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Pyridazine-derived γ-secretase modulators

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ABSTRACT

SAR of a novel series of pyridazine-derived γ-secretase modulators is described. Compound **25** was found to be a potent modulator in vitro, which on further profiling, was found to decrease Aβ42 and Aβ40, and maintain the levels of total Aβ. Furthermore, **25** demonstrated excellent pharmacokinetic parameters as well as good CNS penetration in the rat.

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

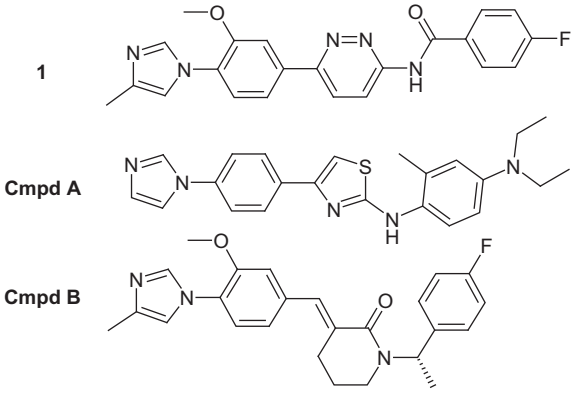

Figure 1. Analogs with phenyl-imidazole fragment.

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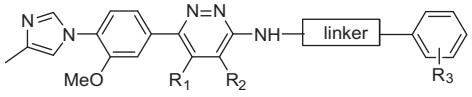
processing, research focus has been shifted to developing γ-secretase modulators (GSMs). Heterogeneous proteolysis of APP by γ-secretase leads to the production of Aβ peptides in various lengths, ranging from 37 to 43 amino acid residues. While GSIs inhibit production of all Aβ peptide species, GSMs shift the cleavage site towards production of shorter non-amyloidogenic 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.

Our research program was aimed at discovering novel, potent and brain penetrant GSMs for AD.[10] A pyridazine amide analog (**1,** Fig. 1) was identified through cross-screening of our internal compound collection. This compound also contains phenylimidazole fragment, a common component of known GSMs (e.g., **A** and **B**).[11] However, compound **1** behaved more alike a GSI due to its potent inhibition of Notch processing (Table 1). A chemistry program was then initiated to optimize this template. Our initial objective was to identify GSMs with selectivity greater than 100 fold over Notch processing favoring inhibition of Aβ42 production. Greater attention was paid to Aβ42 due to the less clear pathological role of Aβ40.

Synthesis. Pyridazine derivative **6** was prepared from aniline 2 (Scheme 1). Bromination with NBS, diazotization, and displacement with KI afforded **3**, which was coupled with 4-methyl-imidazole to provide **4**. Formation of the boronic acid, Suzuki coupling with 3,6-dichloro-pyridazine, followed by Buchwald reaction with (*S*)-1-(4-fluorophenyl)-ethanamine then furnished compound **6**. This synthetic route was utilized to prepare compounds **12**–**25** (Table 1) by using appropriate dichloro-pyridazines and amines.

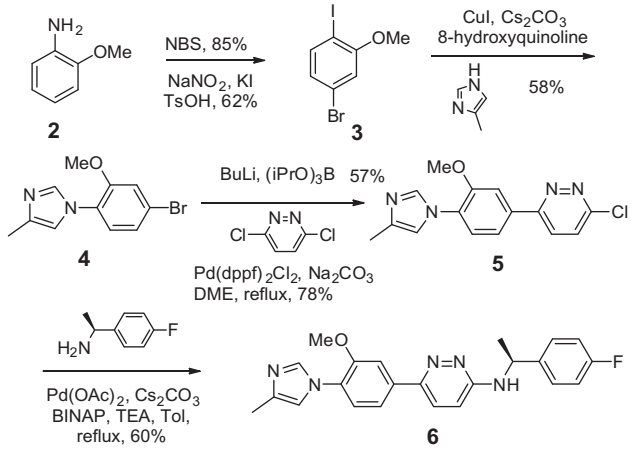
Compound **11** was synthesized from 1-[4-iodo-3-(methyloxy)phenyl]ethanone **7** (Scheme 2). Coupling with 4-methyl-imid-

Table 1
In vitro SAR for pyridazine derivatives

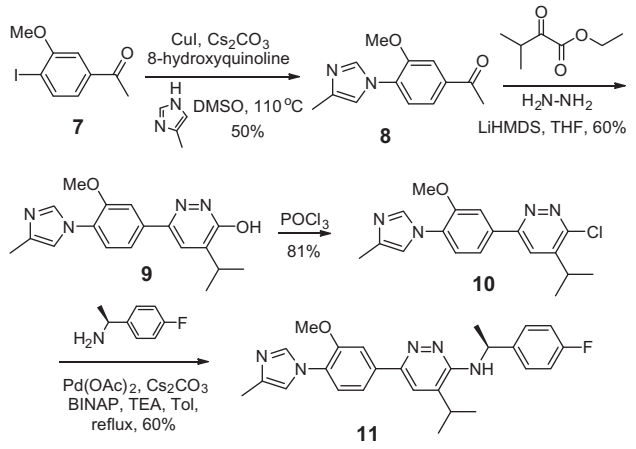


Compd	R_1	R_2	Linker	R_3	Aβ42[a] pIC_{50}	Aβ40[a] pIC_{50}	Notch[a,b] pIC_{50}
1	H	H		4-F	5.6	4.7	6.0
12	H	H	CH_2	4-F	6.5	6.2	<4.7
13	H	H		4-F	7.0	6.1	agonist
14	H	H		4-F	6.2	6.0	<4.7
6	H	H		4-F	7.2	6.7	<4.7
15	H	H		H	7.1	6.8	<4.7
16	H	H		3-F	7.2	6.9	<4.7
17	H	H		2-F	7.3	7.1	5.0
18	H	H		4-OCH_3	6.8	6.8	<4.7
19	H	H		4-CF_3	7.3	6.9	<4.7
20	H	H		4-F	7.3	6.9	5
21	H	H		H	6.8	6.5	<4.7
22	H	H		4-F	7.4	6.8	4.7
23	CH_3	CH_3		4-F	6.5	5.9	<4.7
24	CH_3	H		4-F	6.7	6.3	4.8
25	H	CH_3		4-F	7.2	6.7	5.0
26	H	CN		4-F	6.9	5.8	4.8
27	H	CH_2CH_3		4-F	7.2	6.8	agonist
11	H	$CH(CH_3)_2$		4-F	7.3	7.0	agonist
28	H	CF_3		4-F	7.0	5.6	agonist

[a] Aβ42, Aβ40 and Notch were assayed in the same way as reported previously.[10]
[b] When screened at 10 μM, greater than 30% increase of luciferase activity signal was displayed by agonists in the Notch reporter assay.[10]



Scheme 1. Synthetic Strategy for Analogs **6** & **12–25**



Scheme 2. Synthetic Strategy for Analogs **11** & **26–28**

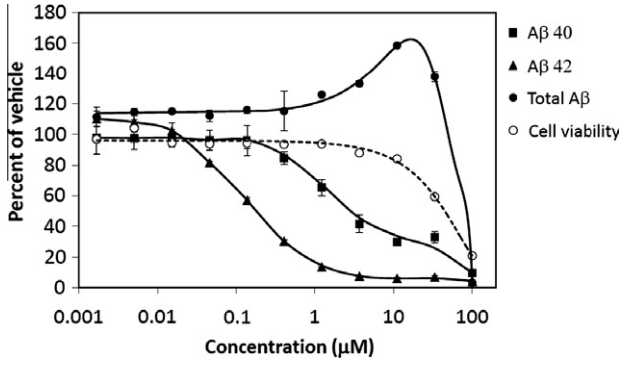


Figure 2. Effect of 25 on Aβ levels and cell viability in SHSY5Y-APPswe cells.

Table 2
Rat PK (iv × po) of 25[a]

CLb (mL/min/kg)	V_{dss} (L/kg)	$t_{½}$ (h)	Fpo (%)
5.1	1.4	3.1	98

[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 .25[a]

Blood Conc. (μM)	Brain Conc. (μM)	Br:Bl
15.998 ± 1.709	9.085 ± 3.257	0.56 ± 0.16

[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.

azole followed by ring formation with ethyl 3-methyl-2-oxobut anoate and hydrazine provided **9**. Chlorination with $POCl_3$ and Buchwald coupling with (*S*)-1-(4-fluorophenyl)-ethanamine then furnished **11**. This synthetic sequence was applied to prepare pyridazine derivatives **26–28** (Table 1) by choosing appropriate α-keto-esters for the ring formation.

Results and discussion. Compound **1** displays comparable potency to inhibit both Aβ42 production and Notch processing and is thus more alike a GSI, although about 10-fold less potent to inhibit Aβ40 production. Replacement of the carbonyl group with a simple CH_2 not only improved potency against Aβ42/40, but also demonstrated more than 60-fold selectivity at Notch (**12**). Di-substitution of the carbon linker (**13**) further improved its potency at Aβ42, but to our surprise, this compound enhanced Notch processing at a high concentration (10 μM). It is very interesting to note that this enhancement was reduced through mono-substitution (**14** and **6**) with the *S*-enantiomer **6** being 10 fold more potent than the *R*-enantiomer **14**. Different substituents on the phenyl ring (**15–19**), ranging from strong electron donating group (OCH_3) to strong electron withdrawing group (CF_3), did not significantly alter potency. A few other chiral linkers were well tolerated (**20–22**).

Having identified several chiral benzyl amines which provided good levels of in vitro potency, we selected (*S*)-1-(4-fluorophenyl)-ethanamino group due to convenient access and fixed this while we varied the substitution of the pyridazine ring. Di-substitution with methyl group (**23**) resulted in loss of potency. Mono-substitution of $R_2 = CH_3$ gave rise to compound **25** with potency similar to **6** but with a CYP inhibition profile much improved (data not shown). Nitrile was also tolerated at this position (**26**). Substitution with larger groups (**27, 11 and 28**) resulted in enhanced Notch processing as noticed previously, although still potent at inhibiting Aβ42.

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 analogues were profiled to assess their potential as time-dependent CYP inhibitors. Based on these data, compound **25** emerged as the most interesting compound for studying mechanism of action (Fig. 2). SHSY5Y-APPswe cells were thus pre-incubated with **25** 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, 37, 38 and 40. Levels of Aβ42 and Aβ40 were decreased in a concentration-dependent manner after treatment (0.01–3 μM), while total Aβ levels were maintained 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. Cytotoxicity was exhibited when **25** was tested at higher concentrations.

The in vivo pharmacokinetic profile of **25** is listed in Table 2. Compound **25** demonstrated low clearance and excellent oral bioavailability in the rat.

Compound **25** (Table 3) also demonstrated good brain penetration and showed that a 30 mg/kg oral dose could deliver brain concentrations of 9.1 μM which is significantly higher than the in vitro IC_{50} (0.06 μM at Aβ42 and 0.2 μM at Aβ40) values indicat-

ing that compound **25** has the potential to deliver in vivo efficacy at low doses. Indeed, greater than 20% reduction of brain Aβ42 was achieved after rats were treated with **25** at 30 mg/kg (6 h post dose).

In summary, we have identified a novel and potent γ-secretase modulator **25** which has good pharmacokinetic properties in the rat. This compound achieved good brain penetration in the rat and is suitable to explore the efficacy of γ-secretase modulator in this species. Data from in vivo efficacy studies will be reported in due course.

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