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LETTER

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Discovery of a Potent Pyrazolopyridine Series of γ-Secretase Modulators

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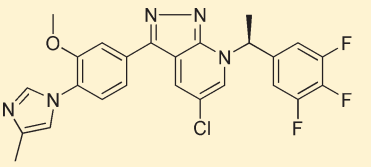
Supporting Information

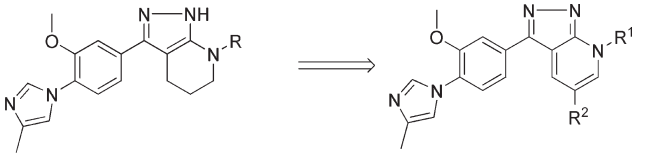
ABSTRACT: The synthesis and structure−activity relationship of a novel series of pyrazolopyridines are reported. These compounds represent a new class of γ-secretase modulators that demonstrate good in vitro potency in inhibiting Aβ_{42} production. Examples with statistically significant in vivo efficacy in reducing the production of rat cerebrospinal fluid Aβ_{42} were also identified.

KEYWORDS: Alzheimer's disease, γ-secretase modulator, amyloid-β peptide, Notch



Despite enormous efforts to develop cures for Alzheimer's disease (AD), there is no solution to this medical problem yet.[1−3] AD is characterized by neuronal loss, neurofibrillary tangle (NFT) formation, and extracellular deposition of amyloid-β (Aβ) peptide plaques. Patients normally show symptoms of cognitive impairment, as well as disturbance in language, memory, movement, attention, and orientation. The Aβ peptides have been hypothesized to be the pathological cause of this disease. The Aβ peptides are generated from sequential cleavage of the amyloid precursor protein (APP) by two key proteases, β-secretase 1 (BACE1) and γ-secretase.[4,5] γ-Secretase cleavage is heterogeneous, resulting in Aβ peptides that range from 37 to 42 amino acids in length. Aβ_{42} is more hydrophobic than other shorter Aβ peptides and is found in disproportionately high amounts in Aβ plaques. Development of γ-secretase inhibitors (GSIs) holds promise for the treatment of AD. Several classes of GSIs have been reported to demonstrate efficacy in reducing overall production of Aβ peptides in plasma, cerebrospinal fluid (CSF), and brain.[6−8] However, recent preclinical experiments demonstrated that inhibition of γ-secretase results in mechanism-based GI toxicity such as thymus atrophy and intestinal goblet cell hyperplasia because γ-secretase has other substrates in addition to APP such as Notch to process, and cleavage of Notch by γ-secretase is important for cellular gene transcription.[9,10] The recent clinical results of GSI candidate semagacestat showed that it not only failed to slow disease progression but also increased the incidence of skin cancer of patients in the treatment group than the placebo group.[11,12] The failure of semagacestat again raised concerns about the efficacy and safety of GSIs. γ-Secretase modulators (GSMs) selectively reduce the production of Aβ_{42} while maintaining other normal functions of γ-secretase, including Notch processing and signaling. 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 Aβ_{38} instead of the toxic Aβ_{42}.[13−18] As a result, GSMs should not cause Notch-related side effects and could potentially provide a better safety profile than GSIs. Different classes of GSMs have been reported to demonstrate in vivo efficacy, including nonsteroidal anti-inflammatory drugs (NSAIDs) with carboxylic acid moiety,[19−24] compounds with noncarboxylic acid scaffold,[25−27] and Eisai compound E2012 currently under clinical investigation, containing 1-(2-methoxyphenyl)-4-methyl-1*H*-imidazole substructure, which is essential part of Eisai compound to maintain the GSM activity.[28−31]

Scheme 1. Design of Pyrazolopyridine Series as γ-Secretase Modulators



We recently reported the discovery of a tetrahydro-pyrazolopyridine series of γ-secretase modulators.[32] An example from this series demonstrated good in vitro potency but only moderate in vivo efficacy in reducing rat CSF Aβ_{42}. This molecule exhibited

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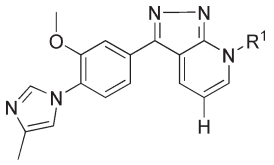
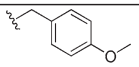
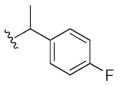
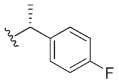
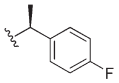
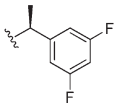
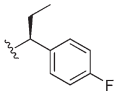
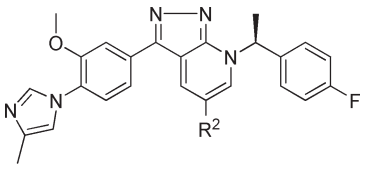
Table 1. Cell Aβ_{42} IC_{50}, γ-Secretase Modulator Selectivity, in Vivo Efficacy, and PK Profile of Compounds 1−6

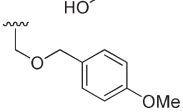


Compound	R^1	$A\beta_{42}$ IC_{50}[a] (nM)	$A\beta_{total}$ IC_{50} /$A\beta_{42}$ IC_{50}[b]	Pl. C_{3h} (μM)	Br. C_{3h} (μM)	CSF $A\beta_{42}$ (-%)
1		168	119			
2		231	87			
3		332	60			
4		97	205	2.88	0.17	n.s.e.[c]
5		117	94	1.96	0.14	n.s.e.[c]
6		140	42			

[a] IC_{50} values are reported as an average of multiple determinations ($n \geq 2$). [b] The ratio represents a measure of GSM selectivity and the ability to reduce the Aβ_{42} without affecting the total Aβ production. [c] n.s.e., no statistically significant efficacy.

Table 2. Cell Aβ_{42} IC_{50}, γ-Secretase Modulator Selectivity, in Vivo Efficacy, and PK Profile of Compounds 7−14



Compound	R^2	$A\beta_{42}$ IC_{50}[a] (nM)	$A\beta_{total}$ IC_{50} /$A\beta_{42}$ IC_{50}[b]	Pl. C_{3h} (μM)	Br. C_{3h} (μM)	CSF $A\beta_{42}$ (-%)
7		70	288	2.74	0.92	32
8		634	32			
9		123	52	0.00	0.00	n.s.e.[c]
10		143	66	0.03	0.00	n.s.e.[c]
11		172	60			
12		85	161	n.a.[d]	n.a.[d]	n.s.e.[c]
13		81	248			
14		182	110			

[a] IC_{50} values are reported as an average of multiple determinations ($n \geq 2$). [b] The ratio represents a measure of GSM selectivity and the ability to reduce the Aβ_{42} without affecting the total Aβ production. [c] n.s.e., no statistically significant efficacy. [d] n.a., not available.

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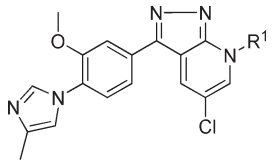
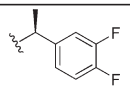
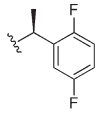
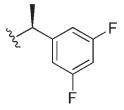
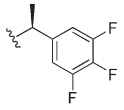
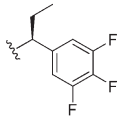
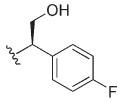
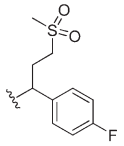
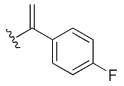
Table 3. Cell $A\beta_{42}$ IC_{50}, γ-Secretase Modulator Selectivity, in Vivo Efficacy, and PK Profile of Compounds 15−22



Compound	R^1	$A\beta_{42}$ IC_{50}[a] (nM)	$A\beta_{total}$ IC_{50} /$A\beta_{42}$ IC_{50}[b]	Pl. C_{3h} (μM)	Br. C_{3h} (μM)	CSF $A\beta_{42}$ (-%)
15		66	301	6.51	0.88	27
16		71	151	2.52	0.74	24
17		38	6			
18		107	186	5.02	1.22	45
19		43	463	7.23	1.61	30
20		168	119	2.15	0.10	n.s.e.[c]
21		316	63			
22		481	42			

[a] IC_{50} values are reported as an average of multiple determinations ($n \geq 2$). [b] The ratio represents a measure of GSM selectivity and the ability to reduce the $A\beta_{42}$ without affecting the total $A\beta$ production. [c] n.s.e., no statistically significant efficacy.

a relatively high efflux ratio, which could be the cause for its limited brain penetration and in vivo efficacy. We decided to explore the pyrazolopyridine core, which might offer better physicochemical properties and efficacy than the tetrahydropyrazolopyridine series (Scheme 1). Herein, we discuss the synthesis and structure−activity relationship (SAR) of this scaffold as a different class of γ-secretase modulators.

The SAR of this pyrazolopyridine series was examined on the R^1 and R^2 substituents. Initially, we varied R^1 while maintaining R^2 as a hydrogen. A series of compounds (**1**−**6**) with a variety of *N*-benzyl groups as R^1 were prepared, and SAR data are presented in Table 1. 4-Methoxybenzyl derivative **1** showed moderate activity ($A\beta_{42}$ IC_{50} = 168 nM).[33] Replacement of the methoxybenzyl group of **1** with a 1-(4-fluorophenyl)ethyl group (**2**) maintained similar potency. After resolving this enantiomeric mixture, the (*S*)-enantiomer **4** was 3-fold more potent than the (*R*)-enantiomer **3**. Compound **5** with a 1-(3,5-difluorophenyl)ethyl group also demonstrated good activity similar to that of **4**. When the methyl substituent attached to benzylic carbon in **4** was changed to an ethyl group (**6**), it slightly reduced the potency. Compounds **4** and **5** were dosed orally in rats at 30 mg/kg, but they did not demonstrate statistically significant efficacy in reducing CSF $A\beta_{42}$. The pharmacokinetics of **4** and **5** revealed that they had limited brain exposures 3 h after dosing (**4**: brain C_{3h} = 0.17 μM; **5**: brain C_{3h} = 0.14 μM).[34] Not surprisingly, analogues **4** and **5** were found to be PGP efflux substrates (**4**: $P_{a\text{-}b}$ = 9 nm/s, $P_{b\text{-}a}$ = 314 nm/s, and *P* ratio = 33.8; **5**: $P_{a\text{-}b}$ = 85 nm/s, $P_{b\text{-}a}$ = 324 nm/s, and *P* ratio = 3.85).

The SAR of R^2 was conducted to further improve the in vitro and in vivo activity, while maintaining R^1 as a (*S*)-(1-(4-fluorophenyl)ethyl group. We prepared a series of analogues (**7**−**14**), for which key data are summarized in Table 2. A variety of functionalities including electron deficient (**7** and **9**−**11**), electron rich (**12**), hydrophilic (**13**), and sterically hindered (**14**)

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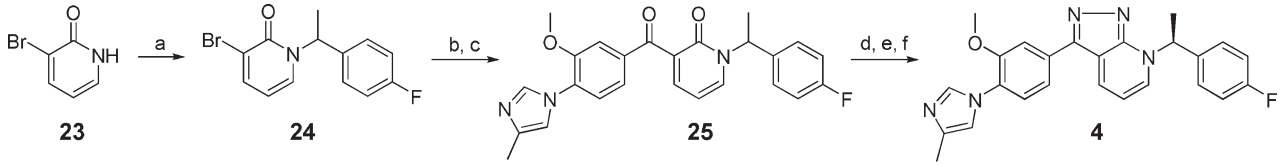
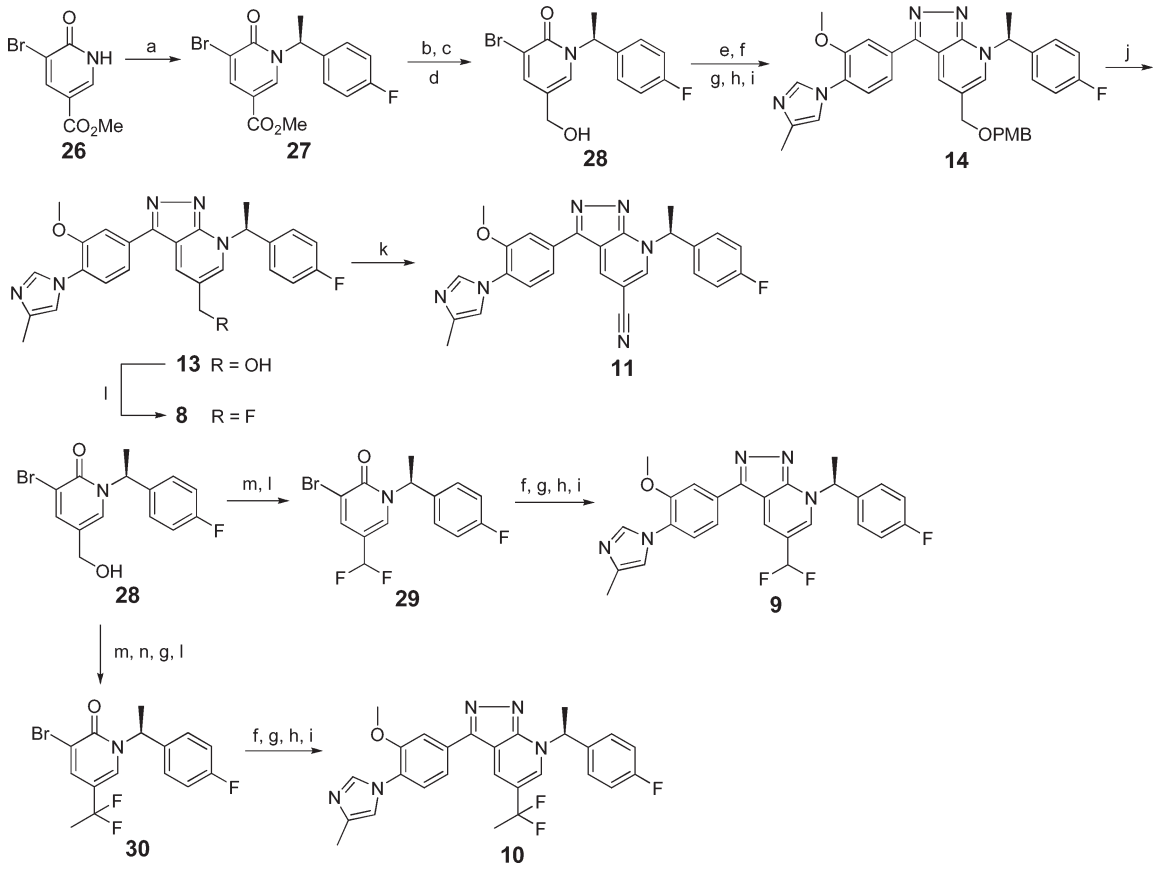
Scheme 2. Synthesis of Compound 4[a]



[a] Reagents and conditions: Method 1: (a) NaH, 1-(1-bromoethyl)-4-fluorobenzene, THF/DMF, 59%. (b) *i*-PrMgCl·LiCl, 3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)benzaldehyde, THF, 48%. (c) Dess−Martin periodinane, CH_2Cl_2, 67%. (d) NH_2NH_2, Py, 60 °C, 39%. (e) $POCl_3$, 71%. (f) Chiral HPLC separation. Method 2: (a) (*R*)-1-(4-fluorophenyl)ethanol, PBu_3, ADDP, THF, 0 → 80 °C, ∼50%. (b−e) The same as method 1.

Scheme 3. Synthesis of Compounds 8−11 and 13[a]



[a] Reagents and conditions: (a) PBu_3, ADDP, (*R*)-1-(4-fluorophenyl)ethanol, THF, 80 °C, 60%. (b) NaOH, H_2O/MeOH, 95%. (c) Cyanuric fluoride, Py, CH_2Cl_2. (d) $NaBH_4$, CH_2Cl_2/MeOH, 81% for two steps. (e) PMBBr, NaH, THF, 81%. (f) *i*-PrMgCl·LiCl, 3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)benzaldehyde, THF, 76%. (g) Dess−Martin periodinane, CH_2Cl_2, 90%. (h) NH_2NH_2, EtOH, 80 °C. (i) $POCl_3$, Py, 50 °C, 25% for two steps. (j) CAN, CH_3CN/H_2O, 76%. (k) NH_3/H_2O, I_2, 60 °C, 10%. (l) DAST, CH_2Cl_2, 15%. (m) PDC, CH_2Cl_2, 98%. (n) CH_3MgBr, THF, 75%.

groups were well tolerated. Compounds with chloride, methyl, or hydroxymethyl group as R^2 (**7**, **12**, and **13**) showed very similar activity to **4**. Analogues bearing larger R^2 substitution such as difluoromethyl (**9**), difluoroethyl (**10**), and 4-methoxybenzyl group (**14**) were slightly less active than **4**. It was also the case for compound **11** with a nitrile group as R^2. One exception was when R^2 was a fluoromethyl group (**8**), which was 6-fold less potent than **4**. Compound **7** (R^2 = Cl) was the most potent derivative from this part of the study ($A\beta_{42}$ IC_{50} = 70 nM). Analogues **7**, **9**, **10**, and **12** were examined in our rat in vivo model, but only **7** demonstrated good efficacy in reducing CSF $A\beta_{42}$ (−32% $A\beta_{42}$, 3 h after 30 mg/kg oral dosing). Not surprisingly, compound **7** had good brain exposure (brain C_{3h} = 0.92 μM), and it was not a PGP efflux substrate, while compounds **9** and **10** had little to no plasma and brain exposure. In the case of **12**, this molecule was a PGP efflux substrate (**12**: $P_{a\text{-}b}$ = 48 nm/s, $P_{b\text{-}a}$ = 412 nm/s, and *P* ratio = 8.50). These SAR data demonstrated that a chloride group was the optimal R^2 substituent for obtaining rat in vivo efficacy.

With chloride established as the best R^2 substituent for in vivo efficacy, we carried out additional SAR at R^1 with the hope of optimizing physicochemical properties such as lipophilicity and brain penetration to positively impact in vivo activity. To this end, a variety of fluorinated *N*-benzyl analogues were then

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synthesized and tested (Table 3). 3,4-Difluorophenyl and 2,5-difluorophenyl derivatives (**15** and **16**) maintained activities similar to that of 4-fluorophenyl analogue **7**. Interestingly, compound **17** with a 3,5-difluorophenyl group was 2-fold more potent than **7** but lost its selectivity as a modulator. Additional fluorination on the phenyl ring (**18**) did not help improve the in vitro activity. Varying the side chain attached to the benzylic carbon from a methyl (**18**) to an ethyl (**19**) group improved in vitro potency 2-fold, but this was not the case for hydroxymethyl (**20**), (methylsulfonyl)ethyl (**21**), or methylene groups (**22**). Analogues **15**, **16**, and **18**−**20** were tested in our rat in vivo model. Compounds **15**, **16**, and **19** were as efficacious as **7** in reducing CSF Aβ_{42}, while **18** was superior (−45% Aβ_{42}, 3 h after 30 mg/kg oral dosing). These compounds demonstrated good plasma and brain exposure. Compound **20** did suffer from poor brain exposure (brain C_{3h} = 0.10 μM). It should be noted that all of the compounds tested in rat did not show efficacy to reduce CSF Aβ_{40} and Aβ_{total} at the dosed level. These compounds did not affect Notch cleavage at concentrations up to 20 μM. The result was consistent with the finding in the Aβ_{total} assay. Notch signaling, assessed by Hes-1 expression, was also not affected in studies conducted in mouse and dog.

The synthesis of **4** is described in Scheme 2. In method 1, alkylation of **23** with 1-(1-bromoethyl)-4-fluorobenzene provided **24**. Coupling of **24** with 3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)benzaldehyde afforded an alcohol intermediate, which was then oxidized to give ketone **25**. Compound **25** was transformed into **4** by hydrazone formation and ring closure, followed by chiral HPLC separation of enantiomers. Alternatively, in method 2, a Mitsunobu reaction of **23** with (*R*)-1-(4-fluorophenyl)ethanol installed the chirality of **4** at an early stage. Compounds **5**, **6**, **15**, **16**, and **18**−**22** were prepared by method 1.[35] Analogues **7**, **12**, and **17** were synthesized by method 2.

Synthesis of compounds **8**−**11** and **13** are presented in Scheme 3. A Mitsunobu reaction of **26** with (*R*)-1-(4-fluorophenyl)ethanol provided chiral ester **27**. This compound was hydrolyzed under basic conditions to give an acid intermediate, which was then activated by cyanuric fluoride and reduced by $NaBH_4$ to afford compound **28**. PMB protection of **28**, followed by coupling with 3-methoxy-4-(4-methyl-1*H*-imidazol-1-yl)-benzaldehyde, gave an alcohol intermediate, which was then converted to **14** by oxidation of this alcohol to a ketone, hydrazone formation from this ketone, and ring closure using $POCl_3$. The PMB protecting group of compound **14** was removed, and the resulting alcohol **13** was converted to compound **8** by DAST treatment. Compounds **9**−**11** were prepared by a similar method to that described for **8**.

In summary, we discovered a series of pyrazolopyridines as potent γ-secretase modulators that demonstrated good in vitro activity for reducing Aβ_{42} production. Several analogues were identified to show statistically significant in vivo efficacy, with compound **18** providing the greatest reduction of CSF Aβ_{42} in rats. This compound underwent additional testing, and the results will be the subject of a future publication.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures for assay protocols as well as synthesis and characterization of compounds **1**−**22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS

AD, Alzheimer's disease; NFT, neurofibrillary tangle; Aβ, amyloid-β; BACE1, β-secretase 1, β-site APP cleaving enzyme 1; APP, amyloid precursor protein; GSIs, γ-secretase inhibitors; GSMs, γ-secretase modulators; CSF, cerebrospinal fluid

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