Bioorganic & Medicinal Chemistry Letters 21 (2011) 4832–4835

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Pyridine-derived γ-secretase modulators

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ARTICLE INFO

Article history:
Received 1 May 2011
Revised 7 June 2011
Accepted 10 June 2011
Available online 17 June 2011

Keywords:
γ-Secretase inhibitor
γ-Secretase modulator
SAR
Pyridine

ABSTRACT

SAR of a novel series of pyridine-derived γ-secretase modulators is described. Compound **5** was found to be a potent modulator in vitro, which on further profiling, was found to decrease Aβ42 and Aβ40, and maintain (or increase) the levels of total Aβ. Furthermore, representative compounds **1** and **5** demonstrated in vivo efficacy to lower Aβ42 in the brain without altering Notch processing in the peripheral.

Alzheimer's disease (AD) is a chronic and relentlessly progressive neurologic disorder, affecting over 30 million people worldwide, and is characterized by specific patterns of neuronal loss leading to significant cognitive and other functional impairments.[1] The deposition of fibril Tau and Aβ within the CNS are the defining neuropathological hallmarks of AD. The latter are thought to be causative of the disease and consist of amyloid-beta (Aβ) peptides of 40–42 amino acids (Aβ40 and Aβ42, respectively).[2] These peptides are produced from amyloid precursor protein (APP) by the sequential action of β-secretase and γ-secretase, thus a significant effort has been invested to identify inhibitors of either enzyme to reduce Aβ production.[3] γ-secretase inhibitors (GSIs) have been shown to successfully reduce brain Aβ dose-dependently in preclinical models.[4] Several GSIs have been progressed to clinical development with LY-450139 as the most advanced. However, its Phase III clinical trial was recently halted due to lack of cognitive improvement in AD patients and higher risk of developing skin cancer upon treatment (Eli Lilly News Release, 2010).

Consistent with the unexpected outcomes of LY-450139, some preclinical studies showed that inhibition of γ-secretase may impair learning and memory[5] and induce skin cancer.[6] Causes of these adverse outcomes are not completely understood. However, it is known that many other substrates are cleaved by γ-secretase in addition to APP.[7] Toxicity may arise through inhibition of the processing of these other substrates. For example, inhibition of cleavage of Notch, which is a known γ-secretase substrate and a transmembrane receptor involved in the regulation of cell differentiation, leads to abnormalities in the gastrointestinal tract and the immune system.[8] To avoid adverse reactions mediated by inhibition of Notch processing, research focus has been shifted to devel-

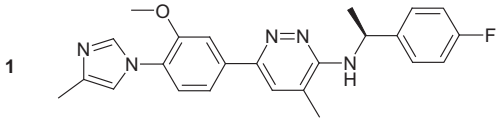
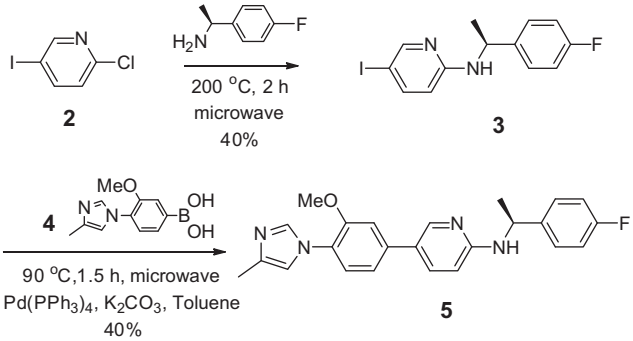


Figure 1. Pyridazine-derived GSM.



Scheme 1. Synthetic strategy for analogs **5** and **11–24**.

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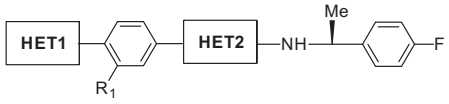
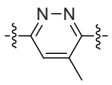
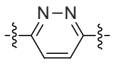
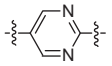
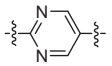
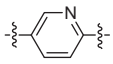
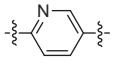
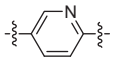
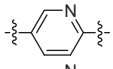
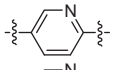
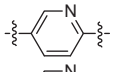
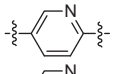
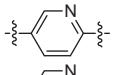
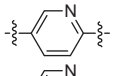
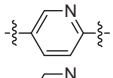
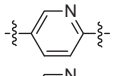
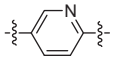
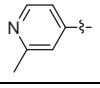
doi:10.1016/j.bmcl.2011.06.042

Table 1
In vitro SAR for pyridine derivatives



Compd	HET1	R_1	HET2	Aβ42[a] pIC_{50}	Aβ40[a] pIC_{50}	Notch[a] pIC_{50}
1		$-OCH_3$		7.2	6.7	5.0
11		$-OCH_3$		7.2	6.7	<4.7
12		$-OCH_3$		6.7	6.0	5.0
13		$-OCH_3$		6.6	5.9	5.0
5		$-OCH_3$		6.9	6.1	5.0
14		$-OCH_3$		6.2	5.0	5.2
15		$-OCH_3$		6.1	4.3	NA
16		$-OCH_3$		7.1	6.2	NA
17		$-OCH_3$		6.5	4.5	NA
18		$-CONHCH_3$		<4	<4	NA
19		$-N(CH_3)_2$		4.3	4.4	NA
20		–Ph		4.8	5.0	NA
21		$-CH_3$		5.2	4.8	NA
22		–Cl		6.4	5.7	NA
23		$-OCH_2CH_3$		6.3	5.3	NA
24		$-OCH(CH_3)_2$		5.5	5.1	NA
10		$-OCH_3$		6.6	5.7	4.8
25		$-OCH_3$		6.4	5.7	4.9
26		$-OCH_3$		6.4	5.3	5.0
27		$-OCH_3$		6.3	5.2	5.2

[a] Aβ42, Aβ40 and Notch were assayed in the same way as reported previously.[10]

oping γ-secretase modulators (GSMs). Heterogeneous proteolysis of APP by γ-secretase leads to the production of Aβ peptides in various lengths, ranging from 37 to 43 amino acid residues. While GSIs inhibit production of all Aβ peptide species, GSMs shift the cleavage site towards production of shorter nonamyloidogenic peptides.[9] Because GSMs selectively inhibit γ-secretase activity, Notch processing is spared and its signaling function is largely intact. Therefore, GSMs are believed to be a better tolerated Aβ-lowering approach than GSIs.[10,11]

Our research program was aimed at discovering novel, potent and brain penetrant GSMs for AD. The previous letter described a novel series of pyridazine-derived GSMs (**1**, Fig. 1).[12] Herein, we describe the subsequent optimization of this series leading to the identification of a novel pyridine series. Proof of in vivo activity

will be disclosed for both pyridazine and pyridine series in this Letter.

Synthesis: Pyridine derivative **5** was prepared from 2-chloro-5-iodopyridine (**2**, Scheme 1). Displacement of chlorine with (*S*)-1-(4-fluorophenyl)-ethanamine provided intermediate **3**. Suzuki coupling with boronic acid **4** then afforded compound **5**. This synthetic route was utilized to prepare compounds **11–24** (Table 1) by replacing **2** and **4** with appropriate reactants. Compound **10** was synthesized from 4-bromo-3-methoxyphenyl 4-methylbenzenesulfonate (**6**, Scheme 2). Formation of boronic acid **7** followed by Suzuki coupling with 5-bromo-3-methylisothiazole provided **8**. Another round of acid (or boronate) formation and Suzuki coupling then furnished **10**. This synthetic sequence was applied to prepare pyridine derivatives **25–27** (Table 1) by selecting appropriate starting materials to replace the isothiazole.

Results and discussion: After discovering pyridazine analog **1** as a potent and brain penetrant γ-secretase modulator, we then turned our attention to exploring the SAR profile of the two heterocycles (HET1 and HET2) and substituent R_1 in order to identify another potent GSM from a different chemical class for in vivo evaluation. Replacement of the pyridazine (HET2) with other 6-/5-membered heterocycles was explored first. Pyrimidine analogs (**12** and **13**) are slightly less potent. Regio-isomers of pyridine exhibit different potency and 2-pyridine (**5**) is more potent than 3-pyridine (**14**). Thiadiazole (**16**) is the most potent among the three 5-membered heteroaryl groups we studied and its potency is similar to that of **1** and **11**.

Having identified several heteroaryl groups which afforded good levels of in vitro potency, we selected 2-pyridine due to its similarity to pyridazine and fixed this while we varied R_1 and HET1. R_1 was investigated next, but very tight SAR was observed. Methyl amide group (**18**), electron rich dimethyl-amino group (**19**) and the bulky phenyl group (**20**) were detrimental to potency. Methyl (**21**), chlorine (**22**), ethoxy (**23**) and isopropoxy (**24**) also resulted in reduced potency when compared with methoxy (**5**). HET1 was studied last. Isothiazole (**10**), pyridazine (**25**), thiazole (**26**) and pyridine (**27**) are tolerated, but less potent than imidazole (**5**). Compound **5** also contains the fragment of 1-(2-methoxyphenyl)-4-methyl-1*H*-imidazole, a common component of known GSMs[11c], variation of the R_1 substitution position (e.g., 2-OCH_3) was not studied at this stage.

Following in vitro potency assessment compounds with a pIC_{50} value $\geqslant 6$ were assessed in terms of in vitro metabolic stability in rat and human liver microsomes and for inhibition of five human CYP isoforms (CYP1A2, 2C9, 2C19, 2D6 and 3A4). Selected analogs were profiled to evaluate their potential as time-dependent CYP inhibitors. Based on these data, analog **5** emerged as the most interesting compound for studying mechanism of action (Fig. 2). SHSY5Y-APPswe cells were thus pre-incubated with **5** for 24 h. Medium was harvested and subject to analysis of Aβ42, Aβ40, total Aβ and cell viability (determined via WST-1). Total Aβ in this assay system includes all Aβ generated by γ-secretase, such as Aβ36, Aβ37, Aβ38 and Aβ40. Since levels of Aβ42, Aβ40 and total Aβ were determined using different assays, the magnitude of reduction of Aβ42 and Aβ40 and the magnitude of increase of total Aβ were not completely matched. The levels of Aβ42 and Aβ40 were decreased in a concentration-dependent manner after treatment (0.01–3 μM), while total Aβ levels were maintained or increased indicating the rise in shorter Aβ species compensating for the decrease in Aβ42 and Aβ40. This is in contrast to the profile of inhibitors such as LY-450139 which decreases levels of all Aβ species and thus the total Aβ. Furthermore, **5** was found not to be cytotoxic and it had no effect on cellular viability up to 3 μM.

The in vivo pharmacokinetic profile of **5** is listed in Table 2. Compound **5** demonstrated low clearance and excellent oral bioavailability in the rat.

Compound **5** (Table 3) also demonstrated good brain penetration and showed that a 30 mg/kg oral dose could deliver brain concentrations of 8.7 μM which is significantly higher than the in vitro IC_{50} (0.126 μM at Aβ42 and 0.8 μM at Aβ40) values indicating that compound **5** has the potential to deliver in vivo efficacy at low doses.

With two brain penetrant and orally bioavailable γ-secretase modulators **1** and **5** in hand, we then evaluated their in vivo efficacy at lowering Aβ42 upon sub-chronic oral dosing. Both compounds were dosed to naive SD rats for 5 days consecutively

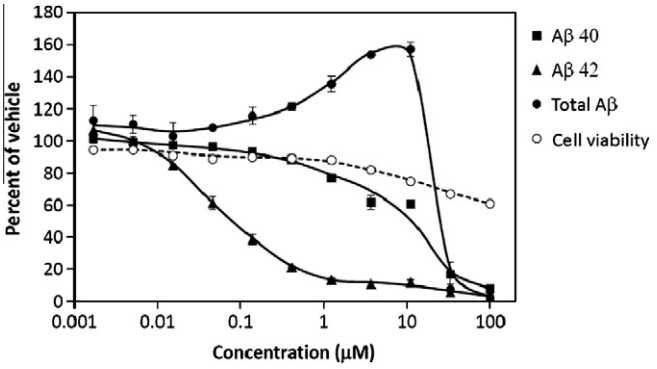
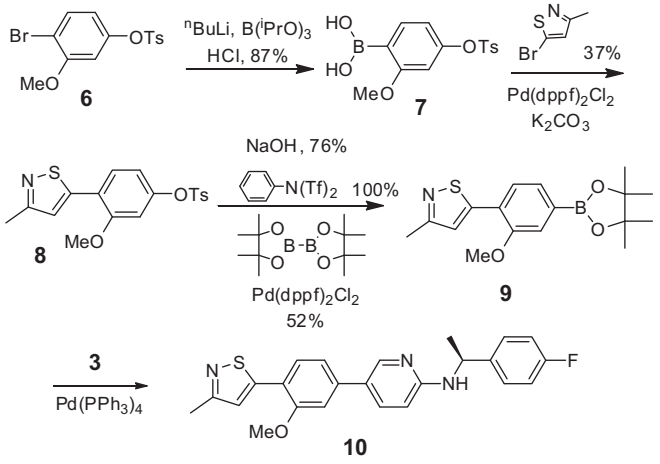


Figure 2. Effect of **5** on Aβ levels and cell viability in SHSY5Y-APPswe cells.



Scheme 2. Synthetic strategy for analogs **10** and **25–27**.

Table 2
Rat PK (iv × po) of **5**[a]

CLb (mL/min/kg)	Vdss (L/kg)	t½ (h)	Fpo (%)
12.2	3.7	3.3	100

[a] Crystalline HCl salts were dosed to SD rats at 1 mg/kg (iv) and 2 mg/kg (po) with vehicle of 1% (w/v) methylcellulose (po) and DMSO solution with 10% HP-β-CD (w/v) (iv).

Table 3
Rat CNS penetration data of **5**[a]

Blood conc (μM)	Brain conc (μM)	Br:Bl
4.983 ± 1.381	8.745 ± 1.802	1.74 ± 0.33

[a] Compound was dosed orally in (30 mg/kg) 1% (w/v) methylcellulose aq. Values are the mean from three rats. Samples were taken 6 h post-dose.

(30 mg/kg/day, oral). Animals were sacrificed 6 h after the last dose. Brain samples were collected and homogenized to analyze the levels of Aβ42 and compound concentration. Reduction of 45.7% ($p < 0.01$) and 27.6% ($p < 0.01$) at Aβ42 and brain concentration of 10.4 and 8.86 μM were achieved by compounds **1** and **5**,[13] respectively. At the end of the same study, measurement of Hes-1 mRNA levels from the ileum and flow cytometric assessment of CD4 and CD8 markers in thymocytes were also carried out. No significant difference from the vehicle control group was observed, suggesting a lack of effect for both compounds on Notch processing, which is consistent with their in vitro Notch inhibition data as shown in Table 1.

In summary, a novel series of pyridines was discovered as potent γ-secretase modulator. Representative analog **5** from the series demonstrated the mechanism of action consistent with that of GSMs. Both pyridine derivative **5** and pyridazine derivative **1** displayed in vivo efficacy to inhibit Aβ42 without altering Notch processing.

Acknowledgment

Helpful discussion with Dr. Jeffrey K. Kerns during manuscript preparation is acknowledged.

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13. Characterization of compound **1**: ^{1}H NMR (400 MHz, MeOD) δ 9.26 (d, J = 1.5 Hz, 1H), 8.43 (s, 1H), 7.88 (s, 1H), 7.77 (s, 2H), 7.67 (s, 1H), 7.55–7.50 (m, 2H), 7.14 (t, J = 8.8 Hz, 2H), 5.25 (dd, J = 7.0, 13.8 Hz, 1H), 4.05 (s, 3H), 2.61 (s, 3H), 2.46 (s, 3H), 1.77 (d, J = 7.0 Hz, 3H) LC–MS m/z 418 $[M+H]^+$; compound **5**: ^{1}H NMR (400 MHz, MeOD) δ 8.98 (s, 1H), 8.25 (d, 1H), 7.96 (dd, J_1 = 2.4 Hz, J_2 = 9.2 Hz, 1H), 7.56–7.52 (m, 2H), 7.46–7.41 (m, 3H), 7.41 (m, 1H), 7.31 (dd, J_1 = 2.2 Hz, J_2 = 8.4 Hz, 1H), 7.06 (t, J = 7.8 Hz, 2H), 6.81(d, J = 8.8 Hz, 1H), 5.03 (q, J = 6.8 Hz, 1H), 3.98 (s, 3H), 2.42 (s, 3H), 1.58 (d, J = 6.8 Hz, 3H) LC–MS m/z 403 $[M+H]^+$.