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Discovery of 4-aminomethylphenylacetic acids as γ-secretase modulators via a scaffold design approach

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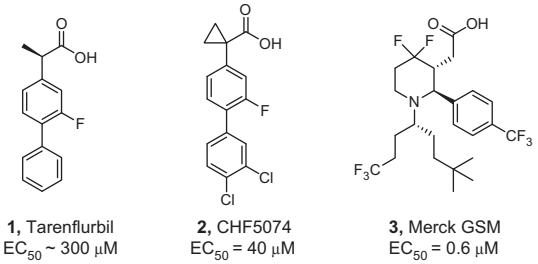
ABSTRACT

Starting from literature examples of nonsteroidal anti-inflammatory drugs (NSAIDs)-type carboxylic acid γ-secretase modulators (GSMs) and using a scaffold design approach, we identified 4-aminomethylphenylacetic acid **4** with a desirable γ-secretase modulation profile. Scaffold optimization led to the discovery of a novel chemical series, represented by **6b**, having improved brain penetration. Further SAR studies provided analog **6q** that exhibited a good pharmacological profile. Oral administration of **6q** significantly reduced brain Aβ42 levels in mice and rats.

Alzheimer's disease (AD) is a neurodegenerative disorder associated with memory loss and behavioral changes. AD affects more than 26 million people worldwide, and it is estimated that this number will reach 106 million by 2050.[1] Unfortunately, there are no disease modifying therapies currently available. It was proposed that the accumulation of amyloid γ-peptides (Aβ) in the brain drives AD pathogenesis.[2] Aβ is produced by the sequential cleavage of amyloid precursor protein (APP) by two aspartic proteases known as β- and γ-secretase. Over the last decade, γ-secretase emerged as a promising target for the treatment of AD. However, γ-secretase is known to process several substrates besides APP (e.g., Notch), and inhibiting its activity was reported to be associated with undesirable gastrointestinal toxicities.[3] γ-Secretase modulation is more desirable than inhibition from a therapeutic perspective and may reduce the risk of mechanism-based toxicities.

In cultured cells, certain nonsteroidal anti-inflammatory drugs (NSAIDs) preferentially decrease the levels of Aβ42, a highly amyloidogenic peptide, and increase the levels of Aβ38, a shorter and less neurotoxic peptide.[4] Compounds showing this Aβ modulation effect in vivo include ibuprofen[5] and flurbiprofen.[6] R-flurbiprofen (**1**, Tarenflurbil, Fig. 1) was advanced into phase III clinical trials, but failed to show cognitive improvement in AD patients.[7] This compound has poor in vitro potency (Aβ42 EC_{50} ~300 μM) and low brain penetration. Therefore, a γ-secretase modulator (GSM) with improved potency and brain permeability is desirable for further evaluation of this class of GSMs as a potential treatment for AD. Major efforts have been made by scientists at Chiesi to improve the potency based on flurbiprofen.[8] Recently CHF5074 (**2**, EC_{50} = 40 μM, Fig. 1) was advanced into phase II clinical trials.[9] Piperidinyl acetic acids, as exemplified by compound **3** (Aβ42 EC_{50} = 0.60 μM, Fig. 1)[10] and similar derivatives[11] have been reported to have sub-micromolar activity for Aβ42 reduction and



1, Tarenflurbil EC_{50} ~ 300 μM
2, CHF5074 EC_{50} = 40 μM
3, Merck GSM EC_{50} = 0.6 μM

Figure 1. NSAID-type γ-secretase modulators.

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promising in vivo pharmacological profiles. Recent work in this field has been reviewed extensively.[12,13] We have disclosed 4-aminomethyl acetic acid-based GSMs in recent publications.[14,15] Herein, we describe the discovery of a novel series of GSMs, represented by **6q**, via a scaffold design and optimization approach.

Phenylacetic acid **4** (Aβ42 EC_{50} = 1.5 μM in APP CHO cells, Fig. 2) was the first compound in the series showing a modulatory profile. This compound decreased the Aβ42 level, increased Aβ38 secretion, and had no effect on Aβ40 in cultured cells. Introduction of a benzylic nitrogen likely reduces the lipophilicity of the molecule, and provides the flexibility for the associated substitutions to adopt favorable hydrophobic interactions. Benzoic acid analog **4a** was three- to fourfold less potent (EC_{50} = 5.4 μM), suggesting that the extended acid functional group is preferred. Compound **4** was subjected to a pharmacodynamic (PD) study in wild-type mice (male CF-1 mice, 50 mg/kg, po), where the percent of Aβ42 level reduction versus a vehicle control was evaluated 4 h post-dose. Unfortunately, this compound showed no significant reduction in brain Aβ42 levels. The concentration of compound **4** was found to be low (0.6 μM) in brain samples, even though the plasma exposure was significant (15 μM).

It was observed that replacement of the aniline by a cyclohexylamine resulted in analog **5a** with an improved brain penetration (brain–plasma ratio ~1:3) and cellular potency (EC_{50} = 0.67 μM). However, introduction of a less lipophilic side chain, such as a trifluoromethylpyridyl group (**5b**, EC_{50} = 5.0 μM), significantly reduced activity. A single dose of compound **5a** in mice (50 mg/kg, po, 4 h) selectively reduced the brain Aβ42 levels by 24%. The brain exposure level was ~7 μM, approximately 10 times higher than the in vitro EC_{50} value. To further increase Aβ42 potency, introduction of additional substituents at the α-position of the phenylacetic acid were explored. This work culminated in compound **5c** (racemic, EC_{50} = 0.12 μM), which was about fivefold more potent than **5a**. However, this gain in in vitro potency was offset by a loss of brain penetration. No Aβ42 reduction was observed when compound **5c** was dosed at 50 mg/kg in mice, the brain exposure level was only 0.7 μM.

Physicochemical properties such as molecular weight (MW) and rigidity are known to greatly affect BBB penetration. Compounds with higher MW are generally less brain permeable. The high MW (573) and increased conformational flexibility of compound **5c** may have contributed to its decreased brain exposure. Unfortunately, analogs with a reduced MW, such as **5d** (MW = 467, EC_{50} = 5.6 μM), were found to be less active. To improve brain penetration while maintaining potency, we decided to increase the molecular rigidity by reducing rotatable bond count, and explore SAR based on unsubstituted acetic acid **5a** with a relatively lower MW. An initial attempt was to keep the basic nitrogen at the benzylic position and relocate the isopentyl side chain of **5a** to the α-position of the benzylamine. This modification provided **6a** with a similar Aβ42 EC_{50} value (0.45 μM) to **5a**, suggesting that substitution is well tolerated at this position. The subsequent replacement of the cyclohexylamine by a cyclic tertiary amine, such as piperidine, led to a structurally more rigid analog **6b** with equipotent cellular activity. Compound **6b** demonstrated enhanced brain penetration and PD response in mice. It achieved about 35% brain Aβ42 reduction (50 mg/kg, po) at the 4 h time point. The brain and plasma exposure levels were 17 and 32 μM, respectively. Compound **6b** exhibited a favorable pharmacokinetic (PK) profile in mice characterized by its excellent oral bioavailability (F = 100%), low clearance (CL = 3.2 ml/min/kg) and a half-life of 2.9 h.

Based on the encouraging results associated with compound **6b**, replacement of the 4-trifluoromethylpiperidine moiety to further reduce the molecular weight while improving or maintaining cellular potency was next examined. A number of piperidines and pyrrolidines were evaluated as these cyclic amines feature less conformational mobility. The presence of an electron-withdrawing substituent on the piperidine ring seemed to be required for potency. As shown in Table 1, the EC_{50} values steadily increased from

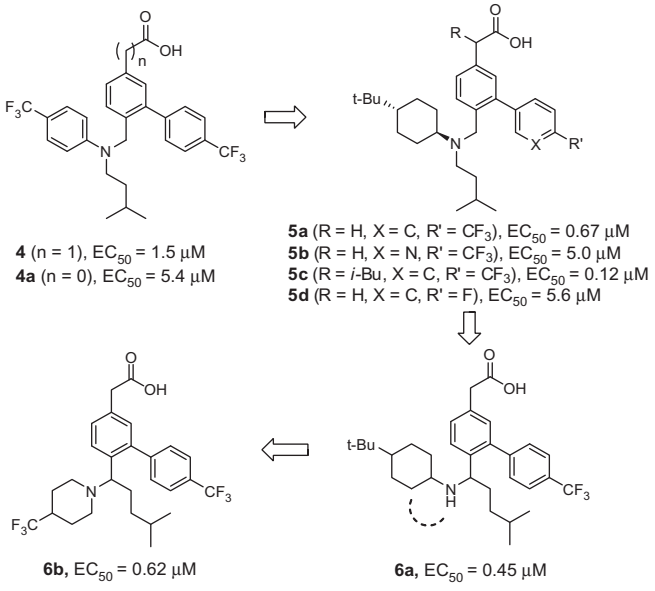
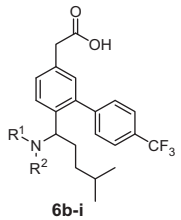
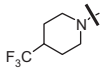
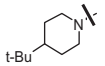


Figure 2. Design and evolution of a phenylacetic acid scaffold as a γ-secretase modulator.

Table 1
In vitro activity of GSM **6b–i** against Aβ42



6b-i

Compd	NR^1R^2	Aβ42 EC_{50} (μM)[a]
6b		0.62
6c		0.52
6d		1.1
6e		10
6f		3.3
6g		3.3
6h		5.9
6i		>10

[a] EC_{50} values were calculated as an average of at least two determinations.

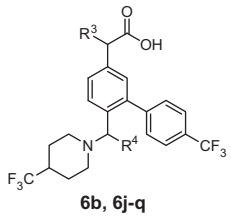
CF_3 (**6b**, EC_{50} = 0.62 μM) to F (**6f**, EC_{50} = 3.3 μM) to electron-donating CH_3 group (**6e**, EC_{50} = 10 μM). On the other hand, increasing lipophilicity was found to be beneficial. Compared to the methyl analog **6e**, compound **6d** (EC_{50} = 1.1 μM), carrying a bulkier *t*-butyl group, displayed a ninefold decrease in its EC_{50} value. The pyrrolidine derivatives were found to be less potent in the cell-based assay (**6h**, EC_{50} = 5.9 μM; **6i**, EC_{50} >10 μM). Among the different substituents explored, trifluoromethyl substituted piperidines exhibited the highest potency. Compound **6c** (EC_{50} = 0.52 μM), a regioisomer of **6b**, was tested as a mixture of four diastereomers and showed a similar cellular potency.

Based on review of the data, it was decided to explore the SAR of 4-trifluoromethylpiperidine analog **6b**, which contains only one stereogenic center, and to investigate the substitution at R3 position for a potential potency gain. As shown in Table 2, compound **6j** (EC_{50} = 0.34 μM), an α-methyl analog of **6b**, was slightly more potent relative to the parent **6b** (EC_{50} = 0.62 μM). The gem-dimethyl derivative (**6k**, EC_{50} = 0.46 μM) offered no significant advantage in cellular potency, whereas the α-isobutyl substitution (**6l**, EC_{50} = 0.21 μM) enhanced activity by threefold. However, brain concentrations tended to decrease with bulkier substituents. Similar to an earlier observation, substituted isobutyl analog **6l** displayed a lower drug concentration in the brain (4 μM) compared to the unsubstituted analog **6b** (17 μM), or methyl-substituted analog **6j** (13 μM). These results correlated well with a decreased PD response of **6l** in the brain. We next focused our efforts on the modification of the isopentyl side chain. Truncation of the isopentyl group resulted in the complete loss of potency (**6m**, EC_{50} >10 μM). Installation of a CF_3-substituted alkyl side chain rendered **6n**, a low micromolar GSM (EC_{50} = 1.4 μM; twofold less potent versus **6b**). Compounds with a bulkier R4 group such as **6o** (EC_{50} = 0.42 μM), **6p** (EC_{50} = 0.54 μM) and **6q** (EC_{50} = 0.63 μM) were well tolerated in the cellular assay. The brain Aβ42 reduction of **6o** and **6p** were weak to moderate (15% and 30%, respectively, 4 h, 30 mg/kg) when dosed orally in PD studies. However, **6q** reduced brain Aβ42 levels by 50% (30 mg/kg, po, 4 h). Considering its moderate in vitro potency, it was not surprising that the brain exposure levels of **6q** in mice were very high (32 μM) with a brain to plasma ratio of ~1. Compound **6q** also showed a dose-dependent lowering of brain Aβ42 (12.5, 25, and 50 mg/kg). At 12.5 mg/kg oral dose, **6q** reduced Aβ42 levels by 25% with a brain exposure level of 9 μM.

In addition to its high brain penetration and good PD/PK correlation in mice, compound **6q** demonstrated favorable ADME properties. It showed good liver microsomal stability (RLM Qh = 31%, HLM Qh = 39%) and good Caco-2 permeability [$P_{app}(A-B) = 16 \times 10^{-6}$ cm/s, *P* ratio ($B-A/A-B$) = 1.9]. Table 3 illustrates the pharmacokinetic parameters of **6q** in rats and dogs. The clearance of **6q** was low in both species (rat = 2.5 ml/min/kg; dog = 0.6 ml/min/kg). Compound **6q** showed no significant hERG activity at 10 μM nor did it inhibit any of the five major human CYP isoforms assayed (3A4, 2C9, 2C19, 2D6, and 1A2; IC_{50} >10 μM).

Chiral chromatographic separation of compound **6q** was performed to obtain two enantiopure isomers, both of which displayed very similar cellular potency (IC_{50} ~0.7 μM) and PD response in rats (25% [enantiomer 1] and 30% [enantiomer 2] Aβ42 reductions in the brain at 30 mg/kg). The isolated enantiomer 2 (absolute configuration not assigned) that achieved a slightly higher brain exposure level (26 μM) and brain Aβ42 reduction

Table 2
SAR summary of GSM **6b**, **6j–q**



6b, 6j-q

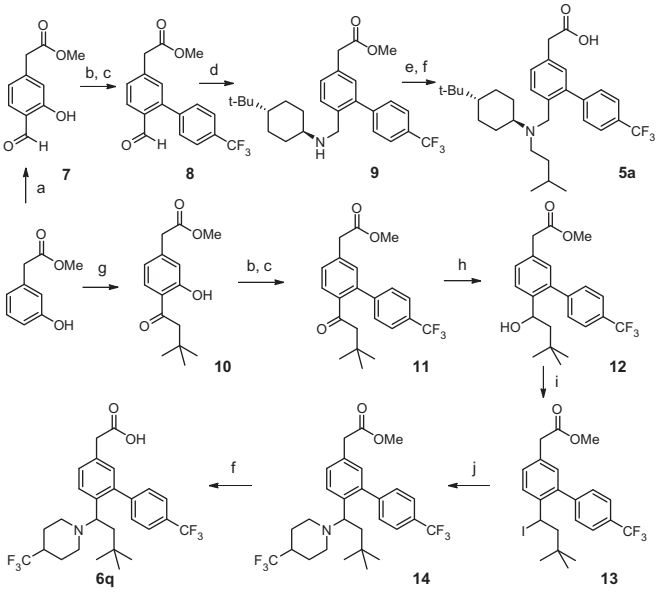
	R^3	R^4	$A\beta_{42}$ EC_{50} (μM)[a]	Brain $A\beta_{42}$ reduction[b]	B/P [c] (μM/μM)
6b	H		0.62	35% (50 mpk)	17/32
6j	Me		0.34	35% (30 mpk)	15/22
6k	Me, Me		0.46	20% (30 mpk)	13/21
6l	*i*-Bu		0.21	21% (30 mpk)	4/14
6m	H	H	>10	ND[d]	ND[d]
6n	H		1.4	ND[d]	ND[d]
6o	H		0.42	15% (30 mpk)	10/23
6p	H		0.54	30% (30 mpk)	18/25
6q	H		0.63	50% (30 mpk)	32/37

[a] EC_{50} values were calculated as an average of at least two determinations.
[b] PD response were measure at 4 h after oral administration of compounds at 30 mg/kg in a solution formulation (ethanol/propylene glycol/10% solutol = 1:1:8).
[c] B/P, brain/plasma concentration.
[d] ND, not determined.

Table 3
Single-dose PK parameters of **6q**

Species	Dose (mg/kg)	AUC/dose ng ∗ h ∗ kg/(ml ∗ mg)	C_{max} (ng/ml)	CL (ml/min/kg)	$T_{1/2}$ (h)
Rat (n = 2)	2	5495	—	2.5	9.6
Dog[a] (n = 2)	0.5	28712	1125	0.6	10.1

[a] From enantiomer 2.



Scheme 1. Conditions and reagents: (a) $MgCl_2$, Et_3N, paraformaldehyde, MeCN, reflux, 9%; (b) Tf_2O, pyridine, CH_2Cl_2, 78–97%; (c) 4-CF_3-phenylboronic acid, $(Ph_3P)_4Pd$, 77–90%; (d) 4-*t*-butylcyclohexylamine, $NaBH(OAc)_3$, 45%; (e) isovaleraldehyde, $NaBH(OAc)_3$, 100%; (f) NaOH, 80–100%; (g) 3,3-dimethylbutanoic acid, EDCI, Et_3N, CH_2Cl_2; $BF_3{\cdot}Et_2O$, 25%; (h) BH_3 in THF, 0 °C, 40%; (i) NaI, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (j) 4-CF_3-piperidine hydrochloride, Et_3N, CH_2Cl_2, 40% for two steps.

(30%) was evaluated for Notch activity by immunoblot analysis of endogenous HES1, a Notch intracellular domain (NICD) regulated transcription product. This enantiomer of **6q** showed no inhibitory activity at 10 μM.

The syntheses of **5a** and **6q** are outlined in Scheme 1. Formylation of methyl 2-(3-hydroxyphenyl)acetate with paraformaldehyde in the presence of magnesium chloride provided a low yield of salicylaldehyde **7**. The phenol **7** was transformed to a triflate intermediate, which was converted to **8** via a Suzuki coupling. Reductive amination with an excess of *cis*/*trans*-4-*tert*-butylcyclohexanamine generated amine **9** as a pure *trans*-isomer. A second reductive amination with isovaleraldehyde was followed by hydrolysis with NaOH to provide compound **5a** in excellent yield.

Synthesis of compound **6q** was accomplished via similar procedures starting with acylation of 2-(3-hydroxy-phenyl)acetate to yield the arylketone **10**. Triflation and a Suzuki coupling afforded key intermediate **11**. A three step sequence that involved reduction to **12**, iodination to **13** and displacement to install the amine moiety of **14** was performed. The direct reductive amination of **11** did not proceed well. Treatment of ester **14** with NaOH proceeded smoothly to afford the corresponding acid **6q**.

In summary, we have discovered a novel series of 4-aminophenylacetic acids as γ-secretase modulators through a scaffold design approach. This has culminated in compound **6q**, which has desirable pharmacokinetic properties in mice, rats and dogs. This compound also exhibits good brain penetration and a robust PD response with selective lowering of brain Aβ42 levels in mice and rats. Furthermore, compound **6q** was shown to be low risk in in vitro hERG and CYP inhibition assessments, and is thus suitable for further evaluation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.047.

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