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Fluorinated piperidine acetic acids as γ-secretase modulators

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ABSTRACT

We report herein a novel series of difluoropiperidine acetic acids as modulators of γ-secretase. Synthesis of 2-aryl-3,3-difluoropiperidine analogs was facilitated by a unique and selective β-difluorination with Selectfluor®. Compounds **1f** and **2c** were selected for in vivo assessment and demonstrated selective lowering of Aβ42 in a genetically engineered mouse model of APP processing. Moreover, in a 7-day safety study, rats treated orally with compound **1f** (250 mg/kg per day, AUC_{0-24} = 2100 μM h) did not exhibit Notch-related effects.

Alzheimer's disease (AD) is a progressive, chronic neurodegenerative disorder characterized by impairments in memory and cognition.[1] In the United States, estimates suggest that an individual develops AD every 72 seconds. By 2050, this rate is projected to increase to one person developing the disease every 33 seconds[2] and the number of people worldwide with the disease is expected to triple by the year 2050, intensifying what is already a serious worldwide public health problem.[3]

Accumulation of amyloid-β (Aβ) peptides and formation of amyloid plaques is a central event in sporadic and familial AD pathology.[4] Proteolytic cleavage of amyloid precursor protein (APP) by two membrane-bound aspartyl proteases, β-secretase (BACE) and γ-secretase, leads to the formation of Aβ. Among the two major Aβ peptides produced by γ-secretase cleavage, Aβ40 and Aβ42, the less common Aβ42 fragment is thought to play the most important role in AD pathology.[4] Pharmacological intervention by inhibiting the function of these two enzymes with a small molecule, and thus reducing Aβ levels, has been and remains an attractive strategy for developing disease modifying AD treatment.

Although inhibiting γ-secretase is an attractive strategy for treating AD, it has led to undesirable adverse events in clinical trials, most notably gastrointestinal toxicity.[5] These side effects are associated with blocking the processing of Notch, a transmembrane receptor signaling protein which is also a γ-secretase substrate.[6] Modulation of γ-secretase was introduced as a strategy to avoid Notch-related toxicity when certain non-steroidal anti-inflammatory drugs were found to selectively inhibit the production of Aβ42 while not affecting the production of Aβ40 or Notch processing.[7,8]

Piperidine acetic acid γ-secretase modulators have been the subject of a recent patent application by our group.[9] In this Letter, we describe modulators within this series where fluorine is incorporated into the piperidine ring as a part of our continuing effort to identify compounds with desirable pharmacokinetic (PK) properties (Fig. 1).

The synthetic strategy employed to prepare 4,4-difluoropiperidine acetic acids with the general formula **1** is outlined in Scheme 1. Enone **4** was prepared by treating 4-methoxypyridine (**3**) with benzyl chloroformate and (*p*-trifluoromethylphenyl)magnesium bromide using the method previously reported by Comins et al.[10,11] Treatment of **4** with LiHMDS in THF followed by quenching with methyl bromoacetate and reduction with L-Selectride® afforded *trans*-ketopiperidine **5**. Subsequent treatment of ketopiperidine **5** with Deoxo-Fluor™ followed by hydrogenation afforded

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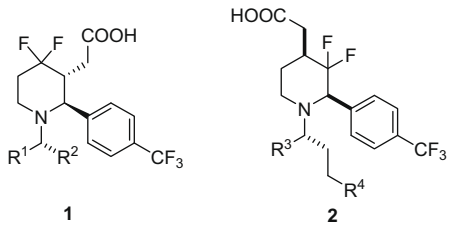
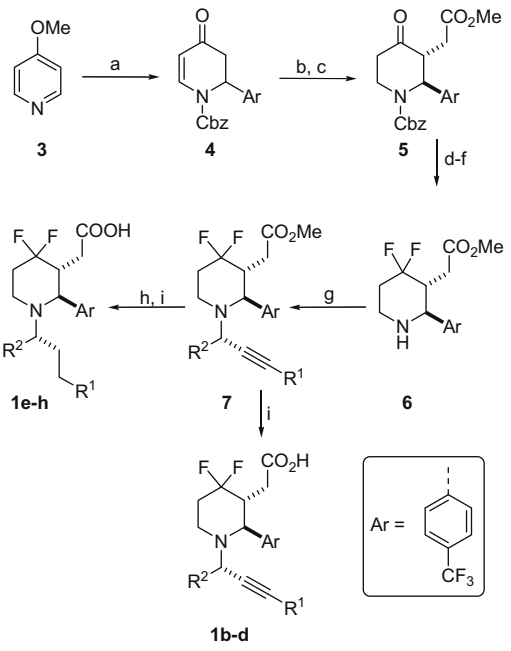


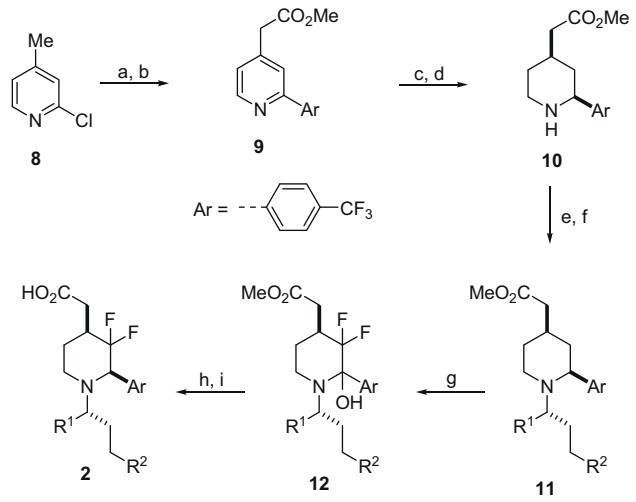
Figure 1. Novel class of difluoropiperidine acetic acids.



Scheme 1. Synthetic strategy for the preparation of 4,4-difluoropiperidine acetic acids (**1**). Reagents and conditions: (a) Cbz-Cl, (*p*-trifluoromethylphenyl)magnesium bromide, THF, −78 °C to 0 °C; (b) LiHMDS, methyl bromoacetate, THF, −78 °C; (c) L-Selectride®, THF, −78 °C; (d) Deoxo-Fluor™, DCM; (e) Pd/C, H_2, MeOH; (f) chiral resolution, HPLC (Chiracel®-AD 10% *i*-PrOH/heptane); (g) alkyne, aldehyde, $AuBr_3$, H_20, 75 °C, μW; (h) Raney-Nickel, MeOH, H_2, 60 psi; (i) 1 M KOH, MeOH.

the methyl 4,4-difluoropiperidineacetic acid methyl ester **6**, which was resolved using a Chiral pak® AD-H column on multi-gram scale. Enantiomerically pure amine **6** could be alkylated with methyl iodide and the ester hydrolyzed to give compound **1a** (not shown). For the preparation of more complex, *N*-branched carboxylic acids, the key step to afford the 4,4-difluoropiperidineacetic acids with the general structure **1** was a three-component gold(III) bromide catalyzed Mannich reaction with the piperidine **6**.[12] The acetylene analogs **7** were reduced with Raney-Nickel at 60 psi H_2 in methanol from 24 to 48 h to afford the fully saturated products. Methyl esters were treated with potassium hydroxide in methanol to afford the desired 4,4-difluoropiperidine acetic acids **1**.

The synthesis of the 3,3-difluoropiperidine acetic acids with the general structure **2** proceeded as outlined in Scheme 2. Pyridine **8** was coupled to 4-trifluoromethylbenzeneboronic acid under Suzuki reaction conditions[13] to afford the biaryl pyridine, which was subsequently acylated with dimethylcarbonate to afford methyl ester **9**. Ester **9** was reduced with Adam's catalyst in methanolic HCl to give the corresponding piperidine, which could then be resolved by recrystallization as its mandelate salt. Enantiomerically pure piperidine **10** was subjected to the three-component gold(III) bromide catalyzed Mannich reaction described above, followed by palladium-catalyzed hydrogenation to afford piperidine **11**. The key transformation in this synthetic sequence was difluorination



Scheme 2. Synthetic strategy for the preparation of 3,3-difluoropiperidine acetic acids (**2**). Reagents and conditions: (a) 4-trifluoromethylbenzeneboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O; (b) Me_2CO_3, LDA, THF, −78 °C to 0 °C; (c) H_2 (20 psi), HCl, Pt_2O, MeOH; (d) L-(+)-mandelic acid, *i*-PrOH; (e) $AuBr_3$ (5 mol %), R_1CHO, R_2CCH, H_2O, 75 °C; (f) H_2 (50 psi), Pd/C (10%), EtOH; (g) Selectfluor®, DMF then H_2O; (h) BH_3·THF, THF; (i) LiOH, H_2O, THF, MeOH.

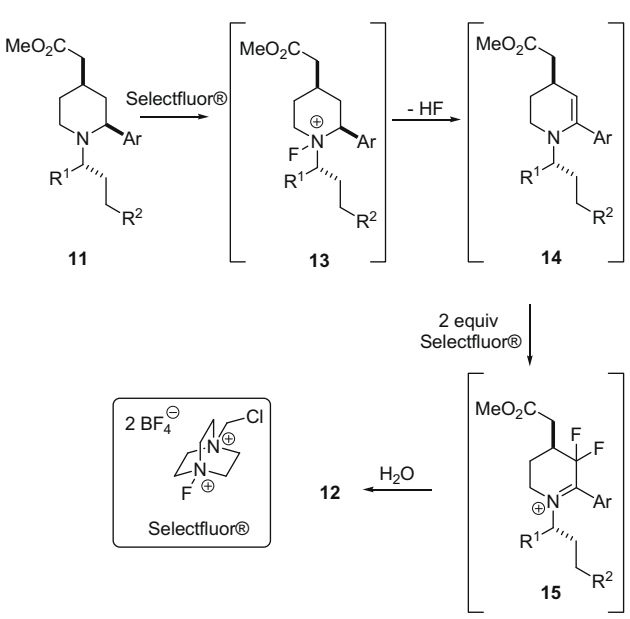


Figure 2. Putative piperidine fluorination mechanism.

of **11** with Selectfluor®, which provided the difluorohemiaminal **12**. The desired γ-secretase modulators of the general structure **2** were obtained by first reducing the hemiaminal to the amine with borane THF complex, followed by hydrolysis of the ester with lithium hydroxide.[14]

A possible mechanism for the difluorination reaction is proposed in Figure 2. Piperidine **11** is fluorinated on nitrogen to give ammonium intermediate **13**, which subsequently eliminates HF to give an iminium ion that can tautomerize to afford enamine **14**. This enamine is difluorinated by Selectfluor® to give iminium ion **15** that can be hydrated upon workup to provide hemiaminal **12**.

The required structural features for potency in these series is the presence of the carboxylic acid at either the 3 or 4 position, the aryl substituent at the 2 position, and functionalization of the piperidine nitrogen. The carboxylic acid moiety at the 3 or 4

position was ideally suited for incorporation of fluorine and led to an attenuation of the pK_a of the nitrogen. The initial SAR utilized racemic material, however, select compounds were further evaluated using the enantiomerically pure amine **6**. The SAR is summarized in Table 1.

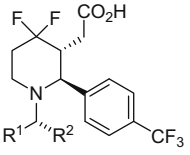
Increasing alkyl substitution beyond the methyl group of **1a** led to an enhancement in potency against Aβ42. The preferred stereochemical requirements of the 4,4-difluoropiperidine acetic acid scaffold was determined based on the results of **1b** and the subsequent enantiomerically pure isomers **1c** and **1d,** indicating that the activity resided in the enantiomerically pure **1d**. Reduction of the alkyne led to compound **1f**. Compound **1f** exhibited an IC_{50} against Notch activity of greater than 10,000 nM (data not shown).[16]

The optimal *N*-alkyl substituents identified in Table 1 were incorporated into the examples of the 3,3-difluoropiperidineacetic acids of structure **2**. The results are summarized in Table 2. The data indicate that 3,3-difluoro- and 4,4-difluoropiperidine analogs have similar activity against γ-secretase.

Compounds **1f** and **2c** exhibited favorable rodent PK (Table 3) and were tested in APP-YAC transgenic mice and non-transgenic rats.

Initial efficacy screening in mice (10 mg/kg po, 7 h, Table 4) demonstrated that **1f** and **2c** led to a selective inhibition of brain Aβ42 levels relative to Aβ40 (Table 4). Exposure levels of **1f** in mouse brain/plasma were 0.58/1.14 μM.

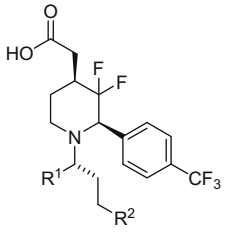
Table 1
In vitro activity of 4,4-difluoropiperidine acetic acids (**1**) against Aβ42 and Aβ40



Compound	R^1	R^2	Aβ42 IC_{50}[15] (nM)	Aβ40 IC_{50}[145] (nM)
1a[c]	H	H	>10,000	>10,000
1b[a]	$CF_3CH_2CH_2$	*t*-BuCC	800	>10,000
1c[b]	$CF_3CH_2CH_2$	*t*-BuCC	1500	>10,000
1d[c]	$CF_3CH_2CH_2$	*t*-BuCC	390	>10,000
1e[b]	$CF_3CH_2CH_2$	*t*-BuCH_2CH_2	1400	>10,000
1f[c]	$CF_3CH_2CH_2$	*t*-BuCH_2CH_2	600	>10,000
1g[c]	$CF_3CH_2CH_2$	$Me_3SiCH_2CH_2$	640	>10,000
1h[c]	*i*-PrCH_2CH_2	*i*-PrCH_2CH_2	880	>10,000

[a] Racemic.
[b] From piperidine enantiomer 1.
[c] From piperidine enantiomer 2.

Table 2
In vitro activity of 3,3-difluoropiperidine acetic acids (**2**) against Aβ42 and Aβ40



Compound	R^1	R^2	Aβ42 IC_{50}[15] (nM)	Aβ40 IC_{50}[15] (nM)
2a	4-CF_3Ph	*i*-Pr	710	>10,000
2b	$CF_3CH_2CH_2$	*t*-Bu	490	>10,000
2c	$CF_3CH_2CH_2$	$Si(CH_3)_3$	230	>10,000

Table 3
Rodent PK for **1f** and **2c**

	1f	**2c**
Dose (iv; po) (mg/kg)	1; 2	1; 2
Cl_p (mL/min/kg)	3.8	9.1
Vd_{ss} (L/Kg)	1.9	5.1
$t_{1/2}$ (iv) (h)	5.1	6.4
%F	160	20
$AUCN_{0-24}$ po (μM h kg/mg)	13	1.2

Table 4
Relative levels of Aβ42 and Aβ40 inhibition in APP-YAC transgenic mouse model for **1f** and **2c**

	Aβ42 (%)	Aβ40	Brain (μM)	Plasma (μM)
1f	−84	N.S.	0.58	1.1
2c	−64	N.S.	N.A.	N.A.

N.S. = not significant.

Further evaluation of **1f** in rats (1, 3, 10, and 30 mg/kg po, 7 h) demonstrated a dose-dependent lowering of Aβ42 (ED_{50} = 5 mg/kg, brain EC_{50} = 1 μM, and plasma EC_{50} = 3.7 μM). Maximum inhibition of brain Aβ42 levels after treatment with **1f** was observed at 7 h (data not shown).

In summary, a novel series difluoropiperidine acetic acids were discovered that showed moderate potency against the γ-secretase complex in vitro and demonstrated robust PK/PD activity in established rodent models. The selective lowering of Aβ42 without effecting Aβ40 or Notch activity is consistent with the mechanism of γ-secretase modulation. Moreover, in a 7-day oral rat safety study with **1f** (250 mg/kg per day, dosing 7 days, five animals, females, AUC_{0-24} = 2100 μM h) no adverse Notch effects were observed. These results further validate that modulation may prove to be a more formidable approach to targeting γ-secretase with a small molecule in order to circumvent the undesired affects associated with Notch.

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