D6 IC_{50}[c] (μM)	Mouse B/P[d]
14		Cl	>10	>399	45	3.5	7.2	
15		Cl	0.72 ± 0.91	>399	497	4.7	9.8	0.10
16		CF_3	0.28 ± 0.19	290	399	>27	2.0	
17		CF_3	0.53 ± 0.04	NA	NA	NA	NA	
18		CF_3	1.83 ± 1.33	>399	306	2.2	1.9	
19		CF_3	1.26 ± 0.96	169	193	4.5	0.70	
20		CF_3	0.21 ± 0.11	324	371	12.2	0.7	
21		CF_3	4.06	122	270	>27	1.80	

[a] Values with standard deviations shown were the average of at least three independent runs.
[b] Compounds were incubated with microsomes for 30 min at a concentration of 1 μM.
[c] Compounds were incubated with microsomes for 5 (CYP3A4) or 10 (CYP2D6) min at a concentration of 3 μM.
[d] Measured at 2 h after 10 mg/kg oral dosing.

a sevenfold loss of potency (**5**). The 1,3,4-oxadiazole analog **6** was almost equipotent. The isomeric 1,2,4-oxadiazole **7** had over a sevenfold loss of potency compared to **1**. Of the three five-membered diazole isomers **8**–**11**, only the 1-imidazole **9** maintained the potency. This compound was metabolically stable but was also a potent inhibitor of CYP3A4 and CYP2D6. Introduction of a methyl group at the 2-position of the imidazole ring resulted in four fold loss of potency (**10**). Using (3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)phenyl present in E2012 (**3**) to replace 4-(1*H*-imidazol-1-yl)phenyl only improved the potency by twofold over **9**.[22]

Of the six-membered heterocycles examined (**12**–**14**), the 4-pyridine analog **12** was slightly less potent than oxazole **1** but had better metabolic stability in rat liver microsomes. Introduction of a 2-methyl group improved the potency by fourfold and reduced CYP inhibitory activity (**15**). Modification of the sulfonamide portion identified the 3,5-bis(trifluorophenyl)sulfonamide **16** with a twofold improvement in potency.

Comparing the structures of **16**, **3**, and **11**, it is reasonable to assume that 2-methyl pyridine in **16** mimics the methyl imidazole group in **3** and **11**. In the GSM literature the methoxy group is ubiquitous. In recent publications where the effect of the methoxy group replacement was studied, the potency was reduced in all cases with the magnitude of the drop dependent upon other portions of the molecule.[12,18,21] We investigated the effect of the methoxy on the phenyl group of **16**. Interestingly the opposite effect was observed (a twofold decrease in potency) (**17**). Moving the methoxy group next to the amine group led to a threefold decrease in potency (**18**). Of the several methyl group replacements prepared (**19**–**21**), only the ethyl group retained potency.

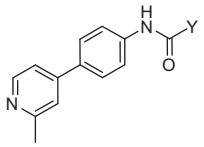
Next we investigated the mouse brain exposure of **15**, two hours after oral dosing at 10 mg/kg. Although the brain/plasma ratio (B/P = 0.10) was slightly better than that of **1** (0.03), it was still considered to be low for an efficacious CNS drug. Three other sulfonamides **6**, **9**, and **12** also showed low B/P ratios (Table 1). This led us to postulate that the polar aryl sulfonamide group might be responsible for the low brain exposure. The corresponding amide of **15** was prepared and had a much higher B/P ratio (1.46) than previous compounds under the same conditions (see compound **22**, Table 2); however, it showed only partial inhibition of Aβ42 production in the cellular assay. At higher concentrations (up to10 μM top concentration) the Aβ42 percent of DMSO control (POC) reached plateau at 58%. Our curve-fitting algorithm automatically assigned an IC_{50} of >10 μM if the minimum POC was >50%. Variation of the substituents on the carboxamide phenyl ring offered little improvement in cellular potency (data not shown). Most compounds showed incomplete inhibition of Aβ42 production. The best compound identified from this SAR effort was 3,5-bis(trifluoromethyl) analog **23**. It exhibited a minimum POC of 52% at 10 μM. The observation of partial inhibition in this class of GSM is consistent with proposed allosteric mechanism (vide infra).

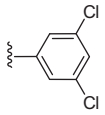
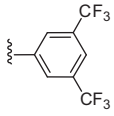
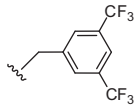
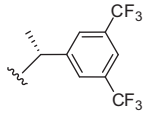
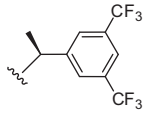
As our next step in establishing the SAR of this GSM chemotype, we examined the effect of introducing a methylene spacer between the amide carbonyl function and the disubstituted aromatic ring. The amide **24** was found to be only slightly less potent than the sulfonamide **16**.

Flexible alignment[23] of both **24** and **23** to the lowest energy conformations of the analogous sulfonamide **16** (predicted from quantum mechanics) provided a qualitative rationale for the increased potency of **24** compared to **23**. The methylene spacer of **24** (Fig. 2, yellow) allowed the pendant, disubstituted aryl ring to better adopt the approximately perpendicular orientation predicted for the sulfonamide species (green) than does an amide lacking the spacing group (pink).

Introduction of a methyl group at the α-carbon of the amide gave two enantiomers **25** and **26**, separated by chiral supercritical fluid chromatography (SFC). The absolute stereochemistry of **25** and **26** were determined via comparison of their predicted and experimental vibrational circular dichroism (VCD) spectra and optical rotations.[24–26] The more active (*R*)-isomer **25** was fivefold

Table 2
Lead generation



Compd	Y	Aβ42 cell IC_{50}[a] (μM)	RLM[b] (μL/min/mg)	HLM[b] (μL/min/mg)	CYP3A4 IC_{50}[c] (μM)	CYP2D6 IC_{50}[c] (μM)	Mouse B/P[d]
22		>10[e]	19	91	>27	>27	1.46
23		>10[f]	14	106	>27	>27	
24		0.46 ± 0.11	22	18	>27	13	
25		0.26 ± 0.10	<14	<14	>27	4.1	2.0[g]
26		>10	<14	<14	>27	2.6	2.0[g]

[a] Values with standard deviations shown were the average of at least three independent runs.
[b] Compounds are incubated with microsomes for 30 min at a concentration of 1 μM.
[c] Compounds were incubated with microsomes for 5 (CYP3A4) or 10 (CYP2D6) min at a concentration of 3 μM.
[d] Measured at 2 h after 10 mg/kg oral dosing.
[e] Maximum 53% inhibition at 10 μM.
[f] Maximum 58% inhibition at 10 μM.
[g] Obtained from rat PD studies.

more potent (Aβ42 IC_{50} = 0.26 μM) than the original HTS lead **1**. It was less active against Aβ40 (IC_{50} = 3.1 μM) than against Aβ42 and about 45-fold selective against Notch (IC_{50} = 11.8 μM).

Compound **25** exhibited low-to-moderate passive permeability (human LLC-PK1 Avg Papp: 7.8 μcm/s) and was not an efflux substrate (efflux ratio: 1.2). Pharmacokinetic analysis of **25** showed that in vivo clearance in rats was 0.58 L/h/kg, V_{ss} was 4.3 L/kg, and $t_{1/2}$ was 6 h following an iv dose (2 mg/kg). The oral bioavailability was 100% based on a 5 mg/kg dose. In cynomolgous monkeys, in vivo clearance was 0.16 L/h/kg, V_{ss} was 3.1 L/kg, and $t_{1/2}$ was 15.9 h following an iv dose (2 mg/kg). Oral bioavailability was 56% based on a 5 mg/kg dose.

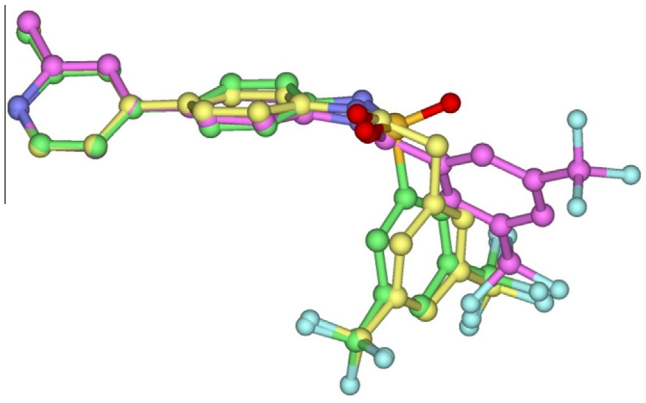


Figure 2. Alignment of **24** (yellow) and **23** (pink) to parent sulfonamide **16** (green).

Due to its low in vivo clearance and good oral bioavailability in rat, we tested **25** in a pharmacodynamic (PD) model of Aβ reduction. It was administered in a single oral dose to naïve Sprague-Dawley rats and Aβ levels were measured in CSF and brain tissue. CSF Aβ42 levels were reduced by 41% versus vehicle control (100 mg/kg po) after 4 h whereas no significant inhibition was observed with **26** (opposite enantiomer of **25**) even though both have similar plasma and brain exposure. In brain homogenate, Aβ42 levels were reduced by 33%. Consistent with in vitro data, no significant lowering of Aβ40 was observed with **25** (data not shown).

To further investigate the time-course of inhibition by **25**, rats were dosed at 100 mg/kg and samples were collected at the indicated time points and analyzed for both drug and Aβ42 levels. There was a clear relationship between **25** plasma concentrations and Aβ lowering in the brain and CSF (Fig. 3). Sustained CSFAβ lowering (>36 hours) was observed (Fig. 3) and the plasma and brain concentrations of **25** were quite stable from 4 h to 36 h post dose. The data were not consistent with $t_{1/2}$ of 6 h obtained with 2 mg/kg iv dose. This might be caused by saturated absorption of **25** at 100 mg/kg. The brain/plasma ratios measured in the PD studies for **25** were 2.0. This represented a significant improvement over the original HTS lead **1**.

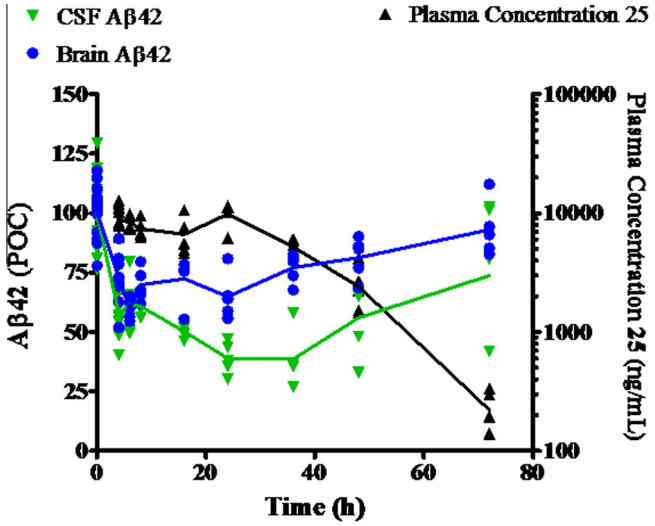


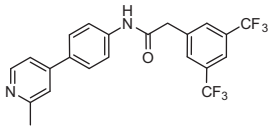
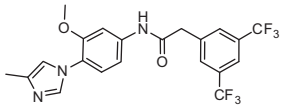
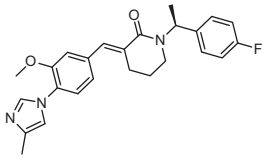
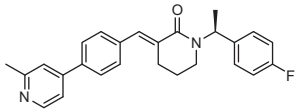
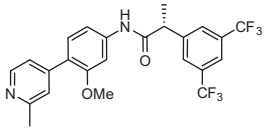
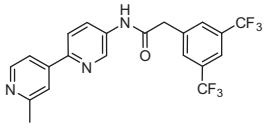
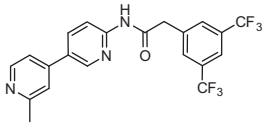
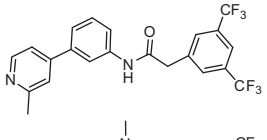
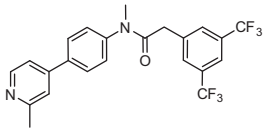
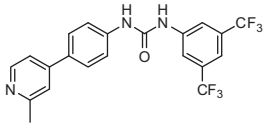
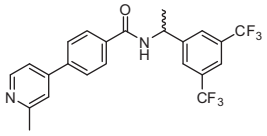
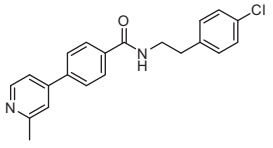
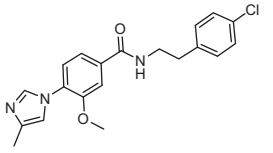
Figure 3. Time course of in vivo brain and CSF Aβ42 reduction with 25 in rats following a single, oral dose of 100 mg/kg and correlation with the plasma concentration of 25.

Having identified **25** as a novel GSM with moderate in vitro and in vivo effects on Aβ42 reduction we were interested to explore whether compounds of this type would cause Notch-related toxicities similar to those observed with earlier generations of GSIs. To assess general and Notch-related toxicity, the compound was dosed orally at 30, 100, and 300 mg/kg in rat for four days. For comparison, a known nonNotch selective GSI, LY411575 (our data Notch IC_{50}: 1.2 nM),[9] was also dosed to a separate group of animals in the same study at 1 and 3 mg/kg. In contrast to LY411575, no Notch-associated effects were identified for **25** (see Supplemental data section for more details).

Having established **25** as a viable lead for further optimization, we studied the SAR of the core modification of the molecule. For ease of synthesis we used its achiral analog **24** as the starting point for comparison (Table 3). In the aniline portion (left hand side), replacement of methylpyridine with commonly used imidazole group resulted in a threefold loss of potency (**27**). This trend was the same as that observed in the sulfonamide series. Replacement of the imidazole group in **3** by methylpyridine resulted in a tenfold drop in potency (**28**). These results clearly demonstrated the different SAR between the amide and E-2012 (**3**) series.

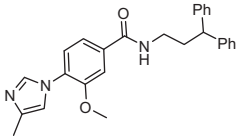
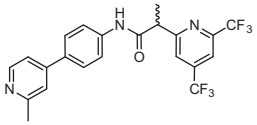
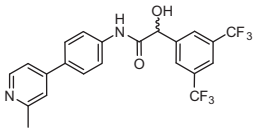
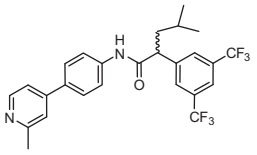
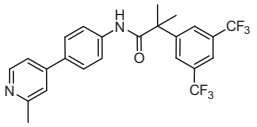
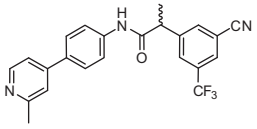
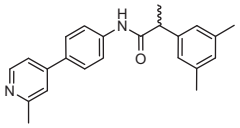
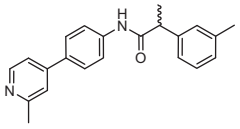
Similar to the sulfonamide SAR, introduction of the methoxy group in the aniline phenyl resulted in slight loss of potency (**29**). Changing the phenyl group to a pyridyl led to threefold loss of potency for one regio-isomer and complete loss of potency for the other (**30** and **31**). The *meta*-isomer of **24** (**32**) was completely inactive. Methylation of the amide NH abolished the GSM activity (**33**). Replacement of the amide $NHCOCH_2$ group by the urea NHCONH group decreased the potency by twofold (**34**). Replacement of the amide NHCO moiety in **25** by the reverse amide CONH resulted in loss of potency (**35**). A small library of 80 reverse amides with different amines was synthesized and the most potent compounds had IC_{50} values in the micro molar range (e.g., compound **36**). Due to the divergent SAR observed between methyl pyridine and imidazole series, we made 55 imidazole phenyl based reverse amides through parallel chemistry. In general, compounds in the series were more potent than the corresponding methyl pyridine analogs (compare **36** and **37**). Lipophilic groups were preferred for improved potency. One of the most potent compounds identified (**38**), had a cell IC_{50} of 0.32 μM but was metabolically unstable (RLM >399 μL/min/mg). At that time we decided not to pursue the series further and instead focused on methylpyridine

Table 3
SAR of compound **25** core modification

Compd	Structures	Aβ42 cell IC_{50}[a] (μM)
24		0.46 ± 0.11
27		1.45 ± 0.44
3		0.16 ± 0.06
28		1.53 ± 0.9
29		0.50 ± 0.15
30		1.35
31		>10
32		>10
33		>10
34		1.11 ± 0.46
35		>10
36		2.12
37		0.42

(*continued on next page*)

Table 3 (*continued*)

Compd	Structures	Aβ42 cell IC_{50}[a] (μM)
38		0.32
39		>10
40		2.13
41		0.82
42		>10
43		7.0
44		0.57 ± 0.07
45		0.54 ± 0.35

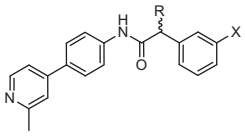
[a] Values with standard deviations shown were the average of at least three independent runs.

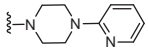
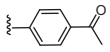
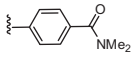
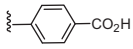
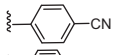
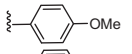
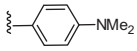
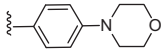
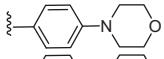
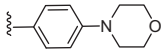
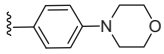
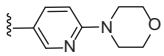
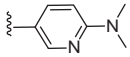
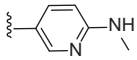
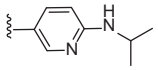
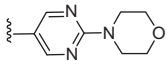
based compounds. Independently a group from Pfizer recently reported similar imidazole-based amides as GSMs.[16]

Subtle modification of the carboxylic acid (right hand side) portion of **25** had dramatic effects on the cell potency. For example, the aza modification of the phenyl group resulted in complete loss of potency (**39**). Replacement of the α-methyl group by an α-hydroxy group decreased the potency by eightfold (**40**). The α-isobutyl group was threefold less active (**41**). The α-dimethyl group completely abolished the cell activity. Replacement of one of the trifluoromethyl groups by cyano group resulted in 27-fold loss of potency (**43**). The replacement of both trifluoromethyl groups by one or two methyl groups only had minor effect on the potency (**44** and **45**). Compound **45** was subsequently used as a lead for further optimization of the carboxylic acid (right hand side) portion (Table 4).

We first investigated the effect of the α-substitution. Only the isobutyl group provided threefold improvement in potency (**50**). Chiral separation of the racemate showed one enantiomer was about 10-fold more potent than the other. Introduction of polar hydroxyl group into the α-alkyl chain abolished the cellular potency (**53**).

Table 4
SAR of the carboxamides



Compd	R	X	Aβ42 cell IC_{50}[a] (μM)
45	Me	Me	0.54 ± 0.35
46	H	Me	2.78 ± 0.29
47	Et	Me	1.05
48	nPr	Me	0.54 ± 0.25
49	nBu	Me	0.49 ± 0.08
50	$_i$Bu	Me	0.17 ± 0.01
51	$_i$Bu single isomer 1	Me	0.18 ± 0.08
52	$_i$Bu single isomer 2	Me	1.38
53	$HO(CH_2)_3$	Me	>10
54	Me	H	6.0
55	Me	1-Morpholine	4.80
56	Me	N-Me-piperazine	>10
57	Me		0.16 ± 0.05
58	Me		0.66
59	Me		0.14 ± 0.03
60	Me		0.26 ± 0.08
61	Me		>10
62	Me		0.25 ± 0.01
63	Me		0.22 ± 0.03
64	Me		0.57
65	Me		0.15 ± 0.05
66	Me		0.12 ± 0.02
67	Me		0.070 ± 0.014
68	(R) Me[b]		0.031 ± 0.008
69	(S) Me[b]		0.070 ± 0.022
70	(R) $_i$Bu[b]		0.020 ± 0.006
71	Me	2-Pyr	>10
72	Me	3-Pyr	1.29 ± 0.09
73	Me	4-Pyr	1.20 ± 0.09
74	Me		0.15 ± 0.10
75	Me		0.17 ± 0.12
76	Me		0.93
77	Me		0.18 ± 0.08
78	Me		0.65 ± 0.24

[a] Values with standard deviations shown were the average of at least three independent runs.
[b] Stereochemistry was randomly assigned for peaks separated by chiral SFC, see Supplemental material for details.

The effect of replacing the methyl group on the phenyl ring was also investigated. Removal of the methyl group resulted in

about tenfold drop in potency (**54**). Replacement with polar groups such as morpholino and *N*-methylpiperazinyl led to over tenfold loss in potency (**55** and **56**). However, *N*-(2-pyridyl) piperazinyl gave a threefold increase in potency (**57**). A variety of substituted phenyls were good replacements of the methyl group. Polar functional groups such as acetyl, cyano, carboxamide, methoxy and substituted amino groups on the terminal phenyl rings were not only tolerated but actually provided potency enhancement (**58**–**67**). The most potent compound identified was the 4-morpholinophenyl analog **68** with IC_{50} of 31 nM. As expected, replacement of the α-methyl by α-isobutyl improved the potency slightly (**70**, IC_{50} = 20 nM).

To reduce the lipohilicity of these more potent GSMs we studied the effect of replacing the terminal phenyl group with a pyridyl. Both 3- and 4-pyridyl analogs were only twofold less potent but the 2-pyridyl was inactive (**71**–**73**). Unfortunately the 3-pyridine analog of **68** was fivefold less potent. The morpholino group could be replaced by the dimethyl amino group (**75**). The monomethylamino group was fivefold less potent than dimethyl amino group (**76**). The potency was restored with the more lipophilic *N*-isopropyl amino group (**77**). Finally the pyrimidine analog of **68** (i.e. **78**) was twenty-fold less potent than **68**. These data were consistent with our findings and literature reports that GSMs tended to be more lipophilic.

Through the modification of the carboxylic acid portion we were able to improve the cell potency of the original lead **25** by over tenfold. We tested some of these more potent GSMs (Table 4) in the in vivo PD model. Unfortunately all showed <30% inhibition of CSF Aβ42 at 100 mg/kg when dosed orally. For example, **70** produced 27% Aβ42 reduction four hours after a 100 mg/kg oral dose. The unbound plasma concentration (290 nM) was significantly higher than the measured CSF concentration (2 nM) (the brain concentration was not measured in the study). The large shift may have been caused by the high lipophilicity (*c*Log*P* 6.86 calculated using ChemDraw) and poor passive permeability (LLC-PK Avg Papp <1 μcm/s).

A major difficulty encountered in our lead optimization of the methylpyridine based GSMs was the so-called 'flat' or 'shallow' SAR, a phenomenon widely described in the literature of allosteric modulators of GPCRs.[27–29] This is characterized by complete loss of activity with minor structural changes and/or very shallow and narrow SAR patterns. Another observation was the presence of 'nonadditive SAR' whereby the substituent effect in one series or molecule was not translatable into another, similar to the observation reported for allosteric modulators of GPCRs. Although we have not carried out any mechanistic studies of our GSMs, several reports suggested an allosteric mechanism of GSM action on γ-secretase.[30–32] This mechanism could also explain the SAR observation mentioned above including the presence of partial enzyme inhibition for certain amides such as **22** and **23** (similar to partial antagonists observed in allosteric modulators of GPCRs).[29]

In summary, starting from an initial oxazole based HTS lead **1** we have identified 2-methylpyridine as a good replacement for oxazole with improved PKDM properties. To improve CNS exposure, the sulfonamide group was replaced by the corresponding carboxamide functionality. Further homologation of the amide group resulted in the lead **25** with significant improvement in the in vitro potency, which could be rationalized by the observed alignment of low energy conformations of the homologated amide to the sulfonamide, unlike the benzamide analog. This lead optimization effort therefore led to a successful replacement of a sulfonamide moiety to improve CNS penetration and in vivo potency. In a rat PD model, **25** demonstrated statistically significant lowering of Aβ42 when administrated orally at 100 mg/kg. Further optimization of the carboxamide portion in **25** led to the identification of morpholinophenyl containing compounds, such as **70**, with a tenfold in vitro potency improvement. These compounds lacked the in vivo PD efficacy achieved by **25**, likely a result of poor permeability and high *c*Log*P*. We also showed the SAR trend of methylpyridine and the widely used methyl imidazole based compounds was different in several cores.

Supplementary data

Supplementary data (synthesis of representative examples, procedures for chiral separation and stereochemistry confirmation, in vitro and in vivo assays, and rat four-day Notch toxicity study) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.09.041.

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